Original Article

Reproductive Toxicity of *Carlina gummifera* L. Incense Inhalation in Adult Male Wistar Rats

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Abstract

Background: Burning incense of *Carlina gummifera* L. is a traditional practice in North African countries for religious or ritual intentions. **Aim:** The aim of this study was to investigate the effects of smoke incense of this plant on the reproductive function in adult male rats. **Setting and Design:** This study was conducted in the Integrated Physiology Laboratory. **Materials and Methods:** Plant roots were collected, dried and finely ground in powder form. Adult Wistar rats were randomly assigned to treated groups exposed daily during 60 min for 15 consecutive days to smoke incense at 2, 4 and 6 g and a control group was subjected to the same conditions in the absence of smoke. **Statistical Analysis Used:** Statistical analysis was performed using one-way analysis of variance followed by Tukey's multiple comparison as the *post hoc* test. **Results:** Exposure to the incense of *Carlina gummifera* L. seriously affected dose dependently the reproductive function in male rats. It was found that in treated groups, the testicle relative weight decreased, while those of seminal vesicles and prostate increased when compared to the untreated group. *Carlina gummifera* L. incense inhalation reduced the total number, viability and mobility of epididymis spermatozoa compared to control. Furthermore, incense exposure induced various histological changes in the testes, prostate and seminal vesicles, including in particular a decrease in the number of gametes in the seminiferous tubes, the reduction of prostatic secretions and the macrophagic resorption of the seminal secretions. The effect of *Carlina* incense on the antioxidant system was evaluated by assaying the two antioxidant enzyme activities catalase and superoxide dismutase as well as thiol group levels in the testicles. Our results showed that fumigation affected these parameters, suggesting that the morphological and functional modifications in the male reproductive system induced by *Carlina gummifera* L. incense may be related, in part, to the alteration of the oxidative balance in the testicle. **Conclusion:** Smoke incense of *Carlina gummifera* L. caused marked reproductive toxicity in adult male rats associated with induced oxidative stress.

Keywords: *Carlina gummifera L., incense, oxidative stress, reproductive toxicity, sperm*

Introduction

Burning incense has been a traditional practice for centuries in large parts of Asia and Africa for religious or ritual intentions.[1] It aims to purify the surroundings of evil spirits, create a pleasant atmosphere or eliminate unwanted odours in an internal environment.[2] Burning incense has been suspected as

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source of indoor air pollution causing adverse effects in exposed individuals.[3]

There are various types of incense such as Oudh (*Aquilaria agallocha* agarwood), Bakhour (sandalwood tree resin, agarwood, etc.) and resin from plants of the genus *Boswellia papyrifera* and *Boswellia carterii*.[4] *Carlina gummifera L*. called also as glue thistle or *Atractylis gummifera L*.[5] is used as incense. The Arabs call it Addad or Dad.[5,6] Indeed, the rhizome of this plant is used to perfume houses and ward off insects, thanks to the release of isovalerian during its combustion.[6] It is known in the Mediterranean region and in the West for its common use in alternative medicine.[7]

Carlina gummifera L. is distributed mainly in the Mediterranean regions of North Africa (Tunisia, Algeria and Morocco) and in southern Europe (Italy, Greece, Spain and Portugal).[8] All parts of the plant are toxic, but the toxicity decreases from root to stem.[6] However, the root is the most dangerous organ as it contains the maximum of the toxicity. It also contains inulin, sugars, asparagine, amino acids, latex, essential oil, flavonoid heterosides and a triglucosyl derivative of luteolin.[7] Two substances are reported to represent the toxic active principles of *Carlina gummifera* L. These are atractyloside (ATR) also known as potassium atractylate and 4-carboxyatractyloside (CATR) also called gummiferin.[9,10]

The toxicity of ATR comes in relation to its structure including aglycone fragments, methylene groups, hydroxyl groups on the glucose fragment, sulphate and isovalerate groups, thus the chemical architecture of ATR creates its characteristic toxic effect.[10,11] It integrates into hepatic biotransformation pathways, which can induce cytolysis even at low doses.[12] In humans, there is a lack of data regarding tissue distribution, routes of excretion and metabolism of ATR.

