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Comparative Evaluation of Methanol Exposure and *Eucalyptus globulus* Extracts on Reproductive Parameters in Rats

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Abstract

Plant-based biopesticides are considered as safer alternatives than synthetic pesticides. *Eucalyptus globulus* is widely recognized for its antioxidant potential, moreover the exposure to methanol raises the risk of possible reproductive toxicity. This study evaluated the effects of methanol and *Eucalyptus globulus* extracts on testicular function of rats. Forty rats were divided into four groups: Control, Methanol (600 mg/kg BW), Methanolic extract (ME, 100 mg/kg BW), and Aqueous extract (AE, 100 mg/kg BW). Treatments were administered orally for 28 days. At the end of the experiment, rats were sacrificed and testes and epididymides were collected. Sperm concentration, motility, and velocity were assessed using a CASA system. Serum testosterone levels and testicular oxidative stress markers (GSH, GPx, and MDA) were determined and histological evaluation of testicular tissue was performed using H&E staining. Methanol exposure was associated with the increase of oxidative stress, seminiferous tubule degeneration, and the reduce of sperm concentration, motility, and velocity. In addition, rats treated with *Eucalyptus globulus* extracts showed more favorable antioxidant status, testicular histology, and sperm characteristics. In conclusion, methanol considerably affected testicular function and reproductive parameters, whereas *Eucalyptus globulus* extracts were associated with more favourable reproductive and oxidative stress profiles.

Keywords: Methanol, Eucalyptus extract, Oxidative stress, Reproductive health, Biopesticide.

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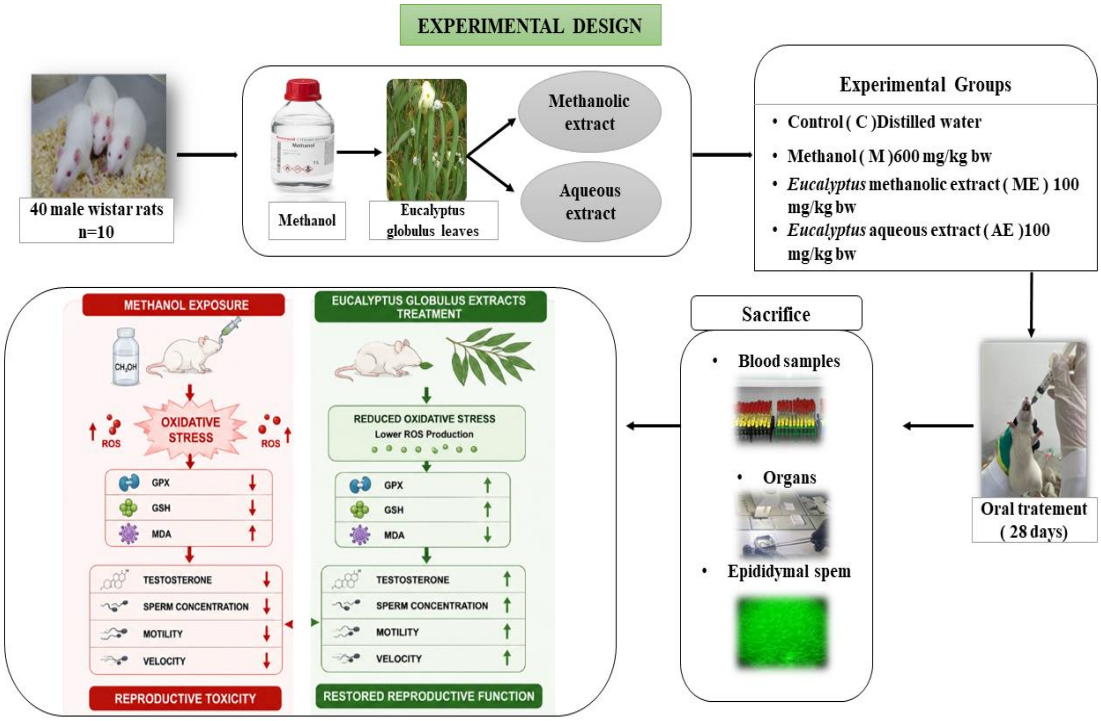
Graphical abstract



1. Introduction

Plant-based biopesticides are increasingly promoted as safer and more sustainable alternatives to synthetic pesticides, whose common use has been linked to environmental pollution and reproductive toxicity in humans and animals (Mnif *et al.*, 2011; Mostafalou and Abdollahi, 2013). Among these natural sources, *Eucalyptus* species are particularly valued for their strong antioxidant and anti-inflammatory properties, which may associate with reduced oxidative stress–related damage in reproductive tissues (Djerrou *et al.*, 2014; El-Sawi and Motawae, 2011).

