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Ameliorative effects of *Achillea millefolium* inflorescences alcoholic extract against nicotine-induced reproductive failure in rat

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ABSTRACT

Background: Nicotine (Nic) is a major risk factor in the development of functional disorders of male reproductive system. *Achillea millefolium*; is highly regarded for medicinal activities, due to its antioxidant and anti-inflammatory properties. This study was carried out to evaluate whether *Achillea millefolium* (*Achm*) inflorescences alcoholic extract could serve as a protective agent in male reproductive male failures during Nic exposure in a rat model.

Methods: Twenty-five adult male Wistar rats were categorized into the five groups. Tests 1 and 3 groups were received Nic at dose levels of 0.2 and 0.4 mg/kg BW/day, respectively by IP injection. Tests 2 and 4 groups were received Nic at the same doses along with *Achm* at dose level of 120 mg/kg BW/day. The study period took forty-eight days for all experimental groups.

Results: Nic groups showed significant decreases in tubule differentiation index (TDI), sperm count, motility, stereological parameters and an increase in dead and abnormal sperms. Moreover, the reduction in total antioxidant capacity (TAC), superoxide dismutase (SOD) activity, serum levels of FSH, LH and testosterone, along with increased serum concentration of LDH were observed in the Nic groups. Total nitrite and malondealdehyde levels increased and total thiol molecules (TTM) levels decreased in testicular tissue in the Nic groups. Notably, *Achm* co-administration caused a contemporary recovery in above-mentioned parameters.

Conclusion: Nic exerts major toxicity in testicular tissue and causes damages in several ways including, oxidative stress, whilst *Achm* imposes protective effect against Nic-induced reproductive failure, which may attribute to its antioxidant capacity.

1. Introduction

Nicotine (Nic) generally disturbs homeostasis of cardiovascular, endocrine and reproductive systems, nevertheless the other systems are also affected by this substance (Carvalho et al., 2006). Though the exact mechanism of Nic noxious effects are uncovered, but more likely, it is due to the liberation of acetylcholine and some other neurotransmitters (Nasehi et al., 2011). According to relevant reports, part of the adverse effects of Nic might be due to the cumulative effects of oxygen radicals (Maritz and van Wyk, 1997). The exposure of men to Nic, not only decreases the motility of sperm, but also it induces a detrimental effect on the process of fertilization and sperm binding to the oocyte (Pekarsky et al., 1995). Nic causes disturbances in functions of leydig cells, thus diminishes the testosterone production. This hormone plays an important role on regulation and maintenance of spermatogenesis (Carvalho et al., 2006). The exposure of rats and dogs to Nic also causes decrease in adult's testis weight and increases sperm abnormalities, genital atrophy including secondary glands, epididymis and the vas deferens (Londonkar et al., 1998; Reddy et al., 1998). Nic in doses 0.2 and 0.4 mg/kg BW for 14 days induced reductions in testosterone levels, changed in gonadal function and sperm maturation and defected in spermatogenesis (Khajeh Jahromi et al., 2011). Nic's effect on testicular germ cell proliferation appears in a dose-dependent way, and duration of exposure could also be effective (Khajeh Jahromi et al., 2011; Nesseim et al., 2011). *In vivo* study revealed that, Nic exerts apoptotic effect on leydig cells in mice. Moreover it is also known that,

Abbreviations: *Achm*, *Achillea millefolium*; Nic, Nicotine; TDI, tubule differentiation index; FSH, follicle-stimulating hormone; LH, luteinizing hormone; LDH, serum lactate dehydrogenase; TAC, total antioxidant capacity; SOD, superoxide dismutase; LDNic, low dose of nicotine; LDNic + *Achm*, low dose of nicotine + *Achillea millefolium*; HDNic, high dose of nicotine; HDNic + *Achm*, high dose of nicotine + *Achillea millefolium*; ROS, reactive oxygen species; NO, nitric oxide; NOS, nitric oxide synthase; TTM, total thiol molecule; MDA, malondialdehyde; PAS, period acid Schiff's; AST, aspartate transaminase; ALT, alanine transaminase

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one of the factors, which can induce apoptosis in the cells, is Nic-induced hormonal changes (Kim et al., 2005).

Achillea millefolium (*Achm*), or "yarrow" plant, is a member of the Asteraceae family (Mitich, 1990). The medicinal properties of *Achm* are worldwide recognized and the plant is native to Europe, North America, southern Australia and Asia (Blumenthal et al., 2000; Lorenzi and Matos, 2002; Mitich, 1990; Sweetman, 2011). Infusions of *Achm* species have an antioxidant capacity, which is consistent with their total flavonoid and phenol contents, thus could be used as a good scavenger of active oxygen species (Konyalioglu and Karamenderes, 2005). The methanol extract of aerial parts of yarrow reduces plasma levels of aspartate transaminase (AST), alanine transaminase (ALT), eliminating congestion and focal necrosis, cell swelling and reduced apoptotic cells in liver of rats with hepatitis induced by galactose amine and lipopolysaccharide (Yaeesh et al., 2006). Protective mechanism is due to increased activity of superoxide dismutase, catalase, glutathione peroxidase and glutathione levels as well as reduced cellular lipid peroxidation (Konyalioglu and Karamenderes, 2005). The effects of plant on the reproductive system are still unclear and the results of the studies in these respects are controversial (Mali et al., 2002). According to the latest report, the low doses of yarrow have no significant effect on reproductive parameters, but high doses can reduce the fertility of male rats (Parandin et al., 2011).