The toxicity of *Carlina gummifera* L. is also due to the action of CATR which inhibits the transport of phosphorylated nucleotides by binding with phosphoryltransferase. It is present in the fresh plant but not in the dried one since when the plant gets old or dissected, the CATR is decarboxylated into ATR. This transformation is still possible by heating or fumigation.[11]

Orally, the lethal dose (LD50) in rats exceeds 1000 mg/kg for ATR and reaches almost 350 mg/kg for CATR. On the other hand and when administered intraperitoneally, the LD50 for ATR is 143 mg/kg and for the CATR reaches 2.9 mg/kg.[13] The use of doses lower than toxic doses, showed that *Carlina gummifera* L. has the ability to lower blood sugar and restore biochemical parameters in diabetic mice.[14] It is also endowed with an antioxidant, antifungal and insecticidal activity.[15]

Therapeutically, *Carlina gummifera* L. is also used in fumigation against colds, dizziness and headaches, as an infusion against haemorrhages during childbirth,[10] as an antisyphilitic and against boils, as a purgative and emetic and as an anthelmintic.[16] The dried root can be chewed or consumed in perfume burners for its insect repellent action.[15]

Although *Carlina gummifera* L. is known for its therapeutic uses, it remains amongst the medicinal plants with the most dangerous toxic potential in terms of number of intoxications, number of deaths and specific lethality.[17] The signs of poisoning are non-specific characterised by damage to various organ functions, general signs such as diarrhoea, vomiting and spasms, followed by neurological signs.[16,17] Renal, hepatic,[18] respiratory[19] and cardiovascular[20] impairment have also been reported. Approximately 24 h after intoxication, death can occur.[16]

Due to its slow and incomplete combustion, the combustion of incense produces continuous smoke, generating pollutants, such as toxic gases and chemical particles, including polycyclic aromatic hydrocarbons, carbon monoxide, benzene and isoprene, which easily accumulate indoors, especially in the presence of inadequate ventilation.[1]

Several researchers have studied possible links between exposure to incense sticks and health problems, including respiratory symptoms,[21] asthma,[22] cardiovascular disease,[23] contact dermatitis and cancer.[1,22] In addition, exposure to frankincense induced significant morphological changes in rat seminiferous tubules.[24]

Long-term exposure to frankincense smoke has been associated with disruption of the male reproductive system, essentially corresponding to cellular alterations, metabolic and hormonal disruptions, spermatogenesis disorders and sperm condition.[25]

Incense can also be classified amongst the agents that harm the male reproductive system and male fertility, but the toxicity of glue thistle smoke on the reproductive system remains little studied.

The aim of this study was to assess the effects of subacute exposure to frankincense smoke from *Carlina gummifera* L. roots on reproductive function in adult male rats and to assess its oxidative potential in relation to this action.

Materials and Methods

Collection of plant material

The plant samples were collected during September 2019 in the North East of Tunisia (locality of Sejnane) and authenticated by Dr. Ridha El Mokni, a botanical expert in Faculty of Pharmacy, Monastir (Tunisia), as roots of *Carlina gummifera* L. Samples were dried and finely ground in powder form before use.

Experimental animals

Wistar male rats weighing 150–200 g were procured from the SIPHAT (Tunisia) and were housed under controlled conditions of temperature (22°C ± 2°C), with 12-h light/dark cycle and a standard diet and water available *ad libitum*.

Animals were cared for in compliance with the Tunisian code of practice for the Care and Use of Animals for Scientific Purposes. The Institutional Ethics Committee (Approval Number 2020-12, dated 10 January 2020) approved the experimental protocols with appropriate minimal sample size (n = 8).

Experimental pattern

The animals are randomly divided into four groups containing 8 rats each. The first group serving as control and the other three groups were exposed to smoke incense by burning 2 (2C), 4 (4C) or 6 (6C) g of incense material for 60 min daily for 15 consecutive days. Each group of rats was housed separately to ovoid the cross exposure to smoke incense. Treated rats were placed in a wooden chamber of dimensions 100 cm × 80 cm × 70 cm, previously filled with smoke of incense.[24,26] The control group (T) was subjected to the same experimental conditions as the treated groups but without receiving the incense smoke. During the experiment, no signs of toxicity were observed.

Animal sacrifice

After experimental period, rats were sacrificed by decapitation. Testes, seminal vesicles and prostate were immediately dissected out, washed, weighed and frozen at −80°C for various biochemical evaluations and tissue samples were fixed in 10% paraformaldehyde for histopathological studies.

