Effect of plant extracts in sperm viability

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Abstract

Medicinal plants have been used since the earliest documented history around the world, as an available and inexpensive therapeutic resource. Plant based bioactive elements play eminent biological roles as antioxidant, anti-inflammatory and antiproliferative agents. It is proved that naturally occurring antioxidants in ethnomedicinal plants are effective in treating various types of diseases.

However, herbal medicine, without any knowledge of their toxicological profile, target organ, and safe dose, is one of the biggest problems of recent health care systems. Any drug or herbal formula which is intended to be used in humans must first be tested in suitable experimental *in vitro* or animal models in order to evaluate its safety. Therefore, it is of interest to evaluate the toxicity of plant extracts to enhance their value for the pharmaceutical and cosmetic industries. Six extracts of plants produced/endogenous in Portugal: *Cistus ladanifer*, *Thymbra capitata*, *Helichrysum italicum*, *Cupressus lusitanica*, *Ocimum basilicum* and *Matricaria chamomilla* were used in order to assess their reproductive toxicity by evaluating the viability of sperm when in contact with the extracts. Hydrolates for each plant were obtained by hydrodistillation of aerial parts. The chemical composition of the hydrolates was determined by GC-MS (gas chromatography mass spectrometry).

Reproductive toxicity was assessed by exposing cryopreserved bovine semen to six different concentrations of each hydrolate (0.2%-0.002% v/v). Methyl methanesulfonate (MMS) was used (0.22nM) as positive control of sperm toxicity. After incubation, sperm viability was determined for each concentration by staining with eosin-nigrosin and counting the number of viable and dead spermatozoa (spz) in a total of 200 spz.

The results show that the hydrolates in test do not compromise sperm viability for all tested concentrations (>25%) when compared with negative control (34%) and in fact, *M. chamomilla* seems to promote sperm viability (39%) at the highest tested concentration.

These results indicate that the extracts at the concentrations used do not seem to have a toxic effect on this evaluated target.

Keywords: Animal biotechnology, Plant hydrolates, Reproductive toxicology, Sperm

Resumo

A utilização de plantas medicinais está documentada desde a antiguidade, como um recurso terapêutico disponível e de baixo custo. Os elementos bioativos das plantas desempenham papéis biológicos importantes, tais como, serem antioxidantes, anti-inflamatórios e antiproliferativos. O uso etnomedicinal dos antioxidantes naturais presentes nas plantas pode ser eficaz no tratamento de vários tipos de doenças.

Contudo, a fitoterapia, sem o conhecimento do perfil toxicológico do elemento medicinal, órgão-alvo e dose segura, é um problema na medicina moderna. Qualquer fármaco ou fórmula à base de plantas que se destine a ser usada em humanos deve primeiro ser testada em modelos experimentais *in vitro* ou animais, adequados para avaliar a sua segurança. Portanto, é de interesse avaliar a toxicidade de extratos vegetais para aumentar o seu valor para as indústrias farmacêutica e cosmética. Seis extratos de plantas produzidas/endógenas em Portugal: *Cistus ladanifer, Thymbra capitata, Helichrysum italicum, Cupressus lusitanica, Ocimum basilicum* e *Matricaria chamomilla* foram utilizados para estimar a sua toxicidade reprodutiva através da avaliação da viabilidade de espermatozóides quando em contacto com os extratos. Os hidrolatos de cada planta foram obtidos por hidrodestilação das partes aéreas. A composição química dos hidrolatos foi determinada por GC-MS (espectrometria de massa por cromatografia gasosa).

A toxicidade reprodutiva foi avaliada pela exposição de sémen bovino criopreservado a seis diferentes concentrações de cada hidrolato (0,2%-0,002% v/v). Foi utilizado metanossulfonato de metil (MMS) (0,22nM) como controlo positivo da toxicidade espermática. Após a incubação, a viabilidade espermática foi determinada para cada concentração por coloração com eosina-nigrosina e contagem do número de espermatozóides viáveis e mortos (spz) num total de 200 spz.

Os resultados mostram que os hidrolatos em teste não comprometem a viabilidade espermática para todas as concentrações testadas (>25% de viabilidade) quando comparados com o controlo negativo (34% de viabilidade). O hidrolato de *M. chamomilla* aumentou a viabilidade espermática (39%) na mais alta concentração testada.

Estes resultados indicam que os extratos nas concentrações utilizadas, não parecem ter efeito tóxico neste alvo avaliado.

Palavras-chave: Biotecnologia animal, Hidrolatos de plantas, Sémen, Toxicologia reprodutiva

Introduction

Disorders in the production of sperm, sex hormones, as well as other factors such as smoking, alcohol consumption and exposure to toxic/chemicals can cause sexual disorders in the male (Jafar and Mawlood, 2020). The consequences on male fertility are known, taking into account the interactions of diet and environment in the spermatogenesis process (Grami *et al.*, 2020).

