RESEARCH ARTICLE

IMPACT OF ALKALOID OF *PEGANUM HARMALA* EXTRACT ON OXIDATIVE STRESS BIOMARKERS AND HISTOMORPHOMETRICS OF TESTICULAR TISSUES IN MALE MICE *(MUS MUSCULUS)*

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ABSTRACT

The study was designed to determine the impact of administration of *P. harmala* total alkaloid extract on testicular oxidative stress biomarkers and histomorphometrics of testicular tissues in male mice. After two weeks of acclimatization, twenty eight Swiss albino male mice (Mus musculus) aged 8 weeks were randomly divided into equal four groups (n=7). Male mice received daily by gavage an alkaloid extract of P. harmala at different concentrations (0, 6.25, 12.5 and 25 mg/kg BW/day in carboxymethyl cellulose vehicle 0.5%) for 35 days. After the sacrifice of male mice, the testes were collected and stored at -80°C, while another set was immediately preserved in 10% formalin after removal from the animals. At the end of the trial, oxidative stress parameters and histomorphometric analyses of testicular tissues were evaluated. A significantly lower value of testicular malondialdehyde (MDA) was observed in the treated groups with the total alkaloid extract of *P. harmala* compared to the control group ($P<0.05$). The mean level of superoxide dismutase increased in the groups treated with the 6.25 mg/ kg and 25 mg/ kg of the extract (0.44±0.07, 0.5±0.04 U/mg protein, respectively) compared to the control (0.4±0.08 U/ mg protein). The results show a significant ($P<0.05$) increase of catalase activity in the group treated with a concentration of 25 mg/ kg. Additionally, the histological pattern of the testes in male mice treated with P. harmala extracts appears normal. The diameter of seminiferous tubules and germinal epithelium thickness appeared to increase in the *P. harmala*-treated groups with different extract concentrations. It should also be noted that luminal height values were higher in male mice treated with a concentration of 25mg/kg than in mice in the control group ($P> 0.05$). Based on our results, the total alkaloids of P. harmala appear to protect testicular tissues against oxidative stress, suggesting their potential as an antioxidant source. Furthermore, the positive effects of *P. harmala* seeds extract, especially at a dose of 25 mg/kg body weight, are interesting in relation to the histomorphometric measures of testes.

Keywords: Male mice, oxidative stress, Peganum harmala, testicular histomorphometrics

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INTRODUCTION

Infertility is a dysfunction of the reproductive system characterized by the inability to achieve pregnancy after a year of unprotected sexual intercourse. It is a global clinical issue with a rising incidence, affecting millions of couples worldwide involving both sexes (WHO 2021). Male infertility is defined as quantitative or qualitative deficit in male reproductive cells, such as the absence of spermatozoa (azoospermia), reduced sperm mobility (asthenospermia), and abnormal sperm morphology (teratospermia) (Al-Tawalbeh et al., 2023). It can also be caused by a genetic disorder, hormonal imbalance, blockage of the reproductive tubules, erectile dysfunction, systematic disease, or caused by poor lifestyle practices, *i.e.* smoking, stress, obesity, drug use, advanced marital age, and exposure to environmental pollutants. These factors can disrupt the oxidative balance of the testes, as spermatogenesis and Leydig cell steroidogenesis are vulnerable to oxidative stress, resulting in lipid and protein peroxidation as well as DNA damage to sexual cells (Aitken and Roman, 2008; Bisht et al., 2017). Consequently, these factors can lead to histological alterations, a reduction in spermatogenesis, and a decrease in the physiological capacity for fertilization (Agarwal et al., 2014).

