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Physiological Investigation of Lycium Barbarum's Protective Effects on Reproductive Hormones and Antioxidant Status Against Sodium Dichromate-Induced Toxicity in Male Rats

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Abstract

This study investigated the protective effects of Lycium barbarum (goji berry) on sodium dichromate-induced toxicity in terms of functional and reproductive aspects of male sexual dysfunction, antioxidant status, testicular reproductive hormones, and fertility impairments in adult male rats. The hormones assessed in the study included testosterone, follicle-stimulating hormone (FSH), and luteinizing hormone (LH), as well as oxidative stress markers (namely, nitric oxide [NO]), antioxidant activity (superoxide dismutase [SOD]), and parameters of renal function (creatinine and urea). Four groups of twenty-four adult male rats were divided (n=6}/group). For four weeks, the first group was given distilled water as a control; for four weeks, the second group received an oral dose of Lycium barbarum (300 mg/kg body weight); for four weeks, the third group received an intraperitoneal injection of sodium dichromate (10 mg/kg body weight); and for four weeks, the fourth group received an injection of sodium dichromate (10 mg/kg body

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weight) followed by an oral dose of Lycium barbarum (300 mg/kg body weight). The results showed that the Lycium barbarum-treated group had better renal function (lowered urea and creatinine), increased SOD activity, decreased NO levels, and significantly increased testosterone, FSH, and LH levels. On the other hand, sodium dichromate intoxication increased oxidative stress (NO) and impaired renal function while drastically lowering levels of reproductive hormones and SOD activity. In summary, Lycium barbarum demonstrated protective benefits against oxidative stress and testicular damage caused by sodium dichromate, indicating its potential as a therapeutic agent to reduce renal and reproductive toxicity.

Keywords:

Lycium barbarum, sodium dichromate, reproductive hormones, antioxidants, oxidative stress, kidney function.

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Introduction

Global fertility rates have experienced a marked decline in recent decades, with male infertility emerging as a critical contributor to this public health challenge (Babakhanzadeh et al., 2020). The testes, integral to spermatogenesis and endocrine regulation, are particularly vulnerable to dysfunction, which may manifest as compromised sperm quality, hormonal imbalances, and ultimately infertility. Etiological factors include environmental toxicants such as heavy metals (sodium dichromate), endocrine disruptors (e.g., diethylstilbestrol, phthalates), and industrial chemicals, with hexavalent chromium (sodium dichromate) compounds representing a significant occupational and environmental hazard (Rim, 2017). Sodium dichromate dihydrate, a prevalent sodium dichromate Cr(VI) compound utilized in electroplating, textile manufacturing, and cement production, is classified as carcinogenic and cytotoxic Occupational exposure occurs primarily via inhalation, while ingestion of contaminated water or food further exacerbates systemic Cr(VI) bioaccumulation, as evidenced by elevated chromium levels in biological matrices of exposed workers (Stohs et al., 2001). Epidemiological studies link chromium exposure to reduced semen quality, sperm DNA fragmentation, and histopathological testicular damage, likely mediated by reactive oxygen species (ROS) generation. Cr(VI)-induced oxidative stress (Lopez et al., 2024) disrupts cellular homeostasis via lipid peroxidation, protein denaturation, and genomic instability, thereby impairing spermatogenic efficiency and steroidogenesis) (Kumar et al., 2005; Yadav & Yadav, 2014; Yu et al., 2023). Occupational exposure to chromium has been consistently associated with increased levels of this element in biological matrices, including blood, urine, and specific tissues. This accumulation is often associated with prolonged or repeated exposure to the element in their work environment, highlighting the potential for systemic absorption and retention of chromium within the body. (Maqbool et al., 2017). Employees in the welding business who were exposed to chromium were more likely to have infertility due to aberrant sperm and decreased semen quality (Maqbool et al., 2017). After intraperitoneal injection, chromium was shown to be too concentrated in the testes, causing damage to the mice's seminiferous epithelium (Saxena et al., 1990). The interaction with proteins and DNA molecules can lead to significant structural and functional alterations, resulting in severe damage that may manifest as various pathological conditions. Such molecular disruptions are often linked to the onset of illnesses and can be influenced by dietary and environmental factors. It can hurt fertility and increase the risk of developing sterility, which can impair reproduction (Lee et al., 2005).