To obtain these bioactive compounds, methanol is frequently used as an efficient organic solvent in extraction procedures. The methanolic extract, free from residual solvent, retains strong antioxidant and anti-inflammatory activities typical of the plant, thanks to its richness in phenolic compounds and flavonoids (Hariram and Soo, 2018). These properties may help counteract oxidative stress–mediated reproductive toxicity, offering potential protective effects on male fertility. Indeed, previous in vivo and in vitro studies have demonstrated the ability of *Eucalyptus* methanolic extracts to reduce oxidative damage in cells through scavenging of free radicals and enhancing antioxidant enzyme activity (Gonzalez-Burgos *et al.*, 2018).

However, methanol itself is well known for its toxicity, especially its adverse effects on the male reproductive system, including impaired spermatogenesis, hormonal imbalance, and reduced fertility (Lee *et al.*, 2010; Madsen and Larsen, 2012). These harmful effects are largely mediated by oxidative stress, characterized by excessive reactive oxygen species (ROS), lipid peroxidation, DNA damage, and germ cell degeneration (Agarwal *et al.*, 2006; Tremellen, 2008).

Although the toxic effects of methanol were reported in several studies, its impact on male reproductive function remains insufficiently investigated. Furthermore, the limitation of the available information regarding the influence of *Eucalyptus globulus* extract on methanol-induced alterations in male reproductive function. Therefore, the present study aimed to evaluate the methanol effects on reproductive parameters and compare these findings with the *Eucalyptus globulus* effects extract administrated in male rats. The novelty of this study is based on the comparative examination of reproductive parameters, oxidative stress biomarkers, testosterone levels and histopathological changes to provide the evaluation of methanol-induced reproductive toxicity and the effects of *Eucalyptus globulus* extract.

2. Materials and methods

2.1. *Animals materials*

This study was conducted on 40 adult male Wistar rats with a mean body weight of 228.2 ± 20 g. The animals were obtained from the Pasteur Institute of Algeria and housed in groups of five per cage, with food and water provided *ad libitum*. They were put at the animal facility of Badji Mokhtar Annaba University, where they were allocated a 6-week acclimatization period under standard conditions. At the end of the experiment, the animals were anesthetized

and then euthanized by decapitation in accordance with ethical guidelines.

2.2. *Chemical material*

The methanol used in this experiment is purity ≥99%, which is a hydroxyl-group compound also known as methyl alcohol or carbinol, with the chemical formula CH_4O, purchased from (aBIOCHEM Chemo-pharma, Montreal Quebec).

3. Plant material and preparation of aqueous extract

3.1. *Plant material*

Fresh *Eucalyptus globulus* leaves were obtained from the University of Badji Mokhtar, Annaba, in early 2023. The species was provided using standard morphological references, however a formal voucher specimen was not performed. After collection, the leaves were washed and dried in the shade, then ground into a coarse powder as part of the standard preparation process.

3.2. *Aqueous extract*

The aqueous extract of *Eucalyptus globulus* was made by decocting method.The dried powdered leaf material was combined with distilled water at a 1:10 (w/v) ratio and brought to a boil at 100°C for 10 minutes. The mixture was then exposed to room temperature and filtered through Whatman No 1 filter paper and the filtrate was stored at temperature degree 4°C until further use.

3.3. *Methanolic extract*

Dried and powdered *Eucalyptus globulus* leaves were cold-macerated in methanol at a solid-to-solvent ratio of 1:10 (w/v) for 24 hours in a sealed container at room temperature with regular stirring. The resulting mixture was then filtered through Whatman No 1 filter paper and concentrated under reduced pressure using a rotary evaporator at 40°C. The obtained extract was dried and stored at 4°C until further use.

4. The experimental design

After the adaptation period, the animals were divided into 4 groups (n=10) according to their body weight, ensuring that the mean body weight of each group was equal, as follows:

Group C (Control): received tap water;

Group M: received 600 mg/kg bw methanol dissolved in distilled water;

Group ME: received 100 mg/kg bw *Eucalyptus globulus* methanolic extract.

Group AE: received 100 mg/kg bw *Eucalyptus globulus* aqueous extract;

At the end of the-28 days of oral gavage treatment, all rats were sacrificed decapitation in accordance with ethical approval and institutional guidelines.

5. Sample collection

5.1. *Hormone parameter assay (Testosterone):*

Plasma testosterone concentration was determined using a competitive ELISA kit (DRG EIA-1559, DRG Instruments GmbH, Germany), following the manufacturer's instructions. The competitive ELISA is based on competition between sample antigen and enzyme-labeled antigen for antibody binding sites in microplate wells. After incubation (1–2 h) and washing, TMB substrate was added to produce a blue color, which was stopped with 1N HCl, turning yellow. Absorbance was read at 450 nm. All samples were analyzed in duplicate, with an intra-assay CV <10%.

