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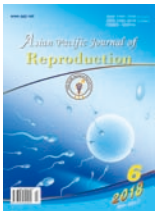

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Effect of paclitaxel and resveratrol on New Zealand rabbit semen

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ABSTRACT

Objective: To examine the effects of paclitaxel and resveratrol on rabbit semen. **Methods:** This study consisted of four groups: control group (40 mL saline), paclitaxel group (5 mg/kg paclitaxel), resveratrol group (4 mg/kg resveratrol) and paclitaxel+resveratrol group (5 mg/kg paclitaxel+4 mg/kg resveratrol). Administrations were *i.v.* (in 40 mL saline) and continued 8 weeks. Sperm motility was evaluated using phase-contrast microscopy. Mitochondrial activity, membrane and acrosome integrity were performed by fluorescence staining. Lipid peroxidation, total glutathione and antioxidant potential levels were determined by spectrophotometry. **Results:** Paclitaxel decreased the sperm motility and fluorescence staining results compared to the control (P<0.05). The paclitaxel and resveratrol group showed better results of the same parameters compared to the paclitaxel group (P<0.05). No significant difference was observed in lipid peroxidation, total glutathione, antioxidant potential and fertility results (P>0.05). Results of this study showed that paclitaxel decreased semen parameters and resveratrol had a protective effect on these parameters. **Conclusions:** Paclitaxel has negative effects on spermatological indicators and biochemical assays, while resveratrol prevents these negative effects of paclitaxel.

1. Introduction

Rabbit semen can be easily collected by artificial vagina. That's why rabbit is the most suitable model for semen evaluation and useful option for biomedical research area including reproduction[1].

Germinal epithelial cells have the ability to quick divisibility, for this reason, they require a high amount of oxygen consumption. However, testicular tissue is in less oxygen cycle, and also there is competition among cells for oxygen. Leydig cell steroidogenesis and spermatogenesis are the targets of oxidative stress and low oxygen tension[2]. Reactive oxygen species formation and membrane lipid peroxidation (LPO) are important problems for sperm viability and fertility[3]. LPO occurs when free radicals attack the cell membrane. Free radicals, eliminating the cell membrane equilibrium, will lead to rapid cell and tissue damage. LPO reduces sperm motility[4].

Spermatogenesis is an event resulting in the formation of approximately 1 000 sperm per second[2]. Chemotherapeutic agents target the rapidly growing cancer cells. Fertility potential declines to its lowest level for about 4-6 weeks after chemotherapy. This suggests that differentiating spermatogonia cells is the most sensitive to chemotherapeutic agents[5].

Antioxidant substances have been adopted to reduce the effects of chemotherapy and radiotherapy by reducing oxidative damage[6]. Antioxidant defense mechanism against oxidative stress has two stages for semen: prevention and stop. The first way (prevention), the antioxidant connects to the metal ions to prevent production of reactive oxygen species. The second way (stop), the structure of the final product is disabled to prevent radical damage. Antineoplastic effect of paclitaxel is to stop mitosis and apoptosis[7]; taxanes

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group drugs connect to the microtubules by inhibiting tubulin from performing separation and disrupting the dynamic equilibrium of mitotic cell division and causing apoptosis[8]. It has been reported that paclitaxel increases oxygen free radicals[9]. Resveratrol (3,4',5-trihydroxy-trans-stilbene) are found mainly in grapes (the bark of red grapes). It is an effective natural antioxidant found in wine and has antiproliferative activities (antimicrobial and antifungal compound). Resveratrol plays a regulatory role in carcinogenesis, further it is an agent having antioxidant, anticyclooxygenase, and lipid and lipoprotein metabolism regulatory activity[10-12].

The hypothesis of this study was that anticancer drugs would have negative effects on reproduction and this effect could be prevented by antioxidant substances. This study was to investigate the effect of resveratrol and paclitaxel on sperm motility, viability, acrosome integrity, mitochondrial activity, biochemical parameters [LPO, total glutathione (t-GSH), and antioxidant potential (AOP)] and fertility results on rabbit semen.

