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Green Nanotechnology: Synthesis of Silver nanoparticles using Taif Rose (*Rosa damascena Mill var.trigentipetala*) and and Evaluation of their Protective Effect on Testes of Adult Male Albino Rats Treated with Dibutyl Phthalate

Raghdh A. Hijji and Najiah M. Alyamani

[1]Department of Biological Sciences, College of Science, University of Jeddah, Jeddah21493, **Saudi Arabia**
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***Correspondence: nmalyamani@uj.edu.sa** **Received: July 13, 2025, Revised: Sep., 02, 2025, Accepted: Sep., 10, 2025 e-Published: Sep., 12, 2025**

Dibutyl Phthalate (DBP) is one of the most used and toxic PAEs, and its primary function as a plasticizer is to increase flexibility and strength. DBP has been suggested to cause several detrimental health effects in the reproductive system. The process of green synthesis involves using biologically active agents like plant materials to create various metal nanoparticles. Green synthesis of nanoparticles is becoming one of the robust techniques that may be a suitable alternative to chemical and physical methods. Taif rose has been shown to have antioxidant, anti-inflammatory, and cardiotonic properties due to the presence of various phytochemical compounds such as alkaloids, phenolic acids, flavonoids, and other phenolic compounds. This study focused on environmentally friendly green synthesis of Silver nanoparticles (AgNPs) using Taif Rosa (*Rosa damascena Mill var. trigentipetala*) and evaluates their protective effect on the toxicity induced by DBP in the reproductive system of adult male albino rats. The results indicated an imbalance in oxidative stress markers after exposure to DBP. On the contrary, the result showed that the green synthesis of AgNPs and Taif Rose positively impacts DBP's toxicity. Also, the silver nanoparticles and Taif Rose showed enhancement in reproductive system hormones, such as testosterone, follicle-stimulating hormone (FSH), and luteinizing hormone (LH). It also showed development and improvement in the levels of oxidative stress resulting from exposure to DBP, including levels of Glutathione (GSH), Lipid peroxides (LPO), Superoxide Dismutase (SOD) and Catalase. An improvement was also shown at the DNA level based on the comet assay or single cell-gel electrophoresis (SCGE) assay.

Keywords: Green synthesis, Silver nanoparticles, Dibutyl Phthalate, Testosterone, Follicle Stimulating Hormone, Luteinizing Hormone, Glutathione, Lipid peroxides, Superoxide Dismutase, Catalase and comet assy.

INTRODUCTION

Green nanotechnology uses the ideas of green chemistry to make, use, and get rid of nanomaterials in a way that is good for the environment (Martins and Kaczerewska, 2021). This method uses biologically active agents like plant extracts, microorganisms, and biowastes (like vegetable scraps, fruit peels, and agricultural residues) to make different metal nanoparticles in a way that is good for the environment and doesn't cost a lot of money (Kumari et al. 2021).

Interest in green nanotechnology is increasing in the medical field as it utilizes natural resources, such as plants, as "eco-friendly nano-factories," thereby promoting research to satisfy rising industrial demand (Kaur, 2018; Khan et al.2025). Green chemistry-derived silver nanoparticles (AgNPs) are promising alternatives to traditional chemical methods because they are more biocompatible and less toxic (Roy et al. 2019).

Plants are especially good for making nanoparticles because their cell walls are nano-textured and rich in silica, which can be changed in vivo, which could lower the cost of cell culture. Biogenic nanoparticles are also cheap to make, safe for the environment, and safe for people (Mukherjee et al. 2024).

In 2018, the world made about 360 million metric tons of plastic. The Hazardous Chemicals in Plastic Packaging project found over 900 chemicals linked to plastic packaging, with phthalates being some of the most dangerous (Groh et al. 2019). Di-n-butyl phthalate (DBP) is a toxic phthalate that is used to make high-molecular-weight polymers more flexible and stronger (He et al. 2022).

A 2020 study found high levels of DBP in toys and childcare products in Saudi Arabia, even though the country has rules against it. This shows that people are still at risk of being exposed (Oteef and Elhassan, 2020).

Herbal medicines are still a cheap way to get primary care, especially in places where regular facilities are hard to find. They are trusted in their cultures, seen as safe, and often have several compounds that work well with the human body. They protect the heart and liver and get rid of free radicals and toxins (Chaachouay and Zidane, 2024).