5.2. *Study of the biology of spermatozoa:*

Sperm analysis was carried out using the SCA® CASA system (Sperm Class Analyzer® Computer-Assisted Sperm Analysis). In order to assess sperm concentration and motility, samples were collected from a small incision in the cauda epididymis. After a 1:8 dilution of semen in 0.9% NaCl physiological solution, a drop was transferred using a micropipette to a GoldCyto 20-µm counting chamber. The preparation was then examined under a microscope (Nikon Eclipse E200) maintained at 37 °C, using ×4 negative-phase contrast combined with a phase-contrast condenser and integrated into the microcomputer.

5.3. *Oxidative stress assays:*

After dissection, the testes were removed, carefully cleaned of fatty tissue, and weighed using a precision balance (Kern PRS 320-3). The tissues were then homogenized in cold phosphate buffer (0.1 M, pH 7.4) using a homogenizer at 4°C. They were then used to determine:

- **Malondialdehyde (MDA) levels** following the method of Ohkawa *et al.*, (1979).
- **Reduced glutathione (GSH) levels** according to Weekbeker and Cory (1988).
- **Glutathione peroxidase (GPx) activity** based on the method of Flohe and Günzler (1984).
- **Total protein concentration** using Bradford's method (1976).

The results were expressed as following:

- nmol/mg tissue for MDA.
- µM GSH/mg protein for GPx activity and GSH.

6. Histopathological study

Formaldehyde-fixed samples were dehydrated through a graded series of alcohols (70% to 100%) using a circulating tissue processor (Thermo Scientific, Microm STP 120, UK) and subsequently embedded in paraffin wax. Paraffin sections of 5 µm thickness were mounted on clean glass slides, stained with hematoxylin and eosin (H&E) (BioLab, Mumbai, India), and examined under a light microscopic at ×40 magnification (Leica DMLB, Wetzlar, Germany) (Hould, 1984).

6.1. *Statistical analysis*

Statistical significance was performed using GraphPad Prism 9 (GraphPad Software). Statistical significance was determined using a one-way analysis of variance (ANOVA) followed by Tukey's post-hoc test. Data are expressed as mean ± Standard Deviation (mean ± SD). Differences was considered significant at $p < 0.05$.

7. Results

7.1. *Reproductive profile*

7.1.1. Testes and epididymis weight

The absolute weights of the reproductive organs (testes and epididymis) are presented in **Table 1**. Exposure to methanol (M) decreased the testicular weight compared with the control group ($p < 0.05$). Similarly, epididymal weight was lower in the methanol-treated group (M) compared with the control group ($p < 0.05$). On the other hand, treatment with the methanolic extracts of *Eucalyptus globulus* (ME) showed higher testicular and epididymal weights compared with the methanol group (M).

Table 1. Absolute weights of testes and epididymis in the different experimental groups (mean ± SD). Values that do not share the same superscript letters are significantly different at $p < 0.05$. C: Control; M: Methanol; ME: Methanolic *Eucalyptus* extract; AE: Aqueous *Eucalyptus* extract.

Groups	Testes weight	Epididymis weight
C	1.850 ± 0,3119 ^{a}	0,6315 ± 0,08520 ^{a}
M	1,581 ± 0,1093 ^{b}	0,3979 ± 0,07878 ^{b}
ME	1,878 ± 0,1733 ^{a}	0.6707 ± 0,04255 ^{a}
AE	1,738 ± 0,1075 ^{a,b}	0,6060 ± 0,07001 ^{a}

7.2. *Sperm concentration*

As shown in **Figure 1**, sperm concentration was reduced in the methanol-treated group in comparision to the control group ($p < 0.05$). Treatment with the eucalyptus methanolic extract (ME) and the aqueous extract (AE) showed a higher sperm concentration compared to the methanol (M) group ($p < 0.05$).

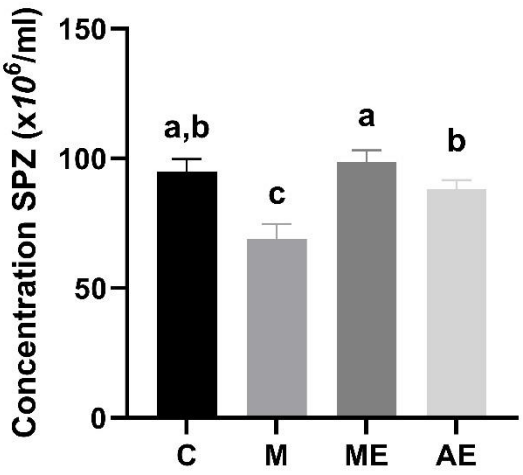


Figure 1. Sperm concentration in the different experimental groups (mean ± SD). Values that do not share the same superscript letters are significantly different at $p < 0.05$. C: Control; M: Methanol; ME: Methanolic *Eucalyptus* extract; AE: Aqueous *Eucalyptus* extract.