Sperm analysis

Epididymal sperm suspensions were prepared by mincing and homogenising two excised cauda epididymis of rat in watch glass containing 1 mL of Earle's medium with 0.2% bovine serum albumin, penicillin (100 units/mL) and streptomycin (1 µg/mL), pH 7.2. The sperm suspensions were incubated for 10 min at 37°C.[27] The sperm concentration and motility were determined according to the method of Besley *et al*.[28] using Neubauer's haemocytometer (Burker, Germany).

Cell viability was checked by the trypan blue (0.2%) exclusion test and was always >95%. Cell counts were performed by using a manual cell counter with an Neubauer chamber.[29]

Oxidative stress biomarkers

Lipid peroxidation (LPO) was expected according to the method given by Buege and Aust.[30] It was estimated from the concentration of thiobarbituric acid (TBA) reactive substance. 400 mg of testes is homogenised in Tris-buffered saline and then centrifuged at 10.000 rpm for 15 min. 500 µL of the supernatant is recovered and resuspended in PBS and TCA-BHT and then recentrifuged at 1000 rpm for 10 min. The supernatant obtained is taken up in HCl and Tris-TBA. The addition of Tris-TBA makes it possible to evaluate the formation of the malondialdehyde (MDA)-TBA complex. Optical density is determined at 530 nm after 10 min of incubation at 80°C.

Thiol groups in proteins can react with reactive oxygen species (ROS). The assay test for these groups is based on the theory of the formation of a relatively stable yellow colour by the sulfhydryl groups with 5,5, dithio-bis-2-nitrobenzoic acid (DTNB).[31]

The level of tissue proteins was estimated by the method of Lowry *et al*.[32] using bovine serum albumin (Sigma, St. Louis, MO) as a standard.

Testicular antioxidant enzyme activities

Catalase (CAT) activity was evaluated by following its decomposition in the presence of hydrogen peroxide (H_2O_2) by spectrophotometer at 240 nm.[33] 25 µL of the sample, diluted to 1/5, was mixed with 925 µL of phosphate buffer and 50 µL of H_2O_2. The solution obtained is read every 15 s for 45 s. Over time, there is a gradual decrease in optical density, which corresponds to the decomposition of H_2O_2 by CAT. CAT activity is expressed in µmoles of H_2O_2 per milligram of protein.

The superoxide dismutase (SOD) activity was estimated according to the method described by Misra and Fridovich.[34] At alkaline pH, $O2^{-\cdot}$ causes the autoxidation of epinephrine to adenochrome, while competing with this reaction, SOD decreased the adenochrome formation. One unit of SOD is defined as the amount of the extract that inhibits the rate of adenochrome formation by 50%. Enzyme extract was added in 2 mL reaction mixture containing 10 µL of bovine CAT (0.4U/µL), 20 µL epinephrine (5 mg/mL) and 62.5 mM sodium carbonate/bicarbonate buffer

pH 10.2. Usually, the absorbance gradually increases over 5 min at 480 nm.

Histomorphological examinations

Serial sections of tissue samples (testis, seminal vesicle and prostate) were prepared according to the method previously described.[35] Small pieces of tissues were fixed overnight at room temperature by direct immersion in 10% paraformaldehyde in 0.1 M phosphate buffer, pH 7.4. The samples were dehydrated with ethanol and toluene and embedded in paraffin wax. Serial sections (5 mm thick) were mounted on gelatin-coated glass slides and stained with haematoxylin and eosin.

Statistical analysis

Statistical analysis was performed using one-way analysis of variance followed by Tukey's multiple comparison as the *post hoc* test. Data are expressed as mean ± standard errors of the mean, and significance of difference between groups was accepted at $P < 0.05$.

Results

Effect of *Carlina* incense on body weight

Treatment by inhalation of the incense of 2, 4 or 6 g glue thistle for 15 days, did not cause significant variations ($P > 0.05$) in the body weight compared to the control animals (data not shown).

Effect of *Carlina* incense on reproductive organ relative weight

Rats treated with glue thistle incense show a dose-dependent decrease in the testis relative weight by 2.6%, 11% and 19%, respectively, in the 2C, 4C and 6C groups compared to the control group. In contrast, the inhalation of incense at the three doses increases significantly the seminal vesicles (36.36%, 29.35% and 37.2%) and the prostate (8%, 11% and 10.76%) indexes, respectively, compared to control [Table 1].