The Damask rose, also known as Taif Rose (*Rosa damascena Mill., family Rosaceae*), came to Europe from Damascus (Galal et al. 2022). Research, encompassing both in vivo and in vitro studies, has elucidated its cardiovascular-modulating, antibacterial, and antioxidant properties (Hamza et al. 2022). These effects are ascribed to diverse phytochemicals, encompassing alkaloids, phenolic acids, flavonoids, and additional phenolic compounds (Galal et al. 2022).

Saudi Arabia is dedicated to environmental protection and well-being through programs like Vision 2030, which are in line with the United Nations' Sustainable Development Goals. The Kingdom is famous for its beautiful Taif roses, which are known for their smell and are used in some of the most expensive perfumes in the world (Kurkcuoglu et al. 2020).

This research examines the physiological, Genetics and histological impacts of green-synthesized silver nanoparticles sourced from Taif Rose (*Rosa damascena Mill*.) on the testes of adult male albino rats subsequent to DBP exposure. We will evaluate reproductive hormones (FSH, LH, and testosterone), oxidative stress indicators, and genetic alterations, assessing the potential ameliorative effects of Taif Rose–derived AgNPs.

MATERIALS AND METHODS

Experimental Animals:

Twenty-four adult male albino Wister rats with body weights from 160±200g and aged from 8 to 10 weeks were used. The experiment take six weeks and was done in the pharmacy college student section at King Abdul-Aziz University (KAU), Jeddah, Saudi Arabia.

Ethics approval:

The use of experimental animals was conducted with strict compliance to the rules and regulations established by the Research Ethics Committee of Faculty of Pharmacy, King Abdul-Aziz University (KAU). (ethics approval: P118-2023).

Chemicals:

Di-butyl phthalate (DBP):

Di-butyl phthalate (DBP) is known as Di-n-butyl phthalate (DnBP). It is a white powder. Also, this material was collected from Sigma-Aldrich St. Louis, MO, USA).

Determination of the Appropriate dose of Di-butyl phthalate:

The selected DBP doses are anchored in well-established toxicological benchmarks. Zhang et al. (2021) administered DBP to pregnant Sprague–Dawley rats at 0, 50, 250, and 500 mg/kg body weight per day via gavage from gestation day 1 to postnatal day 21. They determined a NOAEL for developmental toxicity at 50 mg/kg/day—based on pup body weight and male reproductive system lesions—and recommended a reference dose (RfD) for humans of 500 mg/kg/day.

More recently, Heidari et al. (2022) confirmed that DBP doses of 500 and 1,000 mg/kg resulted in significant embryolethality, evidenced by increased pre- and post-implantation losses. These findings validate the lower dose as a benchmark for safety and highlight the higher dose range for exploring pronounced developmental toxicity.

This dose selection is appropriate because it encompasses both the threshold of no observable adverse effects (50 mg/kg) and the range where clear embryotoxic effects manifest (500–1,000 mg/kg). This design enables comprehensive evaluation of dose–response relationships and ensures that both sub-toxic and overtly toxic outcomes are captured.

Plant Material:

Collection of Rosa *Damascena Mill*.:

The fresh flower of *Rosa Damascena Mill.* Was collecting in June 2022 from local market on Taif city, Sudi Arabia.

Determination of Appropriate Dose of Green Synthesis of Silver Nanoparticles by *Rosa Damascena* Mill.:

The oral study supports that 200 mg/kg/day of AgNPs is non-toxic, making it a safe upper limit for exploring biological effects without overt toxicity (Garcia et al. 2016). The intraperitoneal study shows that at the same dose, neurological and oxidative effects begin to emerge, indicating that this dose is biologically active and relevant for investigating sub-toxic effects (Tarbali et al. 2022).

Therefore, selecting 200 mg/kg for Taif Rose–derived green AgNPs is scientifically valid: it's high enough to elicit meaningful biological responses without causing overt toxicity when administered orally.

Extraction and Preparation:

Green Synthesis of Silver Nanoparticles by *Rosa Damascena* Mill.

Extraction of Taif Rose Flower:

-Harvest fresh Rosa damascena (Taif rose) blossoms. Thoroughly rinse with distilled water to eliminate contaminants.

-Air-dry or oven-dry at around 40–50 °C until moisture is eliminated.
-Macerate desiccated blossoms into a fine powder.
-Measure 10 g of powdered flowers, combine with 100 mL of distilled water (10% w/v), and heat at 80 °C for 1 hour.
-Pass the mixture through Whatman No. 1 filter paper; retain the filtrate (flower extract) at 4 °C for subsequent use.