7.3. *Sperm motility*

Figure 2 shows that sperm motility was reduced in the methanol-treated group compared with the control group ($p < 0.05$).

7.4. *Sperm velocity*

Across the fast sperm category, as presented in **Figure 3**, no differences were observed among all experimental groups ($p > 0.05$). In the medium-velocity category, values were lower in the methanol group (M) compared with the control ($p < 0.05$). Similarly, slow-moving sperm showed a decrease in the methanol group ($p < 0.05$). In contrast, the proportion of immotile sperm was higher in the methanol-treated group compared with the control ($p < 0.05$). Importantly, both (ME) and (AE) groups showed no differences from the control across all sperm velocity categories ($p > 0.05$).

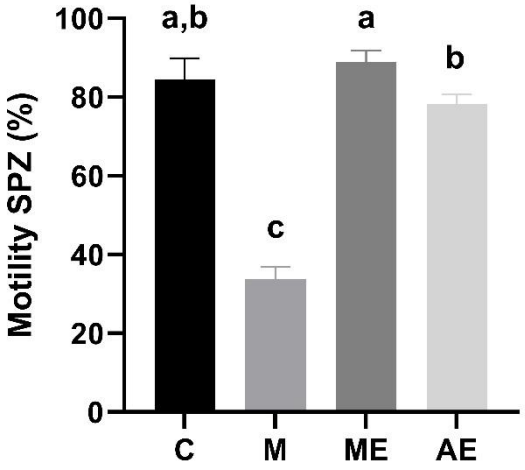


Figure 2: Total sperm motility in rats from the different experimental groups (mean ± SD). Values that do not share the same superscript letters are significantly different at $p < 0.05$. C: Control; M: Methanol; ME: Methanolic *Eucalyptus* extract; AE: Aqueous *Eucalyptus* extract.

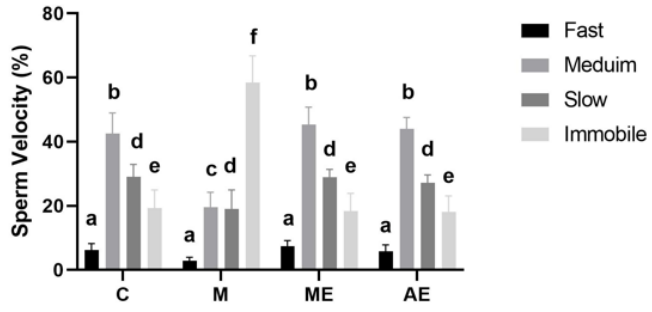


Figure 3. Sperm velocity in rats across the different experimental groups (mean ± SD). Values that do not share the same superscript letters are significantly different at $p < 0.05$. C: Control; M: Methanol; ME: Methanolic *Eucalyptus globulus* extract; AE: Aqueous *Eucalyptus globulus* extract.

7.5. *Testosterone levels*

The testosterone levels (**Figure 4**) were lower in the methanol (M) group compared with the control group ($p < 0.05$).Treatment with the methanolic (ME) and aqueous (AE) extracts of Eucalyptus globulus showed higher testosterone levels compared with the methanol (M) group ($p < 0.05$).

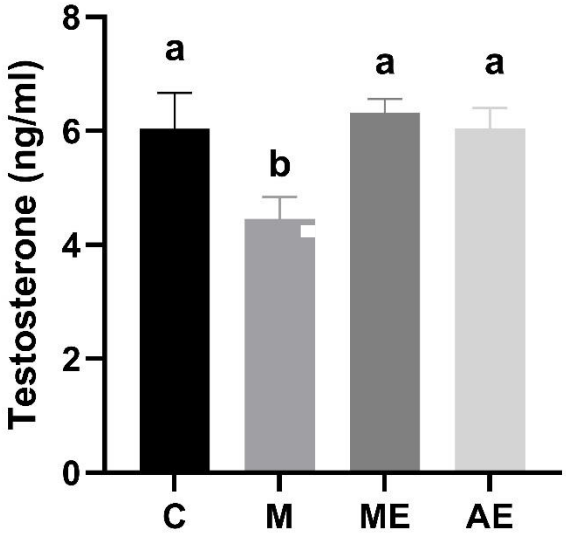


Figure 4. Testosterone levels in rats across the different experimental groups (mean ± SD). Values that do not share the same superscript letters are significantly different at p < 0.05. C: Control; M: Methanol; ME: Methanolic Eucalyptus globulus extract; AE: Aqueous Eucalyptus globulus extract.