Sperm analysis

Table 2 indicates that animals exposed to *Carlina gummifera* L. incense show a dose-dependent decrease in the sperm count and mobility, with a profound reduction in the viability compared to controls.

Testis histology

Microscopic analysis of the testes in the control group shows a normal structure of the seminiferous tubules exhibiting different stages in seminiferous elements loaded with spermatozoa [Figure 1a] and having a well-individualised basal layer [Figure 1b].

The animals exposed to 2 g of *Carlina gummifera* L. incense show seminiferous tubes with little sperm load [Figure 1c] and basal layer remaining clear [Figure 1d]. There is also vascular congestions [Figure 1c] and interstitial

Table 1: Effect of *Carlina* incense on relative weights of testis, seminal vesicles and prostate (g/100 g BW)

	Testis	Seminal vesicles	Prostate
Control	0.539±0.042[a]	0.361±0.021[a]	0.174±0.013[a]
2C	0.525±0.038[a]	0.492±0.028[b]	0.188±0.012[b]
4C	0.480±0.029[b]	0.467±0.031[c]	0.193±0.027[c]
6C	0.437±0.037[c]	0.495±0.025[b]	0.193±0.029[c]

Significant differences are indicated by different letters (a, b, c). Rats received daily smoke from the burnt glue thistle roots for 15 days by inhalation at the rate of 2 g (2C), 4 g (4C) and 6 g of roots (6C). Control animals respire free air. Values means±SEM (n=8) are significantly different (P<0.05) in Tukey's multiple comparison *post hoc* test. SEM=Standard error of the mean

Table 2: Effect of *Carlina* incense on spermogram parameters

	n (×10^6 spz/mL)	Mobility (%)	Viability (%)
Control	193.33±13.33[a]	56.17±2.15[a]	97.52±2.46[a]
2C	53.68±4.18[b]	26±2.10[b]	72.23±2.21[b]
4C	45.31±4.84[b]	23.61±1.87[b]	71.17±1.09[b]
6C	24.67±2.12[c]	15.55±1.21[c]	61.81±1.78[b]

Different letters (a, b, c) indicate statistical significance at P<0.05 in Tukey's multiple comparison *post hoc* test. Rats received daily smoke from the burnt glue thistle roots for 15 days by inhalation at the rate of 2 g (2C), 4 g (4C) and 6 g of roots (6C). Control animals respire free air. Values are represented as mean±SEM (n=8). SEM=Standard error of the mean

oedema [Figure 1d], whereas the seminiferous tubes of animals exposed to 4 g are less loaded with spermatozoa [Figure 1e] and have a basal layer with vacuolated aspect [Figure 1f]. For the animals treated with 6 g, the seminiferous tubules are the poorest in gametes [Figure 1g] and show undeveloped germ cells with basal vacuolated cytoplasm associated with a profound disturbance of spermatogenesis process [Figure 1h].

Seminal vesicle histology

The control group microscopically shows normal seminal vesicles with a secretory epithelium filled with mucosa [Figure 2a], whereas the treated groups show histopathological alterations. In fact, vascular congestion can be distinguished [Figure 2b] in rats treated with 2 g of glue thistle. For those treated with 4 g, there is essentially a macrophagic resorption of the seminal secretions [Figure 2c], while the architecture of the vesicle in the 6C group specifically shows exfoliation of the cells of the coating of the seminal glands in the lumen with macrophagic resorption of the seminal secretions [Figure 2d].

Prostate histology

Our study shows that the prostate in the control group has a normal histology; it contains a lining of the normal prostate glands with blood vessels and other lymphatics [Figure 3a].