Harmel (*Peganum harmala*) is a plant native to the eastern Mediterranean region to East India and belongs to Nitrariaceae family. It is known as Wild rue, Syrian rue and African rue. It is a perennial herb that grows in arid and semi arid regions, steppe areas, and sandy soils (Aslam et al., 2014). *P. harmala* is consumed due to its various nutritional qualities and considered as a universal medicinal plant. It is used to address various problems, including male infertility (Singh et al., 2013). The alkaloid extract of *P.harmala* has a diverse range of pharmacological activities, such as antibacterial (Iranshahy et al., 2019), analgesic (Farouk et al., 2008), anti-inflammatory (Ramadhan et al., 2013), antioxidant (Abbas et al., 2021), hypoglycemic (Ramazani et al., 2014), and antitumor (Zhang et al., 2022). Furthermore, extracts from *P. harmala* seeds have been shown to have positive and varied impacts on male reproductive health. A recent study reported that the alkaloid extract of *P. harmala* seeds grown in Algeria, had an impact on the sexual performance and sperm quality of male mice (Derbak et al., 2014). Moreover, the extracts demonstrated the ability to stimulate sperm motility, preserve membrane integrity, and protect ram spermatozoa against lipid peroxidation (Derbak et al., 2021). However, to the best of our knowledge, the investigation on effect of *P. harmala* seed alkaloids on testicular antioxidant system status and its histological structure have not been reported. Thus, the aim of this study is to determine the impact of administration of *P. harmala* total alkaloid extract on testicular oxidative stress biomarkers (malonaldehyde, superoxide dismutase and catalase activities) and histomorphometrics of testicular tissues in male mice.

MATERIALS AND METHODS

This research adhered to the guidelines for the ethical treatment of laboratory animals. Approval for the proposed experiments was obtained from the Ethics Committee of the Faculty of Natural and Life Sciences, University of Bejaia (Report of Faculty Scientific Council #05 dated 14 December 2016).

Plant material and extraction

Peganum harmala seeds were collected in April 2016 from Ngaoues region (35°32’N, 6°10’E, Batna province, Algeria). The scientific authentication of plant seeds was carried out by the botanist of the Bejaia University. The voucher specimen (PhB080) is kept in the herbarium of the Pharmacy Department (University of Batna, Algeria). 1 kg of air-dried powdered plant material was first exhaustively extracted with ethanol (96%, v/v) in a Soxhlet apparatus (Behr Labor-Technik GmbH, Dusseldorf, Germany) for 8 h. The solvent from the ethanolic extract was completely removed and concentrated using a rotary evaporator (BüchiLabortechnik AG, Flawil, Switzerland). The ethanolic extract was then concentrated and acidified with HCl (2%, v/v), and extracted with petroleum ether (5000 mL) to remove the apolar phase. The acidic aqueous solution was basified three times to pH 9 with ammoniac and extracted with dichloromethane (1000 mL). The dichloromethane layer was evaporated under vacuum conditions to obtain approximately 0.05 % w/w of alkaloids fraction (Kartal et al., 2003).

Animals and preparation

Twenty-eight adult Swiss albino male mice (*Mus musculus*) aged 8 weeks were obtained from the animal house of the Central Faculty (University of Constantine, Algeria). The animals were kept under standard conditions of temperature, humidity, light/dark (23 ± 2°C, 55 ± 5% and 12:12h, respectively). During this period, the mice were fed on a standard diet and given tap water *ad libitum*. All animal procedures were conducted following the recommendations of the International Ethics Committee. (Directive 2010/63/EU, which updated and replaced the Directive of the European Council 86/609/EC).

After two weeks of acclimatization, twenty-eight animals were randomly divided into four groups per seven mice. Mice received a daily gavage of an alkaloid extract of *P. harmala* at different concentrations (6.25, 12.5 and 25 mg/kg BW/ day in carboxymethyl cellulose vehicle 0.5%) for 35 days. Concerning the control group, animals received only carboxymethylcellulose vehicle (0.5%; 10 mL/kg). After the experimental regimen (24 h) following oral administration of the extract, the mice were sacrificed by cervical dislocation under chloroform anesthesia, and their testes were collected. One testicle was preserved at -80°C and another in 10% formalin immediately after removal from the animal.