Formulation of Silver Precursor Solution:

Prepare a 1–5 mM solution of silver nitrate ($AgNO_3$) utilizing deionized water.

Green Synthesis of Silver Nanoparticles:
-Combine the flower extract with $AgNO_3$ solution in a 1:1 volumetric ratio.
-Alternatively, adjust ratios (e.g., 1 mL extract to 1–5 mL $AgNO_3$) based on initial experiments.
-Incubate the reaction mixture at 37 °C in a dark environment. Observe for a discernible color shift (usually to dark brown), signifying the creation of nanoparticles.
-Reaction time might fluctuate—from minutes to hours—based on extract potency and reaction circumstances (Lakkim et al. 2020).

Purification of Silver Nanoparticles:
-Subject the reaction mixture to centrifugation at approximately 10,000 rpm for 10 to 15 minutes to sediment the nanoparticles.
-Eliminate the supernatant and rinse the pellet 2–3 times with distilled water or ethanol to eliminate binding biomolecules.
-Redisperse pure nanoparticles in deionized water for analysis (Lakkim et al. 2020).

Characterization of Nanoparticles:

Atomic Force Microscopy (AFM):
The topography of the surface was studied using an atomic force microscope. The **(Fig.1)** showed two-dimensional image of silver nanoparticles showing in clusters of spherical shapes.
Also **(Fig.2)** showed three- dimensional image of silver nanoparticles revealed a population of homogeneous particles with a regular surface figure (Chicea et al. 2023).

Dynamic light scattering (DLS) analysis:
The particle size distribution (PSD) of synthesized silver nanoparticles is shown on **(Fig. 3)** which the colloidal solution of silver nanoparticles contains particles of different sizes some with average sizes ranging from 15 nm to 21 nm (Chicea et al. 2023).

Zeta potential size analysis (Zeta):
Nanoparticles are very small in size for which they are energetically very unstable. Therefore, the particles undergo agglomeration/aggregation to stabilize themselves. So, there were some potential charges on the surface of the nanoparticles which makes them stable. These charge potential we got from this analysis. Zeta potential (Surface potential) has direct relation with the stability of a form/structure, as mentioned below on **(Fig.4)** the stability of the NPs according to the potential charge was range from -30 mv to -34mv (Clogston and Patri, 2011; Dukhin and Xu, 2025).

X-Ray Diffraction (XRD) analysis:
The XRD pattern **(Fig. 5)** exhibited distinct diffraction peaks near 24°, which correspond to the (800) plane of face-centered cubic silver. These sharp Bragg peaks likely arise from the stabilizing effect of the capping agent on the nanoparticles, indicating strong X-ray scattering centers within the crystalline phase (Ajitha et al. 2016).
The possibility of capping agents crystallizing independently was excluded, as the purification process involved centrifuging the nanoparticles and redispersing the pellet in Millipore water. This suggests that the bio-organic phase crystallizes on the surface of the silver nanoparticles—or vice versa. In general, peak broadening in XRD patterns is attributed to small particle sizes. Broader peaks indicate smaller crystallites and reflect the influence of experimental conditions on the nucleation and growth of crystal nuclei (Ajitha et al. 2016).
-Atomic Force Microscopy (AFM):

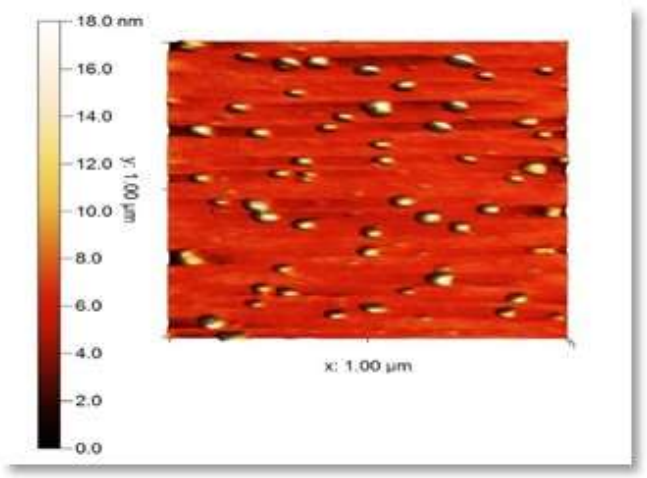

Figure 1: A two-dimensional image of silver nanoparticles was synthesized by Taif rose extract (*Rosa damascena Mill*).