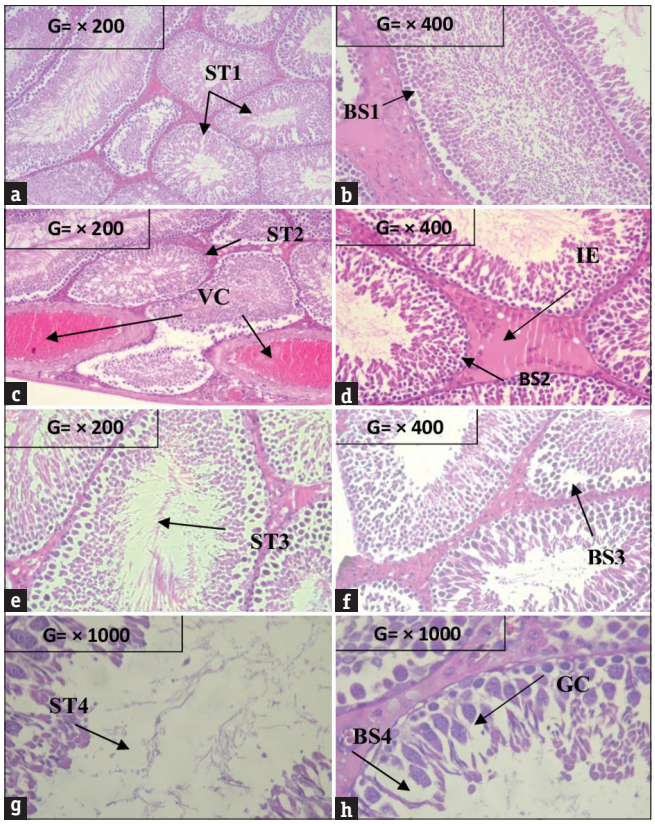

Figure 1: Effect of *Carlina* incense on the testes histology. Photomicrography of haematoxylin-eosin-stained sections of normal rat testes (a and b); testes from rats exposed to *Carlina* root incense at the rate of 2 g (c and d), 4g (e and f) and 6g (g and h). ST1 = Seminiferous tubes loaded with spermatozoa, ST2 = Seminiferous tubules modest loaded with spermatozoa, ST3 = Seminiferous tubes less loaded with sperm, ST4 = Seminiferous tubules poor in spermatozoa, BS1 = Well-individualised basal seat, BS2 = Net basal seat, BS3 = Basal seat with vacuolated appearance, BS4 = Vacuolation of the basal seat, VC = Vascular congestion, IE = Interstitial oedema, GC = Poorly developed germ cells

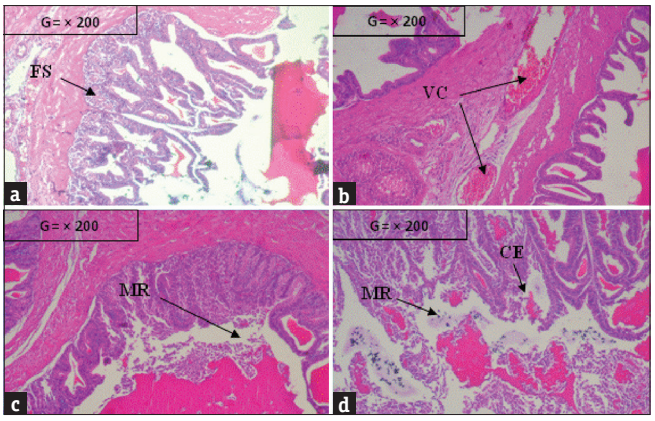

Figure 2: Effect of *Carlina* incense on the seminal vesicles histology. Photomicrography of haematoxylin-eosin-stained sections of normal rat seminal vesicles (a); seminal vesicles from rats exposed to *Carlina* root incense at the rate of 2 g (b), 4g (c) and 6g (d). FS = Folds filled with secretions, VC = Vascular congestion, MR = Macrophagic resorption, CE = Cell exfoliation

Table 3: Evaluation of the oxidative activity of *Carlina* incense in testis

	Protein (mg/mL)	Thiols (mmol)	CAT (µmol/min/mg de protein)	SOD (UIsod/min/mg de protein)
Control	209.48±18.81^{a}	0.13±0.02^{a}	0.17±0.008^{a}	0.12±0.07^{a}
2C	517.17±52.77^{b}	0.27±0.07^{b}	0.18±0.004^{a}	0.07±0.04^{b}
4C	547.66±27.27^{b}	0.43±0.11^{c}	0.23±0.045^{b}	0.018±0.01^{c}
6C	666.46±40.82^{c}	0.46±0.26^{c}	0.20±0.0004^{b}	0.06±0.01^{b}

Different letters (a, b, c) indicate statistical significance at $P<0.05$ in Tukey's multiple comparison *post hoc* test. Rats received daily smoke from the burnt glue thistle roots for 15 days by inhalation at the rate of 2 g (2C), 4 g (4C) and 6 g of roots (6C). Control animals respire free air. Values are represented as mean±SEM (n=8). SEM=Standard error of the mean, CAT=Catalase, SOD=Superoxide dismutase

