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Effects of *Echinacea purpurea* Extract on Sperm Characteristics and Hematology Following Testicular Ischemia-Reperfusion Injury in Rat

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Abstract

Objectives: The aim of the present study was to investigate the effects of *Echinacea purpurea* extract on sperm characteristics following testicular ischemia/reperfusion (I/R) injury in rats.

Materials and Methods: To evaluate this hypothesis, 30 adult rats were randomly divided into 5 groups: sham operations, I/R group and 3 groups of treatment with *E. purpurea* extract (25, 50 and 100 mg/kg). To achieve testicular I/R, torsion (720°) of spermatic cord for 2 hours and reperfusion of the tests for 24 hours were performed. Treatment was done by intraperitoneal injection of 3 different doses of *E. purpurea* extract, 1 hour after ischemia. Then, the sperm count, motility and mobility were determined.

Results: There was a significant increase in sperm count, motility and mobility in treatment groups compared to I/R group ($P<0.05$). Treatment with *E. purpurea* extract (25, 50 and 100 mg/kg) significantly attenuated the adverse effect of testicular I/R on sperm mortality compared to the control group ($P<0.05$). No significant difference was observed in hemograms.

Conclusions: These results confirmed beneficial effects of *E. purpurea* extract on sperm characteristics after testicular I/R injury in rats.

Keywords: *Echinacea purpurea*, Ischemia/reperfusion, Testis, Rat

Introduction

One of the most important disorders in the male reproduction system is testicular I/R injury (1). Torsion of the spermatic cord is a common urological emergency among the infants and adolescents. It needs early diagnosis and surgical intervention to prevent subfertility and infertility (2). The surgical correction of testicular torsion includes detorsion of the spermatic cord and re-establishing bloodstream to the testicles. Reperfusion of ischemic tissues results in the formation of toxic reactive oxygen species (ROS), including superoxide anions, hydroxyl radicals, hypochlorous acid, hydrogen peroxide and nitric oxide– derived peroxynitrite (3). ROS cause subsequent cellular damage via lipid peroxidation in mitochondrial and cell membranes (3,4). Moreover, the repeated succession of ischemia/reperfusion (I/R) injury in testicular cells cause many biochemical and morphological changes which might lead to lipid peroxidation, protein denaturation, DNA damage and apoptosis (5). Based on previous reports, there is a growing interest in using antioxidants because they help to protect the cells against damages induced by ROS generated in oxidative stress-related diseases. There is some evidence that natural products and their derivatives have efficient antioxidative properties, consequently linked to anti-inflammatory activities (6).

Echinacea purpurea is a medical herb which is used for treating acute upper respiratory infections, urinary tract infections, viral infections, cutaneous affections and chronic disease due to a deficiency in immunological responses (7). *E. purpurea* has a high content of polysaccharides, acrylamides, non-saturated acids, glycoproteins and flavonoids (8). The main bioactive constituents of *E. purpurea* extracts (EPE) are cichoric acid, echinacoside, chlorogenic acid, sitosterol, cynarine and caftaric acid. All of them are powerful antioxidant, free radical scavenging which decrease nitric oxide (NO) free radical generation (9). Scarce information exists on the role of *E. purpurea* in sexual activity of male rats (8). In a study, Skaudickas et al reported that administration of *E. purpurea* (50 mg/kg) for 8 weeks decreased the percentage of the testicle in male rats (8). Sitosterol decreases the enlargement of the prostate and affects sexual glands in male rats (8). Although the medical properties of *E. purpurea* and its effects on the male genital system were detected, there is no exact report on the dosage of *E. purpurea*. Therefore, the aim of the current study was to investigate the effect of the EPE on sperm characteristics

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and hematology after I/R injury in rats.

Materials and Methods

Animals

Thirty healthy mature male Wistar rats (250 ± 20 g) were obtained from Razi Vaccine and Serum Research Institute (Tehran, Iran). Rats were acclimated to controlled laboratory conditions for a week and provided with *ad libitum* rodent food and tap water. Then, they were randomly divided into 5 groups including sham operations (group 1), I/R (group 2), I/R + 25 mg/kg EPE (group 3), I/R + 50 mg/kg EPE (group 4), I/R + 100 mg/kg EPE (group 5) (8). Furthermore, purified EPE were purchased from Zardband Co.

Surgery Procedure

A midline longitudinal incision was made to have access to both testes following general anesthesia by intraperitoneal injection of ketamine (90 mg/kg) and xylazine (10 mg/kg) (10). Torsion was created by twisting the left testes 720 in the counterclockwise direction and maintained by fixing the testes to scrotum with a 4-0 silk suture passing through the tunica albuginea and dartos (11). During I/R, 2 hours after ischemia, the suture was removed and left testes were detorted and replaced into scrotum for 24 hours of reperfusion. The sham group was subjected to all operative procedures except vessels occlusion. Groups 3, 4 and 5 received intraperitoneal (i.p) injections of EPE (125, 250 and 500 mg/kg) 1 hour before detorsion of the testis, respectively. At the end of the study, animals were euthanized and orchiectomy of the left testis was performed (12).