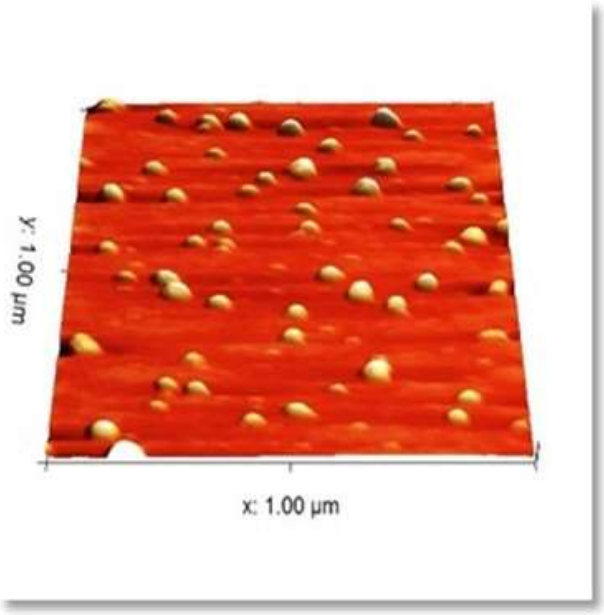

Figure 2:A three-dimensional image of silver nanoparticles was synthesized by Taif rose extract (*Rosa damascena Mill.*)

Dynamic light scattering (DLS) analysis:

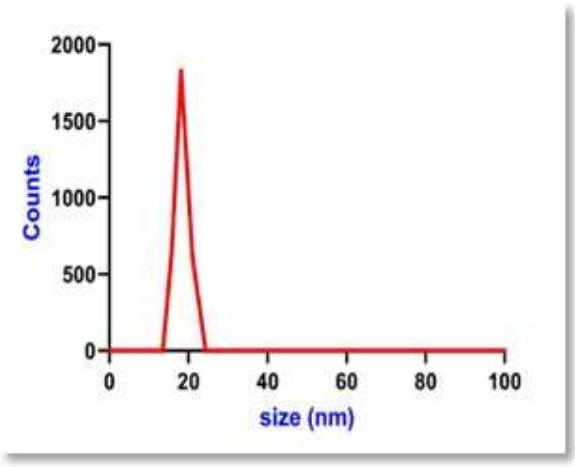

Figure 3: DLS result ratio silver nanoparticles

Zeta potential size analysis (Zeta):

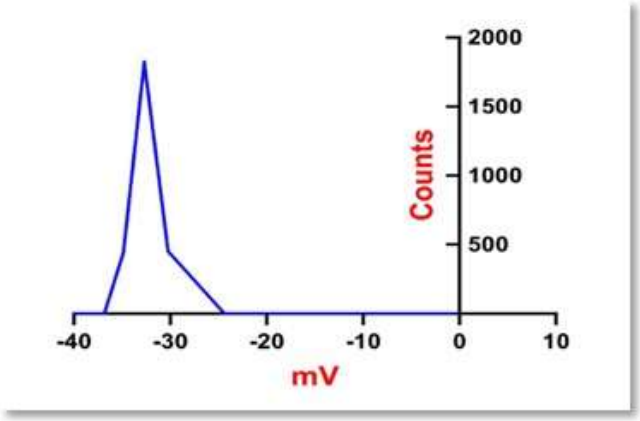

Figure 4: Zeta result: The stability of the NPs according to the potential charge

-X-ray diffraction (XRD) analysis:

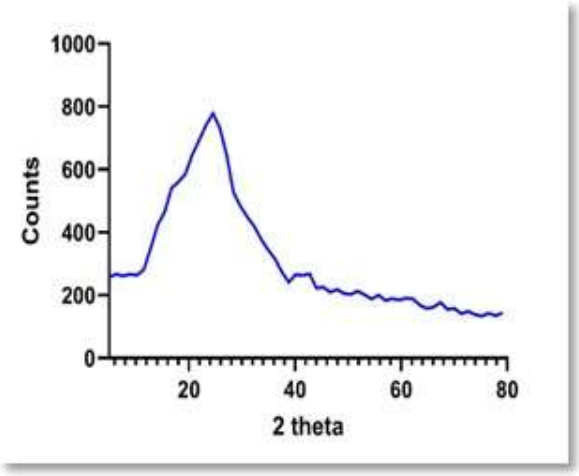

Figure 5: XRD results for silver nanoparticl

Experimental protocol:

24 experimental animals were randomly divided into eight groups and treated as follows:

Group 1: This contains (6 rats) that served as a negative control group that received distilled water by oral administration, 5 days/week for six weeks.