Exposure to incense smoke alters prostate architecture. In the rats treated with 2 g, vascular congestion [Figure 3b] and conservation of the prostate gland coating [Figure 3c] were reported. In addition, the formation of interstitial oedema is noted [Figure 3d]. For a treatment of 4 g, there is an atrophic appearance of the lining of the prostate glands resulting in reduction of prostatic secretions and vascular congestion [Figure 3e]. The prostatic coating, in the group treated with 6 g of glue thistle, shows a flattened atrophic appearance [Figure 3f]. There is also inflammation and focal destruction of the prostate glands with interstitial oedema [Figure 3g].

Testis oxidative stress parameters

Table 3 illustrates that inhalation of *Carlina gummifera* L. incense significantly increases thiol groups content in the testes, respectively, to 110%, 230% and 258% in the 2C, 4C and 6C groups, compared to control group. By correlation with the enhanced concentration of thiol groups, a highly significant increase in tissue protein level is noted in the testes of treated animals. This increase reaches 146.88%, 161.43% and 218.14%, respectively, for the doses 2, 4 and 6 g, compared to the controls. However, testes MDA level decreased in a dose-dependent manner following exposure to incense smoke from 0.210±0.014 nmol/mg protein to 0.164±0.06, 0.075±0.013 and 0.051±0.005 nmol/mg protein, respectively, for control and 2, 4 and 6g treated groups.

Exposure to incense smoke affects the activity of CAT and SOD differently in the testis. Thus, following inhalation of *Carlina* incense, there is an increasing trend of the activity of CAT compared to control animals, while the same treatment causes a remarkable decrease in SOD activity of 41%, 84% and 43%, respectively, for the three doses compared to the controls [Table 3].

Discussion

Few studies have been done on the effects of *Carlina gummifera* L. incense smoke on the body and specifically

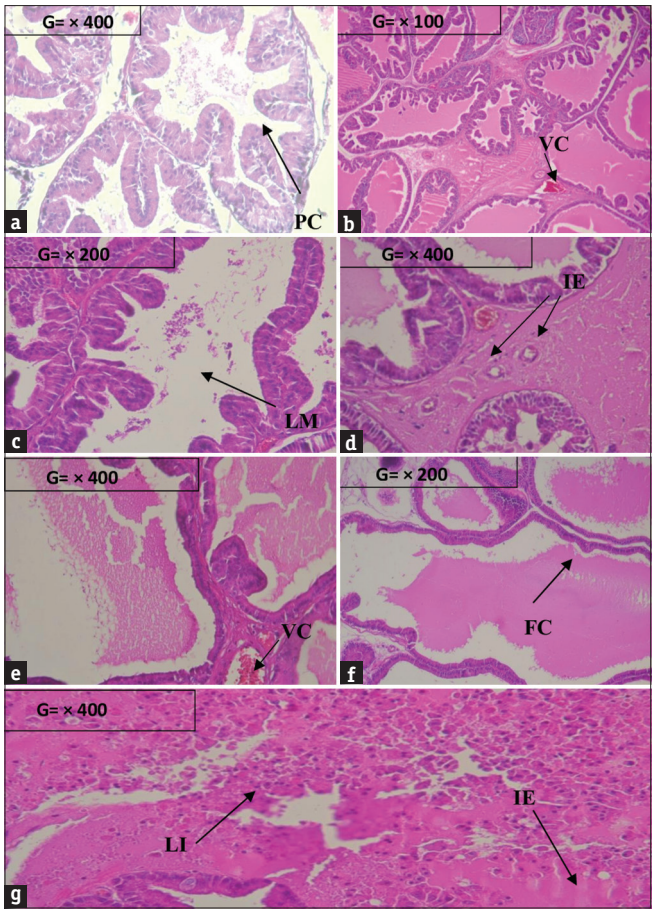

Figure 3: Effect of *Carlina* incense on the prostate histology. Photomicrography of haematoxylin-eosin-stained sections of normal rat prostate (a); prostate from rats exposed to *Carlina* root incense at the rate of 2 g (b-d), 4g (e) and 6g (f and g). P = Prostate coating, VC = Vascular congestion, L = Lumen of the prostate gland, IE = Interstitial oedema, FC = Flattened Coating, LI = Lymphocytic infiltrate

on the reproductive function. In this regard, our research was based on the evaluation of the effect of inhaling the root smoke of *Carlina gummifera* L. (glue thistle) on the reproductive system in adult male rats.