8. Oxidative stress markers

8.1. *Malondialdehyde (MDA) levels*

The MDA levels (**Figure 5**) were higher in the methanol (M) group compared with the control group ($p < 0.05$). In contrast, both the ME and AE groups showed no significant differences compared with the control group ($p > 0.05$).

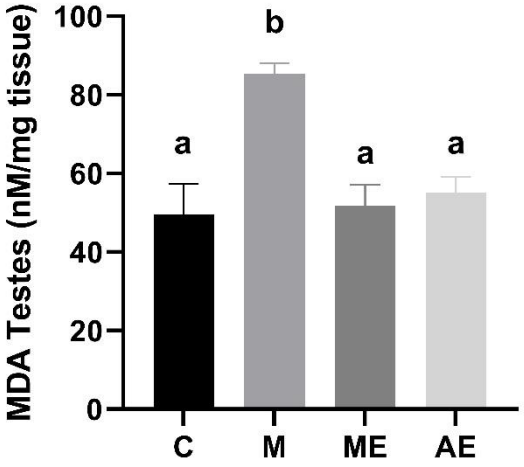


Figure 5. Testicular malondialdehyde (MDA) levels in rats across the different experimental groups (mean ± SD). Values that do not share the same superscript letters are significantly different at $p < 0.05$. C: Control; M: Methanol; ME: Methanolic *Eucalyptus globulus* extract; AE: Aqueous *Eucalyptus globulus* extract.

8.2. *Reduced Glutathione (GSH) content*

GSH levels (Figure 8) were reduced in the methanol (M) group compared with the control group. No significant differences were observed in the ME and AE groups relative to controls ($p > 0.05$).

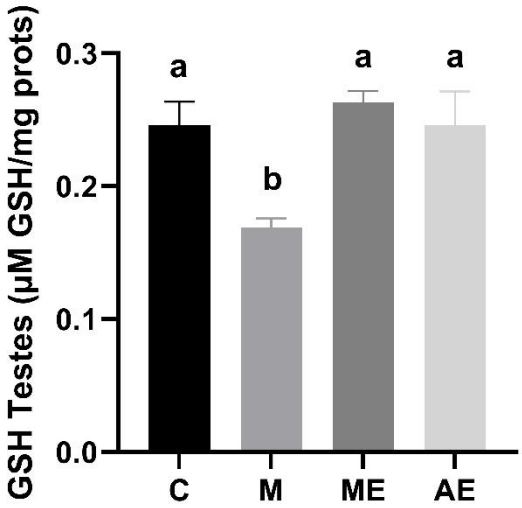


Figure 6. Testicular reduced glutathione (GSH) content in rats across the different experimental groups (mean ± SD). Values that do not share the same superscript letters are significantly different at $p < 0.05$. C: Control; M: Methanol; ME: Methanolic *Eucalyptus globulus* extract; AE: Aqueous *Eucalyptus globulus* extract.

8.3. *Peroxidase Glutathione (GPx) activity:*

Similarly, GPx levels (Figure 7) were reduced in the methanol (M) group compared with the control group. Conversely, higher GPx levels were observed in the groups treated with the aqueous eucalyptus extract (E) and the methanolic eucalyptus extract (ME) compared with the control (C) group ($p < 0.05$).

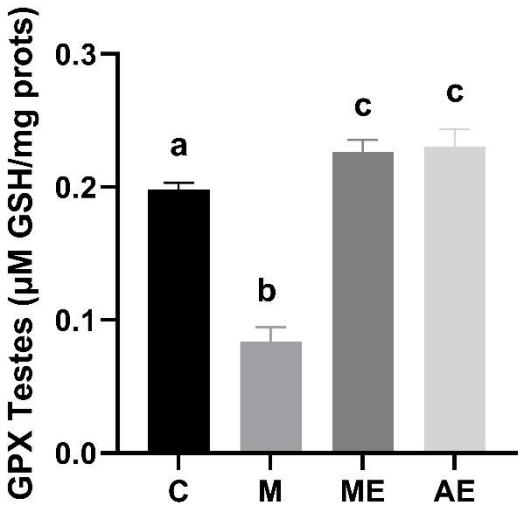


Figure 7. Testicular glutathione peroxidase (GPx) activity in rats across the different experimental groups (mean ± SD). Values that do not share the same superscript letters are significantly different at $p < 0.05$. C: Control; M: Methanol; ME: Methanolic *Eucalyptus globulus* extract; AE: Aqueous *Eucalyptus globulus* extract.

9. Histological study

9.1. *4.1 Testes*

Microscopic examination of testicular sections in the control group revealed normal histo-architecture, with well-organized seminiferous tubules,an intact germinal epithelium and numerous spermatozoa in the lumen. In the methanol treated group, mared histological alterations were observed, including empty lumina, vacuolization of Sertoli cells , and atrophy of seminiferous tubules, reflecting severe disruption of spermatogenesis and testicular degeneration. In contrast, rats treated with themethanolic (ME) and aqueous (AE) extracts of *Eucalyptus globulus* showed nearly normal testicular architecture, with preserved germinal layers and evidence of active spermatogenesis, suggesting a protective effect of both extracts.