In this context, natural phytochemicals with antioxidative and anti-inflammatory properties are increasingly investigated as therapeutic adjuvants (Çetinkaya, 2020; Sungur & Atan, 2020). Lycium barbarum functional food and medicinal herb, has been employed in traditional East Asian medicine for millennia to enhance vitality, ameliorate age-related pathologies, and support reproductive health (Yan et al., 2019). A few physiological characteristics that Lycium barbarum has are highly protective of the liver, has anti-fatigue and anti-aging properties, and decreases blood glucose and cholesterol. They also help with immunological modulation, inflammation, and even tumor growth (Potterat, 2010).

Along with their source of carotenoid zeaxanthin, phenolic acids, vitamins, and polysaccharides make them nutrient-dense, which allows them to perform bioactivities such as scavenging for ROS (reactive oxygen species), immune modulation, and cytoprotection (Xin et al., 2013). The berries of L. barbarum are popular in East Asia as food and have gained fame as a superfood worldwide. These types of plants with essential nutrients and bioactive compounds combining vitamins, dietary fibers, minerals, and many other phytochemicals serve as both food and medicine. Out of the considered species that fall under this genus, particular focus is given to three: Lycium ruthenicum Murray, Lycium barbarum L., and Lycium global, which are primarily known to the East for Horsetail of great medicinal value, and in the West for Herbal Remedies of reputed efficacy for chronic diseases, obesity, and other metabolic syndrome disorders (Jiang et al., 2024). Preclinical studies show that L. barbarum extracts help reduce oxidative injuries to testes, retain the structure of seminiferous tubules, and enhance the quality of sperm in the models of toxin-induced infertility (Qi et al., 2022). Additionally, it serves the purpose of metabolic regulation, hepatoprotection, and neuroprotection which makes it valuable as a multifunctional agent in the management of chronic diseases (Yu et al., 2023). From among the Lycium genus, L. barbarum (red-fruited variant) is well known because of its high carotenoid which helps in eyesight, anti-aging, and boosting fertility. This phytotherapeutic model set the need to evaluate reproductive and other bioactive compounds that can help mitigate manmade reproductive disorders, like many other herbs, the fruit of the Lycium barbarum plant is predicted to promote active mobility, protect the reproductive system, slow down aging, and possibly increase lifespan (Potterat, 2010).

Materials and Methods

Preparation of the Plant

The dried fruits of Lycium barbarum were processed into smaller particles, integrated with the other dietary components, and then compacted into pellet form (Castrica et al., 2020). Each rat consumed its entire daily portion.

Experimental Design

In this study, twenty-four adult male rats, the animals were divided into four groups, with six individuals in each group. The first group, serving as the control, was administered distilled water over four weeks. The second group received oral supplementation of Lycium barbarum at a dosage of 300 mg per kilogram of body weight (BW) for four weeks, in conjunction with their standard diet. In contrast, the third group was subjected to an intraperitoneal injection of sodium dichromate at a concentration of 10 mg per kilogram of body weight (BW). For the same duration of four weeks. The fourth group received an injection of sodium dichromate intraperitoneally and then received Lycium barbarum orally with diet for four weeks, with a half-hour interval between injections.

Sex hormones ASSAY: estimation of testosterone hormone concentration observed by (Mujika et al., 1996), estimation of (FSH) concentration, and estimation of (LH) concentration according to (Uotila et al., 1981).

Oxidant and anti-oxidant Enzymes Assay: Serum nitric oxide measurement (NO), and anti-oxidant enzymes indices assessment of superoxide dismutase activity (SOD) according to (Kavsak et al., 2017).