Our study showed that exposure of rats during 15 consecutive days to the smoke of glue thistle root, did not cause significant variations in body weight for the three treatments (2C, 4C and 6C groups) compared to the control animals. Indeed, the duration of treatment appeared to have no significant effect on body growth. This hypothesis is in agreement with the previous findings of Alokail *et al*.[36] who reported that exposure to the smoke of agarwood incense of 'Oud' (*Aquilaria agallocha*) did not cause significant changes in the weight gain of the rats during the first 8 weeks of treatment, but it subsequently decreased by the 12th week.

Contrarily, inhalation of the smoke from the root of *Carlina gummifera* L. caused a highly significant dose-dependent decrease in the total number and mobility of sperm, with a profound reduction in the viability compared to the non-exposed group. Our results are in line with previous data indicating that exposure to the smoke of *B. papyrifera* and *B. carterii* incense at a rate of 4 g for 48 days induced a significant decrease in the total number, viability, mobility and percentage of abnormal sperm.[24] It has been reported that the decrease in the sperm count was accompanied by a depletion of gonadotrophic hormone and testosterone levels.[37] Moreover, research by Dahamna[38] also showed that oral treatment with rhizome extract of *Carlina gummifera* L. at a rate of 120 mg/kg led to a significant decrease in the total number, viability and mobility of sperm, and suggested that the toxicological effect of *Carlina* incense on the spermatogenesis may be related to the phytochemical composition of the rhizome. Indeed, ATR and CATR are the two major toxic substances of *Carlina gummifera* L. root.[16] By heating, CATR will be decarboxylated into ATR,[10] which creates its characteristic toxic effect even at a low dose.[12] At the mitochondrial level, ATR inhibits oxidative phosphorylation, thus disrupting the metabolism of cells including germ cells (spermatozoa) and therefore their inability to survive. In addition, ATR inhibits dynein, which is an ATP-rich contractile protein necessary for the conversion of chemical energy into mechanical energy for the movement of sperm flagella, which further explains the decrease in sperm mobility.[38]

The maintenance of the structure and function of the male reproductive system are androgen dependent. Testosterone is essential for spermatogenesis process and male fertility.[39-41] Therefore, any alterations and abnormalities observed in this system are probably related to a deficiency in this hormone.[40,42,43] Hence, assessment of reproductive organ weights can be a good indicator to appreciate the level of smoke toxicity. Thus, following exposure to smoke incense of *Carlina gummifera* L., we recorded a significant dose-dependent decrease in the relative weight of the testes, while the same treatment caused a significant increase of the seminal vesicles and the prostate indexes compared to control. The decrease in the testis index is generally associated with the androgen deficiency.[40,43] In this regard, previous findings showed that male sex hormones maintain the structure and function of the gonads and any change in their levels, and particularly the reduction, will cause testicular and accessory sex gland atrophy.[40,44] Furthermore, incense exposure could also disrupt the hypothalamic-pituitary-testicular axis, leading to gonadotropins and testosterone decrease and consequently spermatogenesis damage.

We can postulate that the unexpected increased indexes of prostate and seminal vesicles following inhalation

of smoke incense of *Carlina gummifera* L., could result from the secondary alterations such as the tissue congestion and inflammation with interstitial oedema in these accessory glands as discussed below.

In this regard, a histological study was carried out to better explain the reported relative weight atrophy and semen changes in incense-exposed rats. Microscopic analysis of the testes in the control group showed a normal structure of the seminiferous tubules loaded with sperm. Exposure to all doses of glue thistle incense caused a vascular congestion and interstitial oedema in the testes, a decrease in the number of spermatozoa in the seminiferous tubules and a vacuolated appearance of the basal layer. At the higher dose, seminal tubular sperm depletion, loss of ciliated appearance of the coating, vacuolation of basal cell cytoplasm and decreased germ cell development were also observed. Our results are in accordance with previous data following exposure to various incenses. Thus, Ahmed *et al*.[24] reported that exposure to *Boswellia* incense smoke induced atrophy of the seminiferous tubules, their vacuolation and the widening of the intercellular space, associated with a decrease in the number of Leydig and Sertoli cells and a disruption of spermatogenesis. Exposure to the smoke of *Datura stramonium* induced also a degeneration of germ cells in the seminiferous tubules, vacuolation in the testicular section and cytoarchitectural distortion.[45] Furthermore, oral treatment with the extract of *Carlina gummifera* L. caused, after 48 h, a decrease in spermatogenesis, lysis of the elements of the seminiferous tubes, degeneration of the basal membrane in the tubes and an increase in number of inactive seminiferous tubules.[3]