Preparation of testicular tissue homogenate

The tunica albuginea was manually removed from the testes. Testicular tissues were homogenized in 3 mL of PBS (pH 7.4) for 5 min. Subsequently, the homogenate was centrifuged at 10000 rpm for 10 min at 4 °C. The supernatant obtained was used for the determination of lipid peroxidation, superoxide dismutase, and catalase activity.

Biochemical measurements

Total protein estimation

The protein concentrations of gut samples were determined by the method of Bradford (1976) using Coomassie Brilliant Blue G-250. Bovine serum albumin was used as a standard. The absorbance values were measured at 595 nm using a spectrophotometer.

Measurement of lipid peroxidation

Malondialdehyde (MDA) is the end product of lipid peroxidation, serving as an indicator of oxidative stress. MDA was quantified using thiobarbituric acid (TBA) assay according to Buegeand Aust (1978). Briefly, 1 ml of the stock solution TBA-TCA-HCl containing (trichloracetic acid 15% w/v, thiobarbituric acid 0.375% w/v in hydrochloric acid 0.25 N) was added to one volume of the tissue homogenate. The mixture was kept in boiling water bath for 60 min and then cooled in an ice bath. The suspension was centrifuged at 18000 g for 15 min. The thiobarbituric acid reactive substances (TBARS) were measured using a spectrophotometer (Biotech Engineering Management Co. Ltd. UK VIS-7220G) at a wavelength of 532 nm. The molar extinction coefficient for MDA is 1.56×10^5 M^{-1} cm^{-1}. The value is expressed as nmol of MDA equivalent formed/mg of the testis.

Superoxide dismutase (SOD) activity

Superoxide dismutase (SOD) activity was assayed by the method of pyrogallol autoxidation, according to Marklund and Marklund (1974). Testicular supernatant was prepared in 1440 µL of Tris–HCl buffer (50 mM/L mM EDTA, pH 8.2), 100 µL of pyrogallol solution (15 mM/10 mM HCl). The SOD activity was measured at 420 nm for 3 min. One unit of SOD was determined as the amount of enzyme that inhibited the oxidation of pyrogallol 50 %. The SOD activity obtained was expressed as U/min/mg of protein.

Determination of catalase (CAT) activity

Catalase activity was estimated by measuring the decomposition of hydrogen peroxide (H_2O_2)

at 240 nm absorption (25 °C for 60 s) (Aebi 1984).The reaction mixture consisted of 1255 µL of phosphate buffer (50 mM, pH 7.0), 20 µL of supernatant, and 725 µLof H_2O_2 (54 mM). The molar extinction coefficient for MDA is 43.6 l/mol per cm. One unit of the catalase activity was expressed as the amount of H_2O_2 consumed per minute per milligram of protein.

Testis histology

The testes from mice were fixed in buffered 10 % formalin and subsequently processed for routine histopathology. The processed testes were dehydrated in a gradient of alcohol (70-95%), followed by clearing and embedding in paraffin wax. Serial sections, each 2-3 µm thick, were meticulously obtained using a rotary microtome (Leica RM2125 RTS). Subsequently, these sections were stained with hematoxylin and eosin. Examination of the slides was conducted under a light microscope (X10 magnification), and measurements were recorded, including the diameter of seminiferous tubules, thickness of germinal epithelium, diameter of the lumen in testes, and thickness of the tunica albuginea. All morphometric analyses were performed using Image J software (Version 1.52, NIH, USA).

Statistical analysis

Results were expressed as means ± standard error (SEM). Experimental data were analyzed using GraphPad Prism Graph Pad Prism 5.0 (San Diego, CA, USA). Analysis of variance (ANOVA) was used, followed by Tukey's test. Results were considered statistically significant at $P<0.05$.