Kidney Function Test: a unique kit called Spectrum–creatinine kit, Egypt-IFUFCC10, was used to test the amount of creatinine in serum using a semi-auto chemical analyzer (Dunn et al., 2004) and a spectrophotometer made by Sesil, England. The Semi-auto chemistry analyzer (Jing et al., 2018) was used to test the serum urea using the Egypt-IFUFCC40 Spectrum-urea kit.

Results

Sex Hormones Assay Estimation

The results reported lower significant differences in sex hormones (Testosterone, FSH, and LH) in the sodium dichromate-treated group (4.13±0.23, 4.62± 0.25, and 5.21±0.24) respectively, as compared with other groups. The 3 and 4 groups showed significantly improved sex hormones in Lycium herbarium, and their combination of sodium dichromate with Lycium barbarum compared to sodium dichromate-treated rats, as shown in table (1).

Table 1. Effect of lycium barbarum, sodium dichromate and their combination on some serum sex hormones in adult male rats (Mean±SE)

Groups	Parameters (Mean ± SD)		
	Testosterone (ng/ml	FSH (mIU/ml)	LH (mIU/ml)
Control	6.01 ± 0.32^{a}	3.24 ± 0.43^{c}	4.12 ± 0.32^{cd}
Sodium dichromate (U/L)	4.13 ± 0.23^{c}	3.18 ± 0.23^{c}	3.85 ± 0.37^{d}
Lycium barbarum (U/L)	6.18 ± 0.27^{a}	4.62 ± 0.25^{a}	5.21 ± 0.24^{a}
Sodium dichromate + Lycium barbarum (U/L)	5.35 ± 0.29^{b}	4.12 ± 0.28^{bc}	4.68 ± 0.37^{b}
LSD	0.59	0.47	0.38

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

Oxidant and Antioxidant Enzymes Assay

Serum activities of antioxidant enzyme superoxide dismutase (SOD) is A significant increase was observed in Groups three and four when compared to Group two. Serum NO level is increased in Group 2 (Treatment with sodium dichromate) compared with other groups (Groups 1, 3, and 4), as shown in (table, 2).

Table 2. Effect of lycium barbarum, sodium dichromate and their combination on some antioxidants in adult male rats (Mean±SE).

Groups	Parameters (Mean ± SD)	
	NO (µmol/mg)	SOD (U/mg)
Control	5.2 ± 0.62^{a}	11.8 ± 2.30^{ab}
Sodium dichromate (U/L)	2.5 ± 0.93^{c}	7.5 ± 2.32^{d}
Lycium Barbarum (U/L)	5.7 ± 0.74^{a}	12.1 ± 1.53^{a}
Sodium dichromate + Lycium Barbarum (U/L)	3.8 ± 0.56^{b}	9.6 ± 2.01^{b}
LSD	1.37	2.27

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

Kidney Function

In comparison to the other groups the blood levels of kidney parameters (urea and creatinine) in the sodium dichromate treated group (39.6 ± 3.22, 0.835 ± 0.087) respectively reported higher significant ($p \leq 0.05$) values (Table 3). Furthermore, as compared to the sodium dichromate, the urea and creatinine levels of the Lycium barbarum and combination sodium dichromate + Lycium barbarum groups (three and four) were significantly lower.

Table 3. Effect of Lycium barbarum, sodium dichromate and their combination on some kidney serum function in adult male rats (Mean±SE).

Groups	Parameters (Mean±SD)	
	Urea (mg/dl)	Creatinine (mg/dl)
Control	29.1± 4.74[c]	0.462 ± 0.024[d]
Sodium dichromate (U/L)	39.6 ± 3.22[a]	0.835 ± 0.087[a]
Lycium Barbarum (U/L)	32.4 ± 2.97[b]	0.538 ± 0.045[c]
Sodium dichromate + Lycium Barbarum (U/L)	34.7 ± 2.85[b]	0.721 ± 0.071[b]
LSD	3.42	0.19