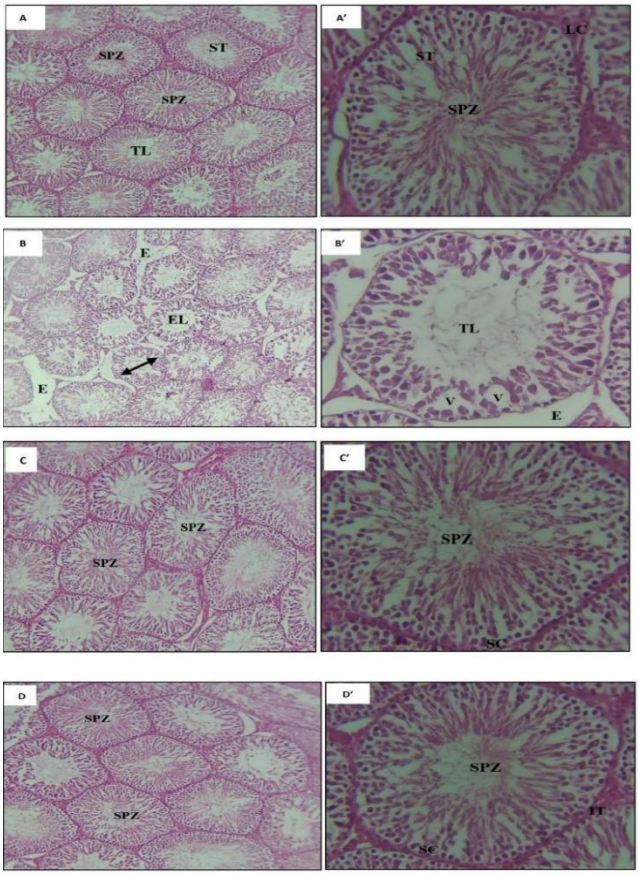


Figure 8. Microscopic observation of histological sections of the testes. (A) Control group; (B) M group; (C) ME group; (D) AE group. H&E staining, magnification ×10 (left) and ×40 (right).ST: Seminiferous tubule, TL: Tubular lumen, IT: Interstitial tissue, LC: Leydig cell, SC: Sertoli cell, SPZ: Spermatozoa, EL: Empty Lemen, E: interstitial edema, V: vacuolization in Sertoli cells. Atrophy of the seminiferous tubules (Black arrow).

9.2. *Epididymis*

Microscopic examination of epididymal sections in the control group revealed a normal histoarchitecture, with well-defined epididymal ducts and lumina densely filled with mature spermatozoa. Conversely, the methanol-treated group displayed marked histopathological alterations characterized by empty lumina and a notable reduction in sperm count, reflecting impaired sperm storage and transport, consistent with methanol-induced reproductive toxicity. In rats treated with methanolic (ME) and aqueous (AE) extracts of , the epididymal epithelium appeared preserve*Eucalyptus globulus*d, and the ducts remained rich in spermatozoa, indicating maintained epididymal function and sperm maturation.

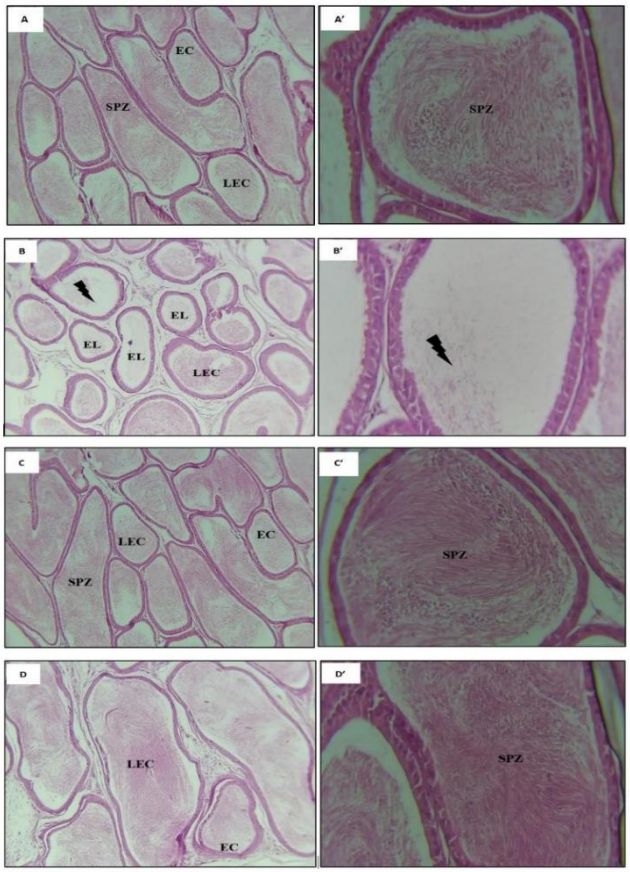


Figure 9. Microscopic observation of histological sections of the epididymis. (A) Control group; (B) Methanol (M) group; (C) Aqueous eucalyptus extract (E) group; (D) Methanolic eucalyptus extract (ME) group. H&E staining, magnification ×10 (left) and ×40 (right).

