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Nicotine-induced reproductive toxicity, oxidative damage, histological changes and haematotoxicity in male rats: The protective effects of green tea extract

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ABSTRACT

Nicotine is an active substance present in tobacco that causes oxidative stress and tissues damages leading to several diseases. Natural antioxidants that prevent or slow the progression and severity of nicotine toxicity may have a significant health impact. We have analyzed the effects of green tea extract (GTE) on nicotine (NT)-induced reproductive toxicity, oxidative damage and haematotoxicity in adult Wistar male rats. Thirty-two rats were randomly divided into four groups: control, nicotine (NT, 1 mg/kg i.p.), green tea extract (GTE, 2% w/v as the sole beverage) and (NT + GTE) group. After 2 months of treatment, blood samples were collected for measuring the haematological and oxidative stress parameters and testosterone level, while the reproductive organs were weighed and used for the semen analysis and histopathology. NT induced oxidative damage as indicated by a significant reduction in the activities of antioxidant enzymes and an elevation in TBARS levels. NT also caused reproductive toxicity as shown by a decline in testosterone levels, the weights of reproductive organs and sperm characteristics; the histological examination of testes revealed atrophy, degenerative alterations and perturbation of spermatogenesis in several seminiferous tubules, together with increased interstitial spaces and reduced number of Leydig cells. Both NT and GTE altered white blood cell count and red blood cells parameters, albeit with somewhat different effect, no protective action being seen upon NT + GTE treatment. On the contrary, GTE played a protective role against NT-induced oxidative stress as well as the reproductive effects by improving the oxidative status, semen quality and the testicular histological damage.

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1. Introduction

Currently, 1.3 billion people worldwide smoke or use other tobacco products. While smoking prevalence has declined in many developed countries, it remains high in others and is increasing among women and in developing countries (WHO, 2010). There is growing evidence that cigarette smoking and tobacco chewing have a profound negative impact on general health associated with higher morbidity and mortality. Nicotine is the most abundant volatile alkaloid extracted from the dried leaves and stems of the *Nicotiana tabacum* and *Nicotiana rustica* and it features prominently among the over 4000 chemicals found in tobacco products (Rustemeier et al., 2002). Nicotine is well absorbed through the skin, the mucosal lining of the respiratory tract and the lungs and it can rapidly reach peak levels in the bloodstream and brain. It is an active compound on the nervous system, including the neuroendocrine axis, as it binds stereo-selectively to nicotinic cholinergic receptors in the autonomic ganglia, the chromaffin cells of the adrenal medulla, the neuromuscular junctions and the brain (Tundulawessa et al., 2010). Besides its pharmacodynamics

Abbreviations: NT, nicotine; GTE, green tea extract; TBARS, thiobarbituric acid-reactive substances; SC, sperm count; SN, spermatid number; DSP, sperm daily production; STR, sperm transit rate; ROS, reactive oxygen species; WBC, white blood cells; RBC, red blood cells; Hb, haemoglobin; Ht, haematocrit; MCH, mean corpuscular haemoglobin; MCHC, mean corpuscular haemoglobin concentration; MCV, mean corpuscular volume.

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activities, nicotine is the main toxic component of cigarette smoking, with genotoxic, immunotoxic as well as reproductive effects in both sexes; it plays a detrimental role in the development of cardiovascular diseases and of respiratory and digestive tract cancers (Polyzos et al., 2009).

Green tea (*Camellia sinensis*) has a long history of use worldwide. The beverage is made from boiling the naturally dried leaves and is considered as the major popular consumed drink other than water in many countries, e.g., Algeria and Egypt. Green tea is a very rich source of a specific kind of antioxidant called polyphenolic flavonoids, the catechins which include catechin, epicatechin, gallocatechin, epigallocatechin, catechin gallate, epicatechin gallate, gallocatechin gallate, and epigallocatechin gallate (Chengelis et al., 2008).

Over the last decade, green tea and polyphenols have attracted a great deal of attention because of its potential health benefits, which include anti-cancer, anti-inflammatory, antioxidant and antimicrobial effects; tea consumption has been linked to a possible risk reduction in some cardiovascular, dermatological, haematological, metabolic and neurological disorders, as well as obesity (Naganuma et al., 2009; Upaganlawar et al., 2009). However, there is currently little evidence about whether green tea may reduce the adverse effects of toxicants. Nicotine is known to generate reactive oxygen species (ROS), while green tea is known by its anti-oxidant activities and ability to scavenge ROS and trap hydroxyl, peroxyl and superoxide anion radicals due to the presence of catechins. Our study was carried out to investigate the possible protective role of Green tea extract against the oxidative stress, haematotoxicity and reproductive toxicity, induced by nicotine in adult Wistar male rats.