2. Materials and methods

2.1. Animals

In this study 32 male and 32 female New Zealand rabbits (approximately 6 months old) were used. Ethical approval for animal experiments was taken form Selcuk University Ethics Committee (approval number: 2012/6). The rabbits, belonging to the University of Selcuk, Faculty of Veterinary Medicine, were maintained under uniform feeding, housing and daily constant 16L : 8D day lighting conditions. Water was available *ad libitum*.

2.2. Experimental design

There were four groups in this research and each treatment group consisted of eight New Zealand rabbits. The four groups were control group (40 mL saline), paclitaxel group (5 mg/kg/day paclitaxel), resveratrol group (4 mg/kg/day resveratrol), paclitaxel+resveratrol group (5 mg/kg/day paclitaxel+ 4 mg/kg/day resveratrol).

All administrations were *i.v.* (in 40 mL saline) from marginal ear vein, using sterile equipment and continued once a week for eight weeks.

2.3. Semen extending

Rabbits were trained to serve the artificial vagina before study. Ejaculates were collected twice a week for 11 weeks using an artificial vagina and semen of each group was pooled in itself. Sperm motility was estimated using phase-contrast microscopy with a hot plate at 37 ℃. A tris-based extender (Trisma 0.25 M, citric acid 88 mM, glucose 50 mM, egg yolk 20% (v/v), pH 6.8) was used as the base extender. The total motility percent was evaluated subjectively using phase-contrast microscopy with a warm stage maintained at 37 ℃ at × 400 magnification. Sperm concentration was determined via hemositometric method.

2.4. Assessment of sperm plasma membrane integrity (viability)

Sperm viability was performed by staining with SYBR-14/ propidium iodide. This protocol was modified from a study of Garner and Johnson[13].

2.5. Assessment of sperm acrosome integrity

Sperm acrosome integrity was evaluated by using fluorescein isothiocyanate conjugated to *Arachis hypogaea* (peanut) and by propidium iodide staining as achieved by Nagy *et al*[14] with modifications.

2.6. Assessment of sperm mitochondrial activity

Semen mitochondrial activity was evaluated with a protocol modified from Garner *et al*[15].

2.7. Oxidative stress parameters

Thawed straws were centrifuged at 800 *g* for 20 min at 4 ℃ to separate the cells. Sperm samples were washed twice with phosphate buffer saline. The supernatant was discarded and completed to 500 μL with phosphate buffer saline. Subsequently, the sperm suspension was sonicated for 10 s on ice.

2.8. Determination of LPO, t-GSH, and total AOP activity

LPO, t-GSH and total AOP activity were determined using commercial kits of LPO-586™ Oxis Research, GSH-420™, and AOP-490™ by spectrophotometry respectively.

2.9. Fertility trials

In the study, pregnancy rates (%) and average number of offspring were determined. Fertility trial was made by using 32 female New Zealand rabbits. In the experimental groups, each male rabbit was allowed to mate for five days by leaving one female in cage.

2.10. Data analysis

The data from all results were presented as mean ± standard error of the mean (mean ± SEM). All results were analysed by analysis of variance, followed by Duncan's *post hoc* test to determine significant differences between the groups. A value of $P<0.05$ was considered to be significant. Analyses were performed using the SPSS 21 package program.

3. Results

Paclitaxel decreased the percentages of sperm motility, sperm concentration (Table 1), sperm viability (sperm membrane integrity), acrosome integrity and high mitochondrial activity (Table 2) compared to the control group after 11 weeks ($P<0.05$). Resveratrol tended to increase the same parameters compared to the control group (Table 1, 2). These increases were not statistically significant ($P>0.05$). The paclitaxel+resveratrol group showed higher percentages of the same parameters compared to the paclitaxel group ($P<0.05$) (Table 1, 2). In terms of biochemical assays results, no significant difference was observed among the groups ($P>0.05$) (Table 3). In fertility results; control, resveratrol, paclitaxel and paclitaxel+resveratrol group (%) pregnancy rates (87.50, 87.50, 75.00, and 87.50) and number of average offspring rate (6.00±1.52, 5.75±1.67, 5.50±1.41 and 5.75±1.67) were determined respectively and no significant difference was observed.