Sperm Characteristics

Caudal part of epididymis was dissected out and chopped in 5 mL of Ham's F10 medium solution. Epididymal sperms were collected following 5 minutes of incubation at 37˚C to allow sperm to swim out of the epididymal tubules. The sperm count, motility and mobility were evaluated by hemocytometer using Neubauer slide (13).

Blood Collections

Samples were collected by vena porta puncture and transferred into EDTA containing tubes and cell count and Red Blood Cell (RBC) were evaluated by an electronic cell counter at the termination of the trial. The percentages of hematocrit (Hct), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC) and differential Red Blood Cell (WBC) count were also calculated by electronic cell counter.

Statistical Analysis

Statistical analysis was performed using SPSS statistical software version 24.0 and parametric data were analyzed using analysis of variance (ANOVA) and presented as mean values ± standard error of mean (SEM). $P \leq 0.05$ was considered significant to compare the differences between treatments.

Results

The effects of EPE on sperm mortality, mobility and sperm count in different groups were shown in Figures 1-3. Sperm counts in control group, I/R and group 5 were 9795000, 3889700 and 7567000 million/mL, respectively. According to this results, intraperitoneal injection of EPE (25, 50 and 100 mg/kg) significantly increased sperm count 24 hours post-operation in a dose dependent manner compared to the I/R group (Figure 1).

As seen in Figure 2, testicular I/R significantly diminished sperm mortality rate compared to control group ($P<0.05$). In addition, treatment with EPE (25, 50 and 100 mg/kg, for 1 hour before detorsion) significantly attenuated the adverse effect of testicular I/R on sperm mortality compared to the rat without treatment ($P<0.05$) (Figure 2).

As seen in Figure 3, the administration of different levels of EPE increased sperm mobility in a dose dependent

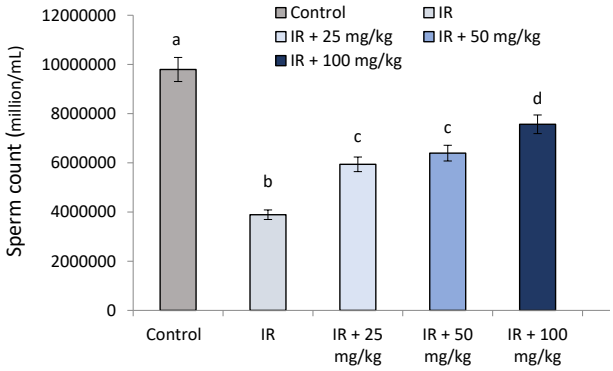

Figure 1. Sperm Count in Rats Treated With *Echinacea purpurea* Extracts Following Testicular I/R injury. There are significant differences between groups with different superscripts in a column ($P<0.05$).

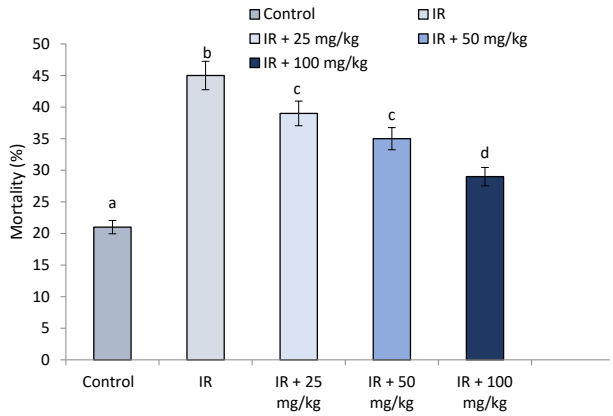

Figure 2. Sperm Mortality in Rats Treated With *Echinacea purpurea* extracts Following Testicular I/R Injury. There are significant differences between groups with different superscripts in a column ($P<0.05$).

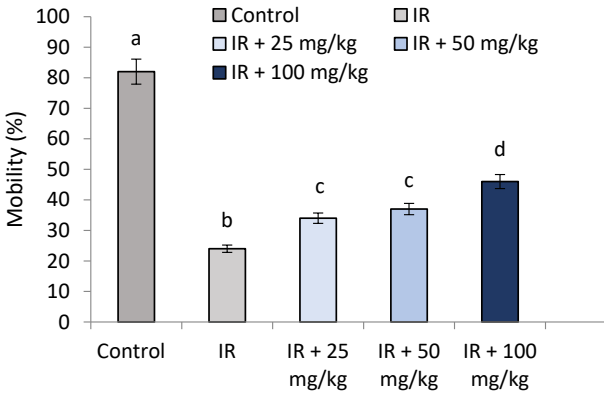

Figure 3. Sperm Mobility in Rats Treated With *Echinacea purpurea* Extracts Following Testicular I/R Injury. There are significant differences between groups with different superscripts in a column ($P<0.05$).

manner ($P<0.05$).