EC: Epididymal canal, LEC: Lumen of the epididymal canal, EL: Empty lumen, BM: Basement membrane, SPZ: Spermatozoa. ( ): Decrease in sperm count.

10. Discussion

Reproductive disorders refer to a range of conditions that impair the ability of the reproductive system to function properly. Numerous studies have shown that exposure to environmental contaminants, especially pesticides, negatively affects the reproductive health of both males and females (Kumar, 2004; Shojaei Saadi and Abdollahi, 2012). International studies have confirmed the reproductive toxicity of various solvents and have identified the testes as primary target organs for toxic substances released into the environment (Fort *et al.*, 2001).

Our findings showed that exposure to 600 mg/kg body weight of methanol over a 28-day period was associated with reduced sperm concentration, motility, and velocity. These results are in agreement with those reported by

Tielemans *et al.*, who observed impaired sperm quality in workers exposed to solvents. Furthermore, several studies have provided experimental evidence of additional impairments in the spermatogenesis process, including disturbances in sperm cell division, reduced motility, and alterations in sperm density and viscosity. Evidence suggests that male fertility may be adversely affected by various toxicants, either through direct damage to testicular tissue or by disrupting the endocrine regulation of male reproductive function (Jeng, 2014). Notably, even short-term exposure may be sufficient to disturb the hormonal balance required for normal spermatogenesis, as reflected by changes in testosterone and other hormone levels. These findings suggest that methanol exposure may affect both hormonal regulation and sperm production in male rats (Cooper *et al.*, 1992).

Methanol intoxication has been associated with mitochondrial dysfunction and increased microsomal enzyme proliferation, both of which contribute to the overproduction of reactive oxygen species (ROS) (Tephly, 1991). The observed reduction in glutathione (GSH) levels may also explain the decreased concentration of vitamin C, as vitamin C is primarily absorbed by cells in its oxidized form and subsequently regenerated by GSH (Briviba and Sies, 1994). Oxidative stress is defined as a significant imbalance between the production of oxidizing agents and the organism's antioxidant defense capacity. This imbalance arises either from excessive generation of reactive oxygen species or from impairments in the antioxidant defense system (Dahmoune *et al.*, 2015; Boyd *et al.*, 2003).

The present study revealed that both the aqueous and methanolic extracts of *Eucalyptus globulus* were associated with increase in GSH and GPx levels, along with decrease in MDA levels in the testes. Glutathione (GSH) plays a vital role in the cellular defense system against toxic compounds of both endogenous and exogenous origin (Meister and Anderson, 1983). Beyond its well-known antioxidant properties, GSH is also involved in protein and DNA synthesis, maintenance of cell membrane integrity, and regulation of enzymatic activities (Meister, 1989). Studies have demonstrated that the enzyme glutathione peroxidase, which functions as an antioxidant, plays a critical role in protecting sperm within testicular and epididymal tissues. A deficiency of this enzyme has been linked to infertility. Located in the plasma membrane of sperm, the sperm nucleus, and epididymal fluid, glutathione peroxidase protects sperm from free radical damage and contributes to the final maturation and development of spermatozoa (Chabory *et al.*, 2009; Drevet, 2006). Moreover, malondialdehyde (MDA), a byproduct of lipid peroxidation, is widely used as a reliable biomarker to assess the level of oxidative stress in biological systems. Elevated levels of MDA indicate increased cellular membrane damage due to reactive oxygen species (ROS), which can contribute to various pathological conditions, including reproductive toxicity (Xiong *et al.*, 2016).

The three tested extracts exhibited significant antioxidant properties, with their potency varying according to the in vitro assay employed. Notably, *Eucalyptus globulus* extracts effectively mitigated H_2O_2-induced oxidative stress by enhancing cell viability, increasing glutathione (GSH) levels and antioxidant enzyme activities, while concurrently reducing reactive oxygen species (ROS) production and lipid peroxidation in SH-SY5Y cells (Tremellen, 2008). Interestingly, *Eucalyptus globulus* mitigated diclofenac sodium-induced alterations in reproductive parameters by enhancing the cellular antioxidant system, leading to a significant increase in glutathione (GSH) concentration within testicular tissue (Mousa *et al.*, 2019). *Eucalyptus globulus* leaf extracts may serve as valuable raw materials for food and pharmaceutical supplements due to their high content of antioxidant compounds, which confer protective health benefits against oxidative stress (Tremellen, 2008).