Table 1

Sperm motility and concenration in diluted rabbit semen (mean±SEM).

Groups	Sperm motility (%)	Sperm concentration ($\times10^6$)
Control	71.82 ± 2.46^{ab}	406.36 ± 76.00^{a}
Paclitaxel	61.36 ± 11.36^{c}	354.09 ± 84.61^{b}
Resveratrol	75.23 ± 1.06^{a}	407.95 ± 70.55^{a}
Paclitaxel+Resveratrol	68.41 ± 7.14^{b}	382.05 ± 68.22^{ab}

a, b, c: Different superscripts within the same column demonstrate significant differences, $P<0.05$.

4. Discussion

Chemotherapeutic drugs cause many side effects in the organism. One of the most important side effects is infertility. In fact, these chemotherapeutic drugs show the same effect in both cancerous and healthy experimental animals.

In the present study, effects of *i.v.* paclitaxel applications on rabbit sperm and oxidative stress parameters were evaluated in rabbits. The study also investigated the protective effect of resveratrol on these additional effects.

In the presence of paclitaxel, microtubules were reorganized to block the cells in the G_2 or M phases of the cell cycle and these cells could not perform a normal mitotic division[8,16–18]. Paclitaxel therapy was associated with the formation of reactive oxygen species[9,19]. Gonadotoxic effects of paclitaxel were determined by many researchers; wistar rats sperm motility[20], sperm concentration and viability[21] were decreased by taxol application.

Resveratrol is a natural polyphenol that is found in various plants, especially in red grape skin[22]. The French paradox is defined as the low incidence of coronary heart disease, although people living in southern France use high amounts of saturated fats. The reason for this is attributed to the high consumption of grapes and red wine in the region[23,24]. Resveratrol inhibits mitochondrial oxidative stress by regulating mitochondrial superoxide dismutase 2 levels and protects cells against oxidative cytotoxic effects[25]. Resveratrol has a positive effect on human and rat sperm motility[26], rat sperm concentration ($\times10^6$/mL)[27] and ram sperm acrosome integrity[28]. Several researchers have investigated the positive effect of resveratrol on LPO and malonyldialdehyde levels in rat sperm[29], rat testicular germ cells[12], and human sperm[26,30].

Kai *et al*[31] found that administration of paclitaxel at 0.6 mg/kg/day and 0.3 mg/kg/day did not have any effect on reproductive parameters in rats. Like our study, no significant differences were observed in this study with similar administration method.

In conclusion, resveratrol provides a protective effect on the negative effects of paclitaxels on spermatological and oxidative stress parameters. Paclitaxel decreases sperm parameters but it does not affect fertility results.

Conflict of interest statement

The authors declare that they have no conflict of interest.

Table 2

Flourescent staining in diluted rabbit semen (mean±SEM)(%).

Groups	Sperm viability	High mitochondrial activity	Acrosome integrity
Control	72.19 ± 4.40^{ab}	73.27 ± 1.80^{a}	67.01 ± 2.37^{ab}
Paclitaxel	62.42 ± 11.49^{c}	70.08 ± 5.32^{b}	58.41 ± 11.19^{c}
Resveratrol	75.33 ± 5.15^{a}	74.50 ± 1.54^{a}	70.87 ± 2.27^{a}
Paclitaxel+Resveratrol	69.59 ± 3.60^{b}	72.57 ± 1.87^{a}	65.40 ± 7.36^{b}

a, b, c: Different superscripts within the same column demonstrate significant differences, $P<0.05$.

Table 3

LPO, t-GSH and AOP levels in rabbit semen (mean±SEM).

Groups	LPO (μM $\times10^9$)	t-GSH (μM $\times10^9$ spz)	AOP (mM $\times10^9$)
Control	136.85 ± 75.19	1.19 ± 0.31	127.59 ± 32.64
Paclitaxel	159.70 ± 64.65	1.14 ± 0.19	142.98 ± 28.42
Resveratrol	108.73 ± 73.00	1.39 ± 0.30	167.68 ± 57.23
Paclitaxel+Resveratol	130.22 ± 55.52	1.24 ± 0.29	151.02 ± 48.71

All $P > 0.05$.

Foundation project

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