RESULTS

The effect of the total alkaloid extract of *P. harmala* on testicular MDA levels in mice is illustrated in Figure 1. A significantly lower value of testicular MDA was observed in the treated groups with 6.25 mg/kg, 12.25 mg/kg, 25 mg/kg of the total alkaloid extract of *P. harmala* (52.93 ±5.47, 55.08 ±3.17 and 58.81±3.16 nmol/mg, respectively) compared to the control group (76.07±4.14 nmol/mg) ($P< 0.05$).

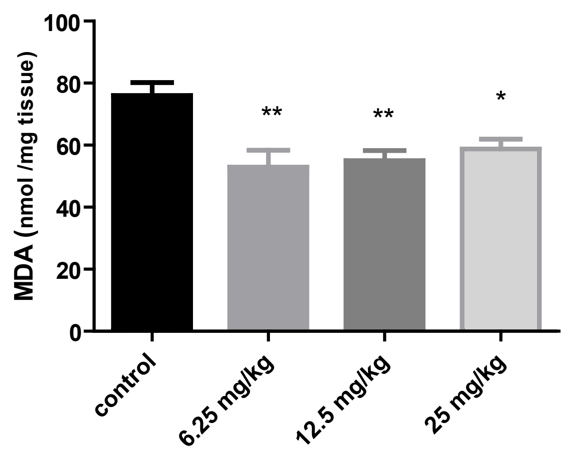

Figure 1 Effect of *Peganum harmala* alkaloid fraction on testicular tissue MDA levels after 35 days of administration. One-way ANOVA followed by Tukey comparison tests was used for statistical significance *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ compared to control group

Figure 2 shows the level of Superoxide dismutase (SOD) in different groups after 35 days of administration of total alkaloid extract *P. harmala*. The mean level of superoxide dismutase increased in the groups treated with the 6.25 mg/ kg and 25 mg/ kg of the extract (0.44±0.07, 0.5±0.04 U/mg protein, respectively) compared with the control (0.4±0.08 U/mg protein). However, this rise was statistically non-significant in the treated group with 25 mg/ kg of total alkaloid extract *P. harmala* ($P> 0.05$).

Figure 3 illustrates the effects of different concentrations of total alkaloid extract *P. harmala* on catalase levels in testicular tissues after 35 days of treatment. Results show a significant ($P <0.05$) increase of catalase activity in the group treated with concentration 25 mg/ kg (33.83± 1.7µmol/H_2O_2/mg prot/min). Mice treated with 6.25 mg/kg and 12.5 mg/kg concentration of total alkaloid extract *P. harmala* showed a slight, non-significant

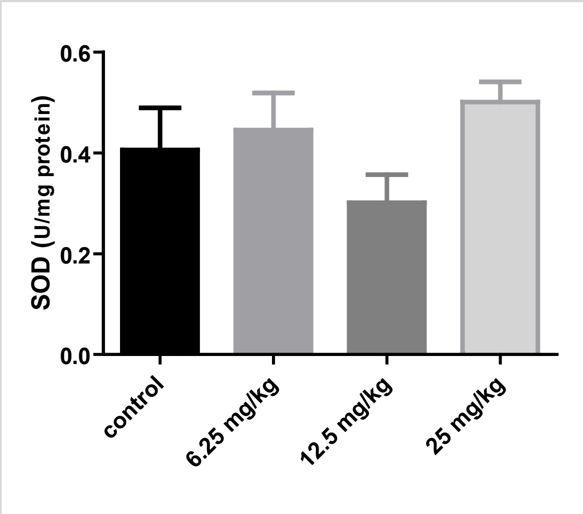

Figure 2 Effect of *Peganum harmala* alkaloid fraction on testicular tissue SOD levels after 35 days of administration. One-way ANOVA followed by Tukey comparison tests was used for statistical significance