Eucalyptus leaves are considered a source of antioxidants, particularly flavonoids, which may contribute to protection against oxidative stress and damage caused by free radicals (Nakhaee *et al.*, 2009).

This study shows that administration of the methanolic extract of *Eucalyptus globulus* was associated with alterations in serum total testosterone levels, as well as improvements in sperm parameters, including sperm count and individual motility. These enhancements are likely attributed to the antioxidant properties of *Eucalyptus*. We hypothesize that *Eucalyptus* supplementation may stimulate the production of reduced glutathione in testicular tissues, thereby protecting spermatozoa from oxidative damage and preserving their motility (Mousa *et al.*, 2019). An increase in testicular weight may occur due to elevated testosterone secretion, stimulation of germ cell proliferation and division, as well as the effects of terpenes and flavonoids present in the extract (Yakubu, 2012) .

Today, there is growing interest in the use of biopesticides as a more sustainable and reliable alternative to synthetic chemical pesticides. Biopesticides are derived from biological sources, including living organisms or substances extracted from natural origins. More broadly, the term encompasses any plant protection product that is not based on synthetic chemical compounds (Yovo, 2010).

Recent studies have demonstrated that exposure to endocrine-disrupting chemicals, including pesticides, mercury, phenols, and phthalates, can interfere with hormone metabolism by altering the synthesis and/or degradation of testosterone, follicle-stimulating hormone (FSH), luteinizing hormone (LH), and other key reproductive hormones. Steroid metabolism primarily occurs in the liver, which is also a major target for many exogenous toxicants. Although numerous scientific publications have explored this topic, a clear causal relationship between such hormonal disturbances and reproductive disorders such as infertility, azoospermia, or gonadal dysfunction has yet to be definitively established *(Queiroz and Waissmann, 2006; Alejandro et al., 2002; Dalgaard et al., 2001)*.

Microscopic examination showed differences in histological features and spermatogenesis among the groups. In the control group and the *Eucalyptus globulus*-

treated groups, the seminiferous tubules generally maintained a normal structure, with ongoing spermatogenesis and the presence of spermatozoa within the lumen. In contrast, methanol-treated rats showed noticeable changes in the seminiferous tubules. With an altered appearance and a reduced presence of spermatozoa in the lumen. An increase in the intertubular space was also observed, along with vacuolization of Sertoli cells. In the epididymis a lower concentration of spermatozoa was observed in the methanol-treated group, together with structural alterations in the epididymal ducts.

The histoarchitecture of the testes in both the control and *Eucalyptus globulus*-treated groups exhibited compactly arranged seminiferous tubules containing germinal layers at all stages of spermatogenesis, Sertoli cells, a normal lumen filled with spermatozoa, intact interstitial tissue, and functional Leydig cells (Mousa *et al.*, 2019)]. Sertoli cells play a critical role in supporting spermatogenesis (Sapori *et al.*, 1986), while Leydig cells are the primary source of androgen production. Both cell types are susceptible to damage from toxicants and chemical drugs (Papadakis *et al.*, 1999).

This study has several limitations that should be acknowledged. First, only a single methanol dose and a 28-day exposure period were evaluated; therefore, the dose-dependent and long-term effects of methanol remain to be investigated. In addition, although oxidative stress biomarkers, reproductive hormone levels, sperm parameters, and histopathological changes were assessed, the underlying molecular mechanisms responsible for the protective effects of *Eucalyptus globulus* were not explored. Future studies incorporating multiple dose regimens, longer exposure periods, and molecular analyses are warranted to further elucidate the mechanisms underlying the protective effects of *Eucalyptus globulus* against methanol-induced reproductive toxicity.

11. Conclusion

This study demonstrates that *Eucalyptus globulus* extracts, particularly the methanolic and aqueous forms, are associated with male reproductive parameters improvement. Both extracts reduced oxidative stress markers, lowered lipid peroxidation, and improved antioxidant enzyme activities, reflecting the strong antioxidant and anti-inflammatory potential of Eucalyptus compounds. The slight increase in testosterone levels observed in treated groups suggests; an effect on testicular functionHowever, the methanol exposure was associated with reproductive toxicity, characterized by level testosterone decrease, this exposure elevated oxidative stress and impaired testicular integrity.

Overall, these findings highlight differences between methanol exposure and *Eucalyptus globulus* extract treatment in reproductive parameters and suggest that further investigation of these extracts may be a topic of interest. Further studies are required to clarify the above mechanisms and determine optimal dosing for practical use.

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