It is clear that the deleterious effects of Nic are because of the increased production of reactive oxygen species (ROS) (Bandyopadhyaya et al., 2008; Sudheer et al., 2008). The ROS damages DNA, proteins, carbohydrates, lipids and disturbs enzyme activity and cellular genetic machinery (Bandyopadhyaya et al., 2008). However, the biological systems own a number of mechanisms to remove ROS, such as the integrated antioxidant defense systems (Bandyopadhyaya et al., 2008; Seema et al., 2007). Indeed, it has been shown that, ROS inhibits steroidogenesis by interfering with cholesterol transport to mitochondria and/or catalytic function of P450 enzymes (Hales et al., 1999). It has also been reported that chronic Nic exposure decreases the level of cytochrome P450 2E1, increases free radical formation, and decreases antioxidant systems, which leads to tissue oxidative damage in rats (Erat et al., 2007). Low levels of ROS are necessary for sperms in the process of movement, capacitation, acrosome reaction and fertilization (Agarwal et al., 2004; Hosseinzade et al., 2009; Rajeev and Rupin, 2005; Sharma and Agarwal, 1996). The ROS invades unsaturated and polyunsaturated fatty acids (PUFAs) in cell membranes, thus lipid peroxidation take place (Chang et al., 2007). In this process, the reactions between free radicals and lipid-chain fatty acid lead to release of free radicals, which subsequently react with molecular oxygen and generates lipid radical's proxyl. One of the compounds of lipid peroxidation is malondialdehyde, which could sustainably be estimated (Hosseinzade et al., 2009; Sharma and Agarwal, 1996).

In contrast to the pathological effects of free radicals, spermatozoa and seminal plasma have the enzymatic and non-enzymatic antioxidants with a series of low molecular weight that generally named total antioxidant capacity (TAC). These antioxidants act as free radical dilatory to protect spermatozoa against ROS (Agarwal and Prabakaran, 2005; Elsayed and Bendich, 2001). Antioxidants available in the seminal fluid and sperm are placed in groups of endogenous antioxidant systems. Possible harmful effects of ROS have inhibited *via* cellular antioxidant protection systems, including enzymes such as superoxide dismutase (SOD). In addition to SOD, which constitutes the main antioxidant present in the sperms, a primary antioxidant enzyme, often in the form of vitamin C, vitamin E, beta-carotene, carotenoids and flavonoids are available (Greco et al., 2005; Limón-Pacheco and Gonsebatt, 2009; McCord, 2000; Sies, 1994; Tarin et al., 1998). These compounds can prevent the sperm membrane lipid peroxidation, and safeguard its integrity. The antioxidants can prevent not only the decrease in motility of sperm, but also enhance its functionality (Wroblewski et al., 2003).

The Nitric Oxide (NO), plays fundamental role in the regulation of many physiologic processes such as blood pressure, neurotransmission, and is a key compound on the immune system (Feldman et al., 1993). NO is generated from the conversion of L-arginine to L-citrulline, by a family of enzymes called nitric oxide synthase (NOS) (Vatish et al., 2006).The nitrosative stress is a process refers to the level of NO that reaches to a concentration, which capable to cause the nitrosation of amine and thiol groups, that would interfere with the function of proteins and the formation of carcinogenic compounds (Colton and Gilbert, 2007). The current study therefore, aimed to investigate the potential detrimental effects of Nic in various dose levels on structure and function of the testis and also protective effects of *Achillea millefolium* methanolic extract on Nic-induced impacts in male reproductive system.

2. Methods

2.1. Plant material

A. millefolium was harvested from their natural habitat around the city of Tabriz in East Azerbaijan province, northwestern Iran during the flowering season (between May and July, 2015). The identification of collected plant was confirmed at the research laboratories of the Department of Agriculture of West Azerbaijan province.

2.2. Preparation of the alcoholic extract

The alcoholic extract of dried inflorescences of the plant was prepared by infusion of the finely dried material in methanol 70% for 36 h. The infusion was filtered and concentrated with rotary evaporator device. The dried extract was diluted in distilled water immediately before use.

2.3. Nicotine

A liquid vial of nicotine was procured from the Sigma–Aldrich Company (N3876, lot no. 0123k 89765. SIGMA), and the working solutions corresponding to two dose levels of 0.2 and 0.4 mg/kg BW/day, were prepared. The prepared solution of nicotine was injected daily *via* intraperitoneal route (Khajeh Jahromi et al., 2011).

2.4. Animal model

For this study, 25 adult male Wistar rats, mean weight of 230.8 ± 3.32 g, were procured from a well-established laboratory animal's house. Animals were divided into 5 groups on random base, and placed in polypropylene cages, maintained under standard conditions of 12 h light and 12 h of darkness, the temperature and humidity were adjusted to 25 ± 2 °C, 50 ± 10, respectively. The animals in all groups were fed with standard rat diet and watered with tap water *ad libitum*. All of the animals were kept in new environment for 15 days without experimentation for sake of adaptation, and their weight were recorded. The animals were grouped as follows:

Control group: Animals were received 1 mL sterile distilled water orally and 0.6 mL intraperitoneally in daily base (the volume of injected water or orally administered solution were adjusted with corresponding volumes of test groups).

Test1 (low dose nicotine: LDNic): Animals of this group were received Nic at dose level of 0.2 mg/kg BW/day, intraperitoneally.

Test2 (LDNic + *Achm*): Animals of this group received Nic at rate of 0.2 mg/kg BW/day, intraperitoneally, and *Achm* at dose level of 120 mg/kg BW/day, orally (gavage).

Test3 (high dose nicotine: HDNic): Animals of this group were received Nic at dose level of 0.4 mg/kg BW/day, intraperitoneally.

Test4 (HDNic + *Achm*): Animals of this group received daily with intraperitoneal injections of Nic at rate of 0.4 mg/kg BW/day, and *Achm* at dose level of 120 mg/kg BW/day, orally (gavage).