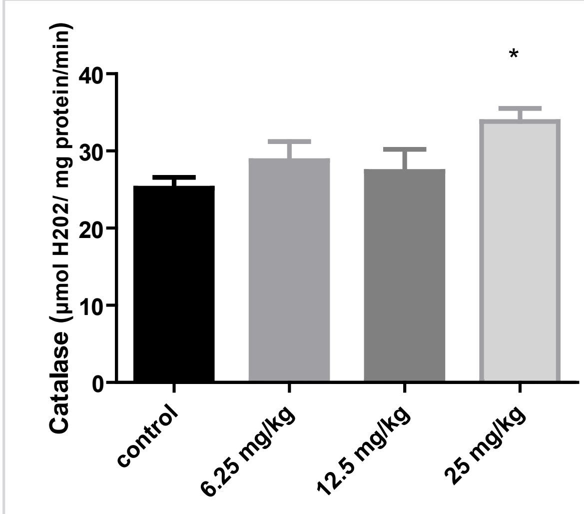

Figure 3 Effect of *Peganum harmala* alkaloid fraction on testicular tissue catalase after 35 days of administration. One-way ANOVA followed by Tukey comparison tests was used for statistical significance *$P < 0.05$, **$P < 0.01$, ***P <0 .001 compared to control group

increase in catalase capacity compared to the control group (25.17± 1.41µmol/ H_2O_2/mg prot/ min).

The histological pattern of the testes of mice treated with total alkaloid extract *P. harmala* appears normal. The spermatogenic cells and Sertoli cells of the seminiferous tubules exhibit regular morphological characteristics. The seminiferous tubules have a regular appearance, lined with well-arranged rows of spermatogenic cells at different stages. Histomorphometric measurements of testicular tissues after 35 days of administration of *P. harmala* extracts in mice were presented in Table 1.

Table 1 Seminiferous tubule diameter, epithelium high, luminal height, and tunica albuginea width of male mice administered with different doses of *Peganum harmala* alkaloid fraction for 35 days. One-way ANOVA followed by Tukey comparison tests was used for statistical significance *$P < 0.05$, **$^P < 0.01$, ***$P < .001$ compared to control group

Parameters	Experimental groups			
	Control	6.25 mg/kg	12.5 mg/kg	25 mg/kg
Seminiferous tubule diameter (µm)	224.9 ± 3.34	237.5 ± 1.3	235.4 ± 3.2	252.2 ± 4.73***
Epithelial height (µm)	67.9 ± 2.1	68.3 ± 1.86	70.7 ± 2.16	79.8 ± 2.95**
Luminal height (µm)	99.1 ± 2.56	99.4 ±4.41	92.4 ± 2.54	100.6 ± 4.
Width of tunica albuginea (µm)	18.12 ± 0.79	17.9 ± 0.92	18.4 ± 2.2	16.2 ± 1.46

The diameter of seminiferous tubules and germinal epithelium thickness appeared to increase in the total alkaloid extract *P harmala*-treated groups with different extract concentrations. Only the group treated with an extract concentration of 25 mg/kg showed a significant difference compared to the control group. It should also be noted that luminal height values were higher in mice treated with concentration 25 than in mice in the control group, but there was no significant difference ($P > 0.05$). Meanwhile, the thickness of the tunica albuginea does not appear to be affected by the different concentrations of total alkaloid extract *P. harmala*.

DISCUSSION AND CONCLUSION

Plant extracts are widely used to treat or alleviate male reproductive diseases as well as to improve fertility, encompassing all aspects of reproductive health. Male reproductive health may benefit from oxidative stress management and prevention. The main defense against oxidation is the consumption of an antioxidant-rich diet. Rich in phytochemicals, plants can provide an extra layer of protection against oxidative stress and are a strong source of natural antioxidants. This investigation aimed to study testicular histology and antioxidant biomarkers in mice after administration of *P. harmala* alkaloid extracts for 35 days, corresponding to the period of mice spermatogenesis.