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

Discussion

The fruit of the Lycium barbarum plant is widely recognized as a natural nutraceutical due to its rich composition of physiologically active compounds. These bioactive constituents have been shown to confer numerous health benefits for both humans and animals. The fruit, in particular, can alter metabolites and hormones. In addition to dramatically raising the blood levels of testosterone, FSH, and LH in adult male rats, the treatment of Lycium barbarum also demonstrated protective effects against testis damage and decreased the testis spermatic injury caused by sodium dichromate. These results provide credence to the theory that goji berries may influence the release of reproductive hormones, which in turn may impact the reproductive performance of rats and other animals. Together with the pituitary gland and hypothalamus, the testis, the central component of the male reproductive system, participates in spermatogenesis and androgen synthesis (Yang et al., 2016). Androgen is primarily made up of testosterone. The primary findings of research evaluating the opposing effects of antioxidants and heavy metals on male fertility. Several animal models have shown evidence of testicular injury following exposure to sodium dichromate. Antioxidants lessen oxidative stress in spermatozoa exposed to sodium dichromate, reducing negative effects on their function and fertility. Oxidative stress elevation represents the main factor underlying the adverse effects induced by chromium exposure (Jobson & Subhashini, 2017). Treatment with sodium dichromate significantly reduced testosterone levels, sperm count, and motility, as well as the weights of the body, testes, and accessory sex organs (Hfaiedh et al., 2014). The mechanism of action in rabbits may involve alterations in pituitary receptors, enhanced secretion from GnRH-secreting neurons, or the production of LH by the pituitary gland (Andoni et al., 2021). Based on these observations, it can be hypothesized that Lycium barbarum may exert regulatory effects on the hypothalamic-pituitary-testicular pathway safeguarding the testis's structure and functionality (Sun et al., 2018). Likewise, demonstrated that the groups receiving low, medium, and high doses of Lycium barbarum had significantly higher levels of serum testosterone, LH, FSH, and sperm count than the model group (Kawanishi et al., 1986). Chromium compounds also cause oxidative stress, which damages tissue (Çetinkaya, 2020). Under physiological conditions, the free radicals in the body and its antioxidant defense mechanisms are maintained in a state of equilibrium. Oxidative stress occurs when the level of free radicals exceeds the cell's ability to protect them through antioxidant actions. Extremely active and unstable, free radicals destroy cells by attaching to proteins, lipids, and DNA. Due to its detrimental effects, it was capable of inducing oxidative stress, which subsequently resulted in an elevated production of reactive oxygen species, this, in turn, caused membrane damage, apoptosis, necrotic cell death, and decreased cell viability (Bagchi et al., 2002). Mohammed and associates (Mohammed et al., 2023) indicated that rats exposed to hexavalent chromium during their prepubescent development may be at risk for developing testes, which could disrupt normal testicular physiology in adulthood. The high biological activity components of Lycium barbarum L. include phenolics, flavonoids, amino acids, vitamins, and trace elements, among other bioactive compounds that are distinguished by their high antioxidant potential, P-coumaric acid, quercetin-glucoside, rutinoside, and rutin are a few of the phenolic found in L. barbarum. These compounds have a very high antioxidant potential (Yadav & Yadav, 2014; Zhang et al., 2024). The other study verified that the administration of Lycium barbarum polysaccharide significantly improved reproductive damage, such as sexual functionality, sperm quality, and serum endocrine hormone levels (Lau et al., 2012). Consuming items derived from L. barbarum may boost immunity, increase fertility, and lower blood lipid concentrations (Xie et al., 2016; Liu et al., 2016). An investigation into the infertility model in mice induced by Baixiaoan, with subsequent treatment using L. barbarum, could identify the most effective initial dosage of L. barbarum for the recovery of spermatogenic function in the testes after exposure to high-dose chemotherapy (Yan et al., 2019).