2. Materials and methods

2.1. Nicotine and green tea extract

Nicotine, *S*-3-(1-methyl-2-pyrrolidinyl) pyridine, ($C_{10}H_{14}N_2$, CAS registry number 54-11-5, purity 98%) was purchased from Sigma Chemical Co. (St. Louis. France). The dose and the administration route were selected according to previous studies (Valença et al., 2004; Jana et al., 2010; Rehan et al., 2012).

The green tea used in this work is a locally grown cultivar usually consumed in Algeria and purchased at Boumerdes market. Green tea extract was prepared from the dried leaves according to Kang et al. (2000) method. Briefly, twenty grams of green tea leaves were infused for 5 min in 1000 ml of boiling water (90 °C), allowed to cool to room temperature and then filtered and stored in a brown bottle. The resulting aqueous extract (2% w/v) from the consumer product is similar to tea brews for human consumption and used as the sole drinking source according to Mehana et al. (2012).

2.2. Animals and experimental design

The local Algerian ethical committee approved the design of the experiment and its compliance with general international principles of laboratory animal welfare. Thirty two adult male Wistar rats aged between 8 and 12 weeks were obtained from Pasteur Institute of Kouba-Algeria (internally bred strain) and acclimatized for 1 week before the experiment. The animals were housed in plastic cages with sawdust bedding and maintained in an air-conditioned animal house at a controlled temperature (22 ± 2 °C) and relative humidity (60 ± 10%) with a photoperiod of 12 h light/12 h dark with free access to pellet feed (purchased from the National Office of Food Livestock, Algeria) and fresh tap water, except groups III and IV (see below). The animals were randomly assigned into four groups of eight animals as follows:

Group I: untreated, served as control (C).
Group II: injected intraperitoneally (i.p.) with nicotine (NT in aqueous solution; 1 mg/kg body weight/day).
Group III: received aqueous green tea extract (GTE) *ad libitum* as the sole drinking fluid at a concentration of 2% (w/v).
Group IV: animals were simultaneously injected NT (1 mg/kg body weight/day i.p.) and given GTE *ad libitum* as the sole drinking fluid at a concentration of 2% (w/v) (NT + GTE).

The animals were treated for 2 months.

2.3. Body and reproductive organs weights

Body weights were recorded weekly during the experimental period. At the end of the experiment, the animals were sacrificed by decapitation under ether anaesthesia. Body weights were recorded before sacrifice; male reproductive organs (testes, epididymes and seminal vesicles) were quickly removed, cleared from adipose and connective tissues and weighed, then relative organ weights were calculated.

2.4. Haematological analysis

At the end of the treatment, blood samples were collected from the orbital sinus and placed immediately on ice. EDTA was used as an anticoagulant agent to determine the selected haematological parameters. Red blood cells (RBC) and white blood cells (WBC) count, haematocrit value (Ht), haemoglobin level (Hb) were measured manually by routine methods, namely haemocytometry and spectrophotometry, respectively. Then, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), and mean corpuscular haemoglobin concentration (MCHC) were calculated.

2.5. Determination of oxidative stress indicators

The content of reduced glutathione (GSH) was determined by the enzymatic method of Jollow et al. (1974). Thiobarbituric acid reactive substances (TBARS) were determined according to the method of Tappel and Zalkin (1959). The antioxidant enzymes, superoxide dismutase (SOD, EC. 1.15.1.1) and Catalase (CAT, EC. 1.11.1.6) activities were measured according to Flohe and Otting (1984) and Aebi (1984), respectively.

2.6. Semen characteristics

2.6.1. Sperm and spermatid number

The left testis and epididymis from each rat were excised. After removal of tunica albuginea, the testis was minced with scissor and homogenized in 10 ml 0.9% NaCl containing 0.5% Triton X-100; the homogenate was gently mixed by using vortex mixer. The number of homogenization-resistant spermatids (spermatids number, SN) was counted by a haemocytometer (Mallassez) chamber; daily sperm production (DSP) was calculated by dividing the number of homogenization-resistant spermatids by 6.1 (Blazak et al., 1993).

The cauda epididymis was cut into small pieces by a disposable blade in 10 ml of 0.9% NaCl containing 0.5% Triton X-100, homogenized and spermatozoa were counted (sperm count, SC) as described above; the epididymal sperm transit rate (STR) was estimated by dividing the epididymal sperm number by the daily sperm production (Amman et al., 1976; Blazak et al., 1993).

2.6.2. Sperm motility and morphology analysis

The right cauda epididymis of each animal was excised and placed in a warmed petri dish containing 2 ml of Hanks's solution at 37 °C. The tissue was cut with scalpel to release sperm and placed in a 37 °C incubator for 15 min prior to determining

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