It is known that antioxidants are utilized extensively these days to halt the oxidative chain reaction (Bilaspuri and Bansal, 2008). In recent years, herbal therapies have emerged as a potential gold standard for treating male infertility. The primary factor likely responsible for the ability of plants to combat infertility may be related to their antioxidant content (Safarnavadeh and Rastegarpanah, 2011). Common Harmel (*Peganum harmala*), widespread in North Africa, is one of the plants that is frequently used in traditional medicine. The results of the present investigation revealed that *P. harmala* total alkaloid extract possessed antioxidant properties, decreasing MDA levels in testicular tissues. Furthermore, the study demonstrates that *P. harmala* extract has beneficial effects on antioxidant enzymes, as shown by the notable elevation of testicular superoxide dismutase and catalase levels in mice administered with the extract. Male infertility is largely linked to oxidative stress, which can affect the immune-privileged testis. It is important to underline that the testicular microenvironment is characterized by high amounts of unsaturated fatty acids and low oxygen concentration (Aprioku, 2013). Lipid peroxidation, also described as the oxidative destruction of polyunsaturated fatty acids, causes a number of membrane-bound enzymes to become inactive and impairs fluidity of the cell membrane (Taso et al., 2019). MDA is one of the main oxidation products of peroxidized polyunsaturated fatty acids (Partyka et al., 2012). Our results are consistent with those previously published, which showed that total alkaloid extract *P harmala* protects sperm *in vitro* from ROS by reducing MDA levels and preserving sperm membrane integrity (Derbak et al., 2021). Likewise, lipid peroxidation has been linked to poor sperm quality and has an impact on the sperm concentration, motility and morphology (Colagar et al., 2013). In another study, Berrougui et al. (2006) suggested that *P. harmala* alkaloids (harmine and harmaline) compounds could be a major source of compounds that inhibit LDL oxidative modification induced by copper. Furthermore, Rezaei et al. (2016) have reported that in rats with Parkinson's disease induced by 6-hydroxydopamine, the aqueous extract of *P. harmala* decreased oxidative stress and the levels of lipid and protein oxidation in the brain. A significant reduction in MDA is explained by a decrease in lipid peroxidation, resulting in high antioxidant enzyme activity. The total alkaloids extract of *P. harmala* have the power to prevent lipid peroxidation and strengthen the antioxidant defense system of testicular tissues. Our findings are in agreement with those of Shokoohi et al. (2019), who found that the flavonoid-rich *Fumaria parviflora* extract, isoquinoline alkaloids,and phenolic compounds protect the testis tissue and sperm quality in diabetic rats from oxidative stress.

It is important to remember that many complex antioxidant defense systems, including enzymes superoxide dismutase (SOD) and catalase (CAT), block the initiation of free radical chain reactions. Free radicals can trigger chain reactions by interacting with proteins, lipids, and nucleic acids, leading to cellular malfunction and even death when they are produced in excess or when the antioxidant defense system within the cell is compromised. Antioxidant enzymes have been widely investigated for the prevention and treatment of diseases resulting from oxidative damage. Many studies have investigated on the antioxidant activity of numerous plant extracts on reproductive function (Iamsaard et al., 2014; Rasyidah et al., 2014), Contino et al., 2023, Akbari Bazm et al., 2019). The present study demonstrated the antioxidant properties of *P. harmala* seeds extract at a concentration of 25 mg/kg in mice by an increase in the enzymes catalase and superoxide dismutase in the testicular tissues. This suggested that the usage of *P. harmala* extracts prevented the decrease in SOD and CAT enzyme, which may be due to the elimination of free radicals by alkaloid total extract. As a result, these antioxidant enzymes are preserved and conserved. Alkaloids are known to have analgesic, antioxidant and anti-inflammatory bioactivity, which help to develop endurance against oxidative stress in animals (Singhai and Patil, 2021; Kumar et al., 2015; Elansary et al., 2020). Our findings are consistent with those reported by Hamden et al. (2008) in an *in vivo* study on male rats, who demonstrated the protective effect of *P. harmala* extract (50 mg/kg) against the negative effects of reactive oxygen species by increasing the activity of the antioxidant enzymes SOD and CAT. It is known that SOD and CAT are primary antioxidants that break down superoxide into hydrogen peroxide and hydrogen peroxide into molecular of oxygen and water, respectively (Weydert and Cullen, 2010). Tissue levels of SOD and CAT reflect the degree of oxidative stress experienced (Singh et al., 2017). As illustrated by the results, the increased levels of SOD and CAT in the testes of mice were enhanced after administration of *P. harmala* alkaloid extracts, and this positive effect may be due to the antioxidant effects of its bioactive compounds. Recently, Abbas et al. (2021) revealed that extracts of *P. harmala* exhibited significant antioxidant activity in both *in vitro* and *in vivo* models, justifying the use of this plant in the traditional medicine. In addition, this bioactivity might be attributed to the presence of harmol, harmine, harmaline, and peganine. In another study, the research team members demonstrated the ability of aqueous extract of *Allium sativum*, rich in alkaloids, to restore the antioxidant status of rats testis *in vivo* (Ayoka et al., 2016).

