Open Access

Research Article Open Access

Abstract

Allium sativum

A. sativum

Keywords: Garlic; Germ cells; Apoptosis; E ector caspases; IAPs (Inhibitor of Apoptosis Proteins); AIF (Apoptosis Inducing Factor)

Introduction

Medicinal plants such as thyme, onion, blackseed, lemon, balm and nettle are intensively used in traditional medicine. Garlic or *Allium sativum* (*A. sativum*) is among the most important of these plants. Garlic is one of the ten commonest herbal medicines used in the United States (ranked in h place), according to recent sales data [1]. Indeed, *A. sativum* displays therapeutic e ects such as in the treatment of hypercholesterolemia [2], prevention of arteriosclerosis [3] and some cancers [4], and presents anticoagulant [5] and antihypertensive [6] properties. For example, non-pharmacological treatment with garlic preparation is suggested to reduce blood pressure in hypertensive individuals [7]. e side e ects, particularly on male reproduction, of such a chronic treatment are poorly investigated. To date, it has been reported that heated garlic juice was e ective in recovery of testicular function a er experimental testicular hypogonadism [8] but other laboratories have reported that powder [9] or crude [10] garlic preparations impaired testicular and male reproductive tract functions. Moreover, garlic metabolites such as diallyl trisul de have been reported to have spermicidal e ects [11,12]. e mechanisms of garlic action on male reproduction function, and particularly on spermatogenesis, however, remain unknown. In the present study, we tried to identify the cellular and molecular targets of crude garlic administrated in various doses to adult male rats.

In this work, we were interested to study the apoptosis on somatic and germ cells. We focused on the last step of apoptosis, before the irreversible cleavage of crucial proteins and endonuclease activation, which is under the control of e ector caspases named Caspase 3, 6 or 7. Caspase 3 (CASP3) has been studied extensively and is known to be synthesized as a pro-enzyme which needs cleavage to be active [13]. Activation and/or activity of CASP3, 7 or 9 are regulated by the Inhibitors of Apoptosis Proteins (IAPs). IAPs share a common domain known as BIR (baculovirus IAP repeat), that binds to and inhibitdcrare-CASP3) lsotivation, male rats.

*Corresponding author:

Received Published

Animals and treatment

A number of 24 adult male Wistar rats (Pasteur Institute of Tunis, Tunisia), whose average weight ranged between 200 g and 250 g, were used for the study. e animals were housed with proper aeration at 25 ± 2°C, and were given tap water *ad libitum*. e rats were allowed to acclimatize in the laboratory for a period of 1 week before the beginning of the study.

e rats were randomly assigned into the di erent groups (of six animals each) using a hazard permutation table. Control animals received a standard pellet diet (group 1). e other groups received a diet supplemented with 5 g, 10 g and 30 g/100 g of standard diet (for groups 2, 3 and 4, respectively). All rats were weighed daily. A er 30 days of treatment, rats were killed by decapitation. Testis were weighed and dissected. For each animal, the rst testis was frozen at -80°C for Western Blotting analysis and the other was xed in a 10% formaldehyde solution, for histo-pathological studies.

All studies on animals were conducted in accordance with current regulations and standards approved by the Faculty of Medicine of Tunis animal care committee.

TUNEL (TdT-mediated dUTP-X nick end labeling)

Para n sections (5 µm) of formaldehyde- xed testicular tissues were mounted onto Superfrost Plus slides. e sections were handled as previously described [21]. For each rat testis at least 100 random seminiferous tubules were numbered. e results were expressed as the apoptotic rate, i.e. the number of TUNEL positive cells per number of Sertoli cells.

Immunohistochemistry

Para n sections were incubated for 20 min at 93°–98°C in citric bu er (0.01 M, pH 6) and le to cool for 20 min at room temperature. e sections were rinsed twice for 5 min in osmosed water, and washed twice for 5 min in Tris bu ered saline (TBS) containing 0.1% Tween-20. e Envision+ kit was used for detection of anti-cleaved CASP3 antibody (dilution 1/50) according to the manufacturer's recommendations. e antigen-antibody complexes were stained with DAB which generated a brown color at the site of peroxidase activity. e sections were rinsed twice for 5 min in osmosed water, counterstained with hematoxylin for 5 min and mounted in Faramount®.

Western blotting analyses

Proteins were obtained from testicular tissues as previously described [10]. Proteins (15-40 µg) were resolved on a 10%-15% sodium dodecyl sulfate/polyacrylamide gel. Proteins were electrophoretically transferred to a nitrocellulose membrane using 25 mM Tris-185 mM glycine bu er (pH 8.3) containing 20% methanol at

touch with apoptosis. e number of apoptotic germ cells in rat testes increased a er treatment with *A. sativum* in a dose-dependent manner (Figure 2E). A signi cant (p<0.05) increase was observed in the rats treated with 10 g and 30 g of *A. sativum*.

Detection of CASP3 in germ cells

By immunostaining, cleaved CASP3 was detected in spermatocytes and spermatids from rats fed with 10 g (G3; Figure 3A) and 30 g of *A. sativum* (G4; Figure 3B). ere were no stained somatic cells detected in all rats.

e cell death process induced in spermatocytes and spermatids from rats fed with *A. sativum* was probably an apoptotic mechanism, since cleaved CASP3 expression was increased in a dose dependent manner in the testicular tissues from rats fed with *A. sativum* with a signi cant increase in G3 (p<0.05) and G4 (p<0.05) (Figures 3C and 3D). In contrast, *A. sativum* -feeding did not modify the expression of cleaved CASP6 (Figure 2C).

Effects of *A. p*

of testicular hypogonadism by heat is prevented in part by di erent types of garlic preparation (raw, heated garlic juices, dehydrated garlic powder of the more potent aged garlic extract) [8]. Aqueous extract of garlic [23] or the metabolites diallyl sul de [24] and diallyl tetrasul de [25] o er a protection against cadmium-induced testicular damages. Garlic is also e ective in restoring the testicular histology altered by EDTA [26]. e antioxidant activities of garlic extract were shown to decrease the toxic e ects of free radicals induced by testicular torsion and detorsion [27]. While several studies show that *A. sativum* has a protective e ect as an adjuvant, other studies show that it modi es spermatogenesis. Indeed, daily administration of 50 mg garlic powder over 70 days induced a spermatogenetic arrest at spermatocyte I stage [9]. Moreover, aqueous garlic extract [11] or the metabolite diallyl trisul de [12] has spermicidal e ects. In contrast, a 90-day administration of 100 mg/kg aqueous extract of garlic has been shown to increase the number of spermatozoa [28]. In this context, to assess the positive or negative e ect of garlic, it was important to analyze its action on the di erent testicular cells through the study of germ and somatic cell apoptosis.

In this context, this study has focused on the e ects of