It is suggested that these gonad histopathological alterations might result from the ATR toxic principle of *Carlina gummifera* L. roots. Indeed, according to Daniele *et al*.,[46] ATR is responsible for the opening of the transitional pores containing the adenine nucleotide translocator at the level of the mitochondrial membrane, which causes a partial permeabilisation of the inner membrane and complete permeabilisation of the outer membrane. Therefore, there will be a release of mitochondrial intermembrane proteins including cytochrome C, resulting in the activation of cell apoptosis,[45-47] which may be the reason for the decrease in the total and living number of sperm. On the other hand, ATR is integrated into the hepatic biotransformation pathways causing cytolysis with cellular degeneration and alteration of the structure of the seminiferous tubules with apparent abnormalities such as oedema and congestion.[45]

We extended the histological examination by cytological observations at the level of the accessory glands. In treated groups and at the level of the seminal vesicles, vascular congestion, macrophagic resorption of seminal secretions and exfoliation of cells covering the seminal glands were observed. In the prostate, exposure to the smoke incense caused vascular congestion, interstitial oedema, reduction of prostatic secretions and focal destruction, inflammation and a flattened atrophic aspect of the glandular mucosa.

According to Ahmed *et al*.,[48] the male reproductive system may also be affected by an estrogenic or anti-androgenic mechanism. The latter can result from the direct effect of the smoke emissions or indirectly through the installation of oxidative stress.[45]

Carlina incense smoke, which is increasingly being recognised as a potential environmental contaminant due to its recently found negative health effects, has not been tested for its ability to induce oxidative stress. It will then be important to evaluate the oxidative activity of *Carlina* incense on testis. The present study indicated that the inhaled glue thistle smoke caused a significant increase in the level of testicular proteins, a dose-dependent increase in the level of thiol groups and a significant decrease in the level of MDA in the testes. According to the work of Al-Attas *et al*.,[49] ROS could lead to chronic inflammation and to tissue damage leading to the release of testicular proteins. By correlation, thiol groups can also be affected by ROS in oxidative stress by increasing their level.[50]

In addition, ROS can also trigger LPO usually resulting in the increase of the MDA levels.[51] Following the work of Ahmed *et al*.,[24] exposure of rats to smoked *B. carterii* and *B. papyrifera* resulted in increased testicular MDA concentration. Similar results were found after treatment with Bakhour and Oudh incense smokes.[52] Our results go against these findings. It seems that the production of ROS is related to the nature of the toxic compounds of the plant. In this regard, frankincense particles can alter the functions of mitochondria and NADPH oxidase, releasing excess ROS and thus a second wave that will amplify any damage.[23,53] According to Daniele *et al*.,[46] one of the active substances of *Carlina gummifera* L., ART, can induce toxicity by an oxidative process involving its methylene group, which produces a free radical unknown until now amongst the ROS.

Our data showed that Carlina incense altered the testis antioxidant system by decreasing the activity of SOD and increasing slightly that of CAT. Increased CAT levels upon exposure to *Boswellia* smoke were shown to enhance the antioxidant activity of this enzyme against increased ROS.[24] A decrease in the SOD activity has also been noted, following exposure to Bakhour incense

smoke. This decrease can result from the depletion of the enzyme during intense oxidative stress or inhibition of certain pro-oxidant substances.

Conclusion

Our study is the first study that investigated *Carlina gummifera* incense-induced reproductive toxicity. To our knowledge, the present study is the first to investigate the male reproductive toxicity of *Carlina gummifera* insencex inhalation caused oxidative stress in the testes, which can be the indirect cause of the profound alterations of gametogenesis. These alterations could also be induced by a direct toxic action of the active substances released in the smoke. However, further study is needed to characterise the incense emissions.

Data availability statement

The data sets in this study are available with the corresponding author upon reasonable request.

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Conflicts of interest

There are no conflicts of interest.

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