It is also important to mention that spermatocytogenesis in mice starts at age of 30 days. The presence of spermatocytes and spermatids were considered as signs of spermatocytogenesis in the seminiferous epithelium. Histological findings of the present study indicate that alkaloid extract of *P. harmala* has no harmful effect on spermatogenesis and Sertoli cells in mice. The proliferation of spermatogenic cells observed in mice treated with different concentration of *P. haramala* extract was normal. Regarding the histological appearance of seminiferous tubules, epithelial cell layers, the testes of the control group were comparable to those of the treated animals. It has been reported that *Withania somnifera* provokes spermatogenesis in male Wistar rats based on histological observations (Abdel-Magied et al., 2001). However, Benbotta et al. (2018) reported that *P. harmala* alkaloids induced testicular toxicity at higher doses (80, and 120 mg/k) and disturbed the function of hypothalamic pituitary testis axis. It mainly included stop of spermatogenesis, deformation in seminiferous tubules structure, damage to Leydig cells, and absence of Sertoli cells. In another investigation, Adisa et al. (2014) reported that alkaloid extract of *Telfairia occidentallis* leave had the potential for reducing male sexual function considering the observed histological presentation of testis. It may be testiculotoxic, as indicated damage on testicular cells, such as cellular degeneration, hemorrhage, interstitial space exudations and cellular necrosis. These differences may be due to administration dose, culture or growth conditions, and geographical variations of plants. In addition, the extraction method can alter the total alkaloid content. In this study, the results of the treated mice with high doses of *P. harmala* total alkaloid extract (25 mg/kg) revealed a significant increased seminiferous tubule diameter, epithelium height, and epithelium area. Our results are in accordance with those previously published (Olawuyi et al., 2019), which showed that *Lawsonia inermis* aqueous leaf-extract led to statistically significant changes in the percentage of seminiferous tubular and seminiferous ductal diameter male Wistar rats. Likewise, Akang et al. (2015) reported that *Telfairia occidentalis* protected the seminiferous epithelium, reduced oxidative stress and promoted spermatogenesis mature male Sprague–Dawley rats, according to cross-section testicular histology. It has also been reported that animals treated with *Cassia abbreviata Oliv* showed an increase in seminiferous tubule diameter and germinal epithelial height with all the stages of spermatogenesis (Msiska et al., 2021). In another study, Azu et al. (2010) investigated the short-term effects of *Kigelia africana*, known to be rich in flavonoids and alkaloids, fruit extract on testicular histomorphometric changes in Sprague-Dawley rats, which significantly improved the testicular weight/volume, diameter somniferous tubules and transverse surfaces. This may be due to a direct effect of bioactive components of *P. harmala* on histomorphometric parameters of testis. Our results could be explained by androgen-stimulating activity, which then stimulates spermatogenesis, increasing the number of spermatogenic cells (Smith and Walker, 2014).