Sperm production is a complex process that involves the correct functioning of the reproductive system, which is made up of the testes, epididymis, hormones and attached glands (Bariweni *et al.*, 2019). The time of spermatogenesis (the process in which thousands of sperm are produced daily in the seminiferous tubules of the testis) varies according to the species, being 60 days in bulls while in rats it is 30 days (Komsky-Elbaz *et al.*, 2019). It is a dynamic process that occurs within the seminiferous tubules of the testis and depends on hormonal regulation (testosterone) and Sertoli cell physiology (Bariweni *et al.,* 2019).

The biological antioxidant, anti-inflammatory and antiproliferative effects of plant-based bioactive elements is known. Natural antioxidants from native plants are known to be effective in treating various diseases (Ijaz *et al.,* 2020). Jafar and Mawlood (2020) refer that in the treatment and prevention of some diseases, as well as for the improvement of reproductive performance, the use of medicinal plants and phytotherapeutic compounds containing phenols is increasing. It was also mentioned that in several animal species, reversible effects were observed at the sperm level when using phytochemicals from same plants (Bariweni *et al.,* 2019).

Currently, throughout the world, the use of medicinal plants, and/or compounds isolated from medicinal plants, as a supplement or for the treatment of diseases is recurrent. On the other hand, there are reports of adverse effects with the use of medicinal plants in animals and humans, so it is important to study the toxicity of these and/or their isolated compounds (Oyinleye *et al.,* 2021).

The aim of this study was to evaluate the reproductive toxicity of plant extracts by assessing the viability of sperm when in contact with them.

Methodology

Six extracts of endemic/produced plants in Portugal, namely: *Cistus ladanifer, Thymbra capitata, Helichrysum italicum, Cupressus lusitanica, Ocimum basilicum* and *Matricaria chamomilla* were used in order to assess their reproductive toxicity by assessing the viability of sperm when in contact with the extracts. Hydrolates for each plant were obtained by hydrodistillation of aerial parts (including flower, stem and leaves). For the determination of their compounds, the hydrolates were subjected to a liquid-liquid extraction (LLE) with an organic solvent (hexane), the aqueous phase was separated from the organic phase. The chemical composition of the organic phase was determined by GC-MS (mass spectrometry by gas chromatography). The hydrolate samples were analyzed after passing through this process, in triplicate, with a determined volume for each species (Ferraz *et al.,* 2022).

The experimental procedure used was the one described to assess sperm viability in (Modified in vitro Sperm Comet Assay). ECVAM DB-ALM Protocol nº 126 – ReProComet Assay" (European Centre for the validation of Alternative Methods (ECVAM) 2009), as graphically represented in Figure 1. Briefly, the reproductive toxicity was assessed by exposing cryopreserved bovine semen to six different concentrations of each hydrolate (0.2%-0.002% v/v) in PBS (phosphate buffered saline solution). Methyl methanesulfonate (MMS, Sigma-Aldrich, USA) was used at 0.22nM as positive control of sperm toxicity (Cordelli *et al.,* 2007). The negative control consisted of PBS only.

After incubation, sperm viability was determined for each concentration by staining with eosin-nigrosin and counting the number of viable and dead spermatozoa (spz) in a total of 200 spz (Abdul Razak *et al.,* 2019; Komsky-Elbaz *et al.,* 2019).

Results and discussion

The reproductive toxicity of plant hydrolates was evaluated using the sperm viability study.

Overall, the sperm viability (%) obtained in this study is in accordance with the results described by Cordelli *et al.* (2007), where the viability in the positive control was above 32%.

The results obtained in this study show that the studied plant hydrolates do not compromise sperm viability for all tested concentrations (>25%) when compared with negative control (34%) and indicates that extracts do not seem to have a toxic effect on this evaluated target, sperm.

and in fact, *M. chamomilla* hydrolate seems to promote sperm viability (39%) at the highest tested concentration (Figure 2). Bisabol Oxide A is the predominant compound in *M. chamomilla* hydrolate, as showed in Table 1, where the major component of plant extracts as determined by GC-MS analysis is presented.

In literature, we found that this compound has an important biological activity, such as: anti-inflammatory, anti-irritant, antibacterial and non-allergenic properties (Komsky-Elbaz *et al.*, 2019; Grami *et al.*, 2020; Jafar and Mawlood, 2020). However, and most interestingly, in previous studies, it was found that this compound can provide chemoprotection of fertility. Specifically, in a group of animals treated with daunorubicin, the fertilization capacity was reduced substantially, while the fertilization capacity in the groups receiving chamomile essential oil was maintained (Kamatou and Viljoen, 2010).

Conclusions and future perspectives

The beneficial effects of the various phytochemical compounds of medicinal plants, such as the antioxidant and anti-inflammatory activities, also include the fact that they contain precursors that promote sperm production as well as increase the level of testosterone (Fahmy *et al*., 2020).

We conclude that some plant extracts, namely *M. chamomilla* hydrolates, may have the ability to promote sperm viability which can lead to a reduction in the impairment of testicular function caused by several factors, such as: stress, poor diet, chemotherapy, among others, by targeting testicular oxidative stress and inflammation and improving testicular steroidogenesis. The use of these extracts, within the tested concentrations, seems to be safe, not toxic, for sperm.