The results of hematological analysis of the treatment group are shown in Table 1. No significant differences were observed in the total hemograms between experimental groups and control group ($P>0.05$).

Discussion

To the best of our knowledge, this is the first report on the effect of *E. purpurea* extract on sperm characteristics and hematology after testicular I/R injury in rats. The obtained results indicated that treatment with EPE (intraperitoneal injection) significantly increased sperm count, sperm mortality and mobility after testicular I/R.

Regarding testes, oxidative stress can disrupt the steroidogenic capacity of Leydig cells as well as the germinal epithelium in the differentiation of normal spermatozoa (14). In order to prevent oxidative stress in the testicle, strong antioxidants are needed. Several studies have reported the anti-inflammatory, antioxidant and radical scavenging activity of *E. purpurea* (15,16). The antioxidant activity of the stems, leaves and roots of *E. purpurea* was compared with purified cichoric acid and alkamides. Cichoric acid is an efficient scavenger of free radicals and comparable to flavonoids for the reaction with the stable radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) (16). Moreover, caftaric acid content of the *Echinacea* spp. protects sperm synthesis from xenotoxins i.e. synthetic pyrethroids (lambda-cyhalothrin), which increases oxidative damage to testicles by elevated levels of malondialdehyde, catalase, superoxide dismutase, glutathione-S-transferase and sperm abnormalities. In addition, oxidative damage can decrease testicular glutathione concentration, sperm count, sperm mortality and motility (17). On the other hand, phenylethanoids in *Echinacea* spp. reduces tumor necrosis factor and apoptosis which improves neuroprotection (18).

It is suggested that cyproterone acetate in *E. purpurea* minimizes oxidative stress in testes (19). Previously, researchers investigated the effect of *E. purpurea* against oxidative stress on renal I/R injury and revealed that the administration of *E. purpurea* decreases malondialdehyde and increases superoxide dismutase levels in both kidney and liver (18). However, it is reported that supplementation of *E. purpurea* during a period of 4 weeks had no effect on the antioxidant status of the spleen in male rats (20).

Conclusions

In conclusion, the results of the present study showed that EPE has protective effects on sperm characteristics 24 hours after testicular I/R injuries in rats. This feature is probably due to its bioactive components such as cichoric acid and echinacoside which have anti-inflammatory, antioxidant and radical scavenging properties.

Conflict of Interests

Authors declare that they have no conflict of interests.

Table 1. Hematologic Analysis in Rats Treated With *Echinacea purpurae* Extracts Following Testicular I/R Injury (mean ± SE)

Parameters	Group 1	Group 2	Group 3	Group 4	Group 5
RBC (10^6/μL)	7.47 ± 0.94	7.68 ± 0.33	7.56 ± 0.81	7.02 ± 0.7	7.32 ± 0.66
WBC (10^3/μL)	6.22 ± 0.66	6.52 ± 0.46	6.08 ± 0.88	6.31± 0.76	6.14 ± 0.54
Hb (gr/dl)	11.43 ± 0.25	12.67 ± 0.10	12.45 ± 0.10	11.12 ± 0.12	12.45 ± 0.52
Hct (%)	41.42 ± 0.60	40.44 ± 0.66	38.62 ± 0.58	41.23 ± 0.70	37.65 ± 0.70
MCV (fl)	50.11 ± 1.16	48.70 ± 3.97	48.36 ± 0.89	51.92 ± 1.32	52.50 ± 0.16
MCH (pg)	18.71± 0.41	19.10 ±1.78	20.26 ± 0.24	18.28 ± 0.22	17.32 ± 0.24
MCHC (g/dL)	31.13 ± 0.42	29.80 ± 0.57	32.65 ± 0.49	30.57 ± 0.90	29.61 ± 0.86
Neutrophil (%)	26.00 ± 0.55	25.54 ± 0.41	24.10 ± 0.54	24.52 ± 0.55	29.62 ± 0.53
Eosinophil (%)	3.17 ± 1.10	2.49 ± 0.18	1.81± 0.20	1.83 ± 0.30	1.79 ± 0.22
Basophil (%)	0.50 ± 0.20	0.60 ± 0.18	0.79 ± 0.20	0.50 ± 0.20	0.48 ± 0.17
Lymphocyte (%)	62.18 ± 0.96	61.45 ± 0.57	67.31 ± 0.20	67.87 ± 0.70	67.43 ± 0.80
Monocyte (%)	2.65 ± 0.16	2.62 ± 0.26	2.22 ± 0.24	2.67 ± 0.30	2.34 ± 0.42

Abbreviations: RBC, red blood cell; WBC: white blood cell; Hct, hematocrit, MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration.
Sham operations (group 1), I/R (group 2), I/R + 25 mg/kg EPE (group 3), I/R + 50 mg/kg EPE (group 4), I/R + 100 mg/kg EPE (group 5).

Ethical Issues

All the experiments were performed in accordance with the guidelines established by the Islamic Azad University, Faculty of Veterinary Medicine for the care and use of laboratory animals and were approved by the Ethics Committee (Ethic code: 84359).

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