Based on our results, the total alkaloids of *P. harmala* protect testicular tissues against oxidative stress, suggesting it as a potential antioxidant source. Furthermore, the positive effect of *P. harmala* seed extract (especially at a dose of 25 mg/kg body weight) is interesting on the histomorphometric measures of testes. Additionally, the present research showed that the histological architecture of the testes was preserved in its physiological form. However, it would be necessary to undertake *in vitro* and *in vivo* toxicological studies on organs such as the liver and spleen as well as hematological parameters.

AUTHOR CONTRIBUTIONS

Conceptualization, writing—original draft preparation, resources, H.D. and A.A.; technical assistance, H.D. and A.C.B.; data validation, review and editing, O.B., E.H.B. and A.A.; supervision, writing—review and editing, A.A. All authors have read and agreed to the published version of the manuscript.

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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UTJECAJ ALKALOIDA EKSTRAKTA *PEGANUM HARMALE* NA BIOMARKERE OKSIDATIVNOG STRESA I HISTOMORFOMETRIJU TESTIKULARNOG TKIVA KOD MUŽJAKA MIŠA *(MUS MUSCULUS)*

SAŽETAK

Istraživanje je provedeno sa ciljem evaluacije utjecaja primjene alkaloida ekstrakta *P. harmale* na biomarkere testikularnog oksidativnog stresa i histomorfometriju testikularnog tkiva kod mužjaka miševa. Nakon dvije sedmice aklimatizacije, dvadeset i osam Švicarskih albino miševa mužjaka (*Mus musculus)* starih 8 sedmica su metodom slučajnog uzorka podijeljeni u četiri jednake grupe (n=7). Mužjaci miševa su svaki dan putem sonde primali ekstrakt alkaloida *P. harmale* u različitim koncentracijama (0, 6.25, 12.5 i 25 mg/kg TT/dnevno u 0.5% karboksimetil celulozi kao nosaču) u trajanju od 35 dana. Nakon žrtvovanja mužjaka miševa, testisi su ispreparirani, pri čemu je jedan iz para uskladišten na -80°C, a drugi odmah prezerviran u 10% formalinu. Na kraju istraživanja su evaluirani parametri oksidativnog stresa i izvršene su histomorfometrijske analize testikularnog tkiva. Od tretiranih grupa, uočena je signifikantno niža vrijednost testikularnog melondialdehida (MDA) kod grupe koja je primala ukupni alkaloid ekstrakta *P. harmale* u usporedbi sa kontrolnom grupom (P<0.05). Srednja vrijednost superoksid dismutaze je povišena u grupi tretiranoj sa 6.25 mg/ kg i 25 mg/ kg ekstrakta (0.44±0.07, 0.5±0.04 U/mg proteina) u usporedbi sa kontrolom (0.4±0.08 U/mg proteina). Rezultati pokazuju signifikantan (P <0.05) porast aktivnosti katalaze u grupi tretiranoj sa koncentracijom od 25 mg/ kg. Osim toga, histološka slika mužjaka miševa tretiranih sa ekstraktima *P. harmale* se *čini* normalnom. *Čini* se da se dijametar sjemenskih kanalića i debljina germinativnog epitela povećavaju u grupama tretiranim sa ekstraktima *P harmale* u različitim koncentracijama. Također je zabilježeno da su vrijednosti visine lumena veće kod mužjaka miševa tretiranih sa koncentracijom od 25mg/kg u odnosu na miševe iz kontrolne grupe (P> 0.05). Naši rezultati pokazuju da ukupni alkaloidi *P. harmale štite* testikularno tkivo od oksidativnog stresa, sugerirajući njihov potencijal kao izvor antioksidansa. Osim toga, pozitivni učinci ekstrakta sjemena *P. harmale*, posebno u dozama od 25 mg/kg tjelesne težine, su zanimljivi u smislu histomorfometrijskih mjera testisa.

Ključne riječi: Mužjaci miševa, oksidativni stress, *Peganum harmala*, testikularna histomorfometrija