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Table 1- Major component of plant extracts as determined by GC-MS analysis. The proportion of the predominant compound in each extract is also showed, as well as their chemical class.

Plant	Major component	Distribution (%)	Class
C. ladanifer	4-Hydroxy-3-methylacetophenone	21.58	Alkyl-phenylketones
T. capitata	Carvacrol	98.11	Monoterpenes
C. lusitanica	Terpinen-4-ol	38.93	Monoterpenes
H. italicum	L-α-Terpineol Carvacrol	30.55 29.58	Monoterpenes
O. basilicum	Eugenol Linalool	52.53 38.34	Phenylpropanoid
M. chamomilla	Bisabolol oxide A	80.67	Sesquiterpenes

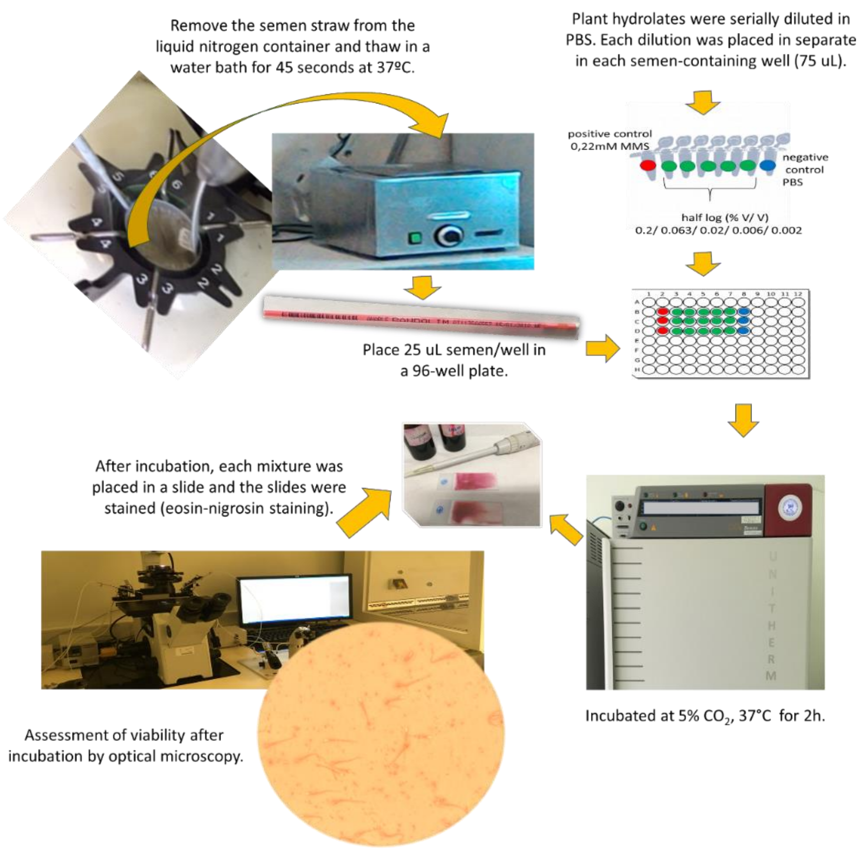

Figure 1- Experimental design of the protocol used to assess sperm viability**.**

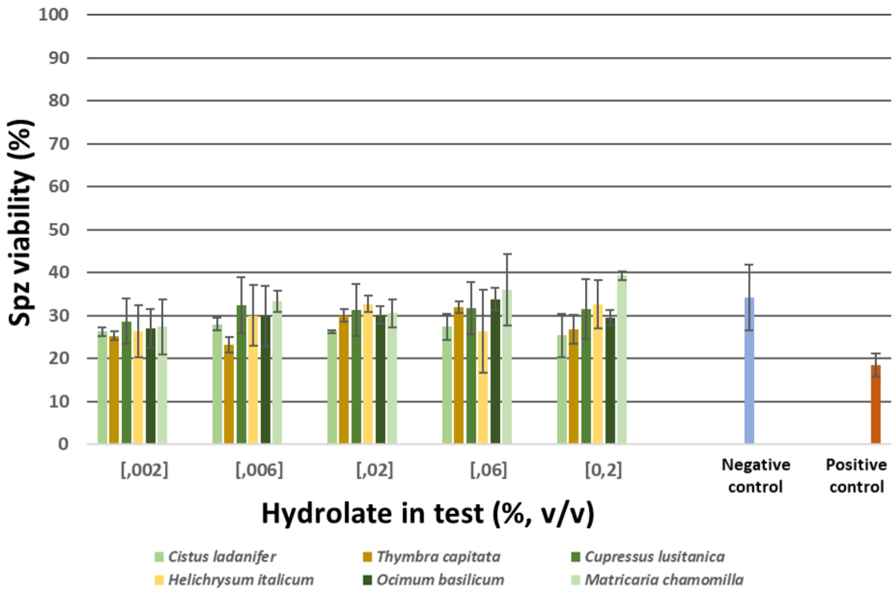

Figure 2- Spz viability (%) in PBS supplemented with each hydrolate (%, v/v). Negative control: PBS only. Positive control: 0.22 mM MMS. The results represent three independent experiments.