Additionally, the study explored the mechanisms by which polysaccharides derived from Lycium barbarum can mitigate neuronal damage induced by oxidative stress. These bioactive compounds were found to enhance the activity of antioxidant enzymes while effectively scavenging free radicals, thereby providing a protective effect against oxidative damage, and potentially aiding in the healing of testicular spongy nerve damage (Zhao et al., 2016). Its toxic damage to the testis is primarily shown by disrupting the body's oxidation and antioxidation balance, and generating an excessive amount of oxygen free radicals, these conditions are marked by a significant rise in lipid peroxidation products, such as nitric oxide (NO), accompanied by a notable reduction in the activity of superoxide dismutase (SOD) and other antioxidant enzymes. Rather than directly generating free radicals, chromium indirectly induces the formation of reactive species, including superoxide, peroxynitrite, nitric oxide, and hydroxyl radicals. These reactive species play a pivotal role in causing cellular damage, a hallmark of oxidative stress (Zhao et al., 2016; Pritchard Jr et al., 2000).

Rats fed L. barbarum plants at a dose of 300 mg/kg considerably lower NO content by boosting SOD activity, delaying cell death, and reducing spermatogenic cell damage by opposing the side effect. It is a naturally occurring plant that is essential for eliminating excess lipid peroxides and (MDA), Enhancing the antioxidant capacity of cells and mitigating damage can be achieved by increasing the intracellular levels of (GSH) and (SOD). Through its antioxidant activity, L. barbarum can help male rats recover their reproductive function and reduce the oxidative stress brought on by diethylstilbestrol (Xiao et al., 2014). It is hypothesized that the fruit may influence the testis's hormone output and lower the oxidative state of the reproductive organs. These studies suggest that Lycium barbarum lessens the ability of testis endocrine cells to withstand degenerative damage caused by Cr VI.

The study's findings demonstrated that following a sodium dichromate injection, chromium entered the liver and kidney tissues and damaged them. Renal function status is often indicated by blood urea and creatinine levels. Both are nitrogen-containing metabolic byproducts; Creatinine is a metabolic end product generated through the catabolism of creatine in muscle tissue, serving as a key indicator of renal function. Blood urea represents the primary nitrogenous waste product resulting from the breakdown of dietary proteins and the turnover of tissue proteins (Walker et al., 1990). The polysaccharides present in L. barbarum were hypothesized to exert a protective effect on the kidneys of diabetic rats by suppressing inflammatory factors, including (IL-2), (IL-6), (TNF-α), proteinuria, and blood urea nitrogen concentration (Du et al., 2016). The study discovered that L. barbarum polysaccharides have renoprotective properties, this mechanism may offer a potential protective effect against hyperuricemia in mice by promoting the renal excretion of uric acid (Yu et al., 2020). It was proposed that the protective effects of Lycium barbarum polysaccharides (LBP) against renal damage induced by a high-fat diet in rats may be mediated through the regulation of lipid metabolism, augmentation of anti-inflammatory responses, and mitigation of renal injury linked to modulators of lipid metabolism (Liao et al., 2019). Cupo & Wetterhahn, (1985) demonstrated that after an intraperitoneal injection of sodium dichromate and chromium(lll) chloride, chromium was attached to kidneys are the primary organs responsible for excreting internal metabolic waste and external nutrients, while nuclear proteins, DNA molecules, and ribonucleoproteins (RNP) play important roles in liver and kidney tissues. Foods from herbs that have been proven to have impactful medicinal properties offer kidney support due to having high micronutrient concentrations for antioxidative and anti-inflammatory activities.

Conclusions

In the L. barbarum flower alone, a multitude of positive results can be extracted such as increased fertility resulting from improved testicular function. These results suggest that L. barbarum supplementation modulates the hormonal profile by increasing testosterone, FSH, and LH which is associated with positive changes in reproductive readiness and improved fertility. L. barbarum supplementation also decreased nitric oxide levels, and increased antioxidant defense systems, improved renal function, decreased concentration of creatinine and urea in adult male rats. All these findings above for L. barbarum provide the scientific grounds for the claimed benefits of L. barbarum on the male reproductive health and other physiological conditions of adult male rats.

Author Contributions

All Authors contributed equally.

Conflict of Interest

The authors declared that no conflict of interest.

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