Group 2: This contains (6 rats) that serve as a positive control group that received DBP by dose (500mg/kg/day) by oral administration, 5 days/week for six weeks (Howdeshell et al. 2007; Morova et al. 2020).

Group 3: This contains (6 rats) that received *Rosa damascene* Mill. Silver nanoparticles by dose (200mg/kg/day) orally, 5 days/week for six weeks (Perona et al. 2021).

Group 4: Which contain (6 rats) that received *Rosa damascene Mill.* Silver nanoparticles by dose (200mg/kg/day) and DBP by dose (500mg/kg/day) by oral administration, 5 days/week for six weeks.

Methodology

Blood sample collection:
After the end of the experiment, at six weeks, the blood sample was immediately collected from the retro-orbital sinus in an anticoagulated (heparinized capillary) tube. The blood serum was then centrifuged at 5000 rpm for 15 min for isolate the serum. Clear serums were carefully separated using posture pipettes and kept in a deep freezer at -20 °C until the biochemical analysis (Parasuraman et al. 2015). The ELISA technique was used to detect the level of hormones: Luteinizing hormone (LH), follicle-stimulating hormone (FSH), and Testosterone (Ongaro et al. 2021). Also, to detect the oxidative stress for superoxide dismutase (SOD), and catalase (CAT). Lipid peroxidation (LPO) and glutathione (GSH) by using a specific ELISA kit for rats (Kelechi et al. 2024).

- Detection of DNA damage by comet assay:
Comet Assay Kit (3-well slides) (ab238544) is a fast and sensitive kit for the measurement of cellular DNA damage. The Assay is a single-cell gel electrophoresis assay (SCGE) for simple evaluation of cellular DNA damage. It is a convenient way to screen for general DNA damage, regardless of the source or nature of the damage. Kits include Comet Slides, reagents, and a fluorescent dye to visualize cells under an epifluorescence microscope (Cui et al. 2023).

- Histopathological examination:
The testicular tissue samples were subjected to fixation using a 10% formalin solution for 48 hours. Following fixation, the samples underwent processing for paraffin sectioning. The resulting sections were subsequently stained with Ehrlich's haematoxylin and counterstaining with eosin was applied for histopathological examination, according to (Bancroft and Stevens, 1990).

-Statical analysis:
Statistical analyses were performed using the IBM SPSS Statistics for Windows (version 21, SPSS® Inc., USA), 2013, The mean values ± SE was used for the presentation of the data. the analysis was performed by *t*-test, statistical significance was established, to be indicated by $P<0.05$.

RESULTS

Physiological result:
Data in **Table 1** and **(Fig. 6,7 and 8)** showed *t*-test of the effect of daily administration of Taif rose Nanoparticles (250mg/kg) and Taif Rose Nanoparticles (2000mg/kg, oral) + DBP (500mg/kg, oral) on male reproductive hormones: Testosterone, FSH and LH.

Table 1: *t*-test of the effect of daily administration of Taif rose Nanoparticles (250mg/kg) and Taif Rose Nanoparticles (2000mg/kg, oral) + DBP (500mg/kg, oral) on Testosterone, FSH and LH Hormone levels in serum of male albino rats.

Parameter / Groups	Level of Testosterone Mean ± S.E. ng\dl	Level of FSH Mean ± S.E. mIU/mL	Level of LH Mean ± S.E. IU/L
G1: Control	285.80 ± 5.34	2.14 ± 0.19	2.77 ±0.19
G2 Control DBP	144.00 ± 10.56 [a]	9.58 ± 0.54 [a]	12.70 ± 0.78 [a]
	-50%	348%	358%
G3 Taif Rose Nanoparticles	353.5 ± 17.56 [a]	2.41 ± 0.23	3.60 ± 0.31 [a]
	24%	13%	30%
G4 Taif Rose Nanoparticles + DBP	241.33 ± 10.57 [a,b]	2.38 ± 0.41 [b]	2.97 ± 0.15 [b]
	68%	-75%	-77%

Data expressed as Mean ± S.E for six animals. Statistical analysis *t*-test $P<0.05$. *: statistically significant at ($P<0.05$) compared to the Control G1. a: statistically significant compared to the Control G1. b: statistically significant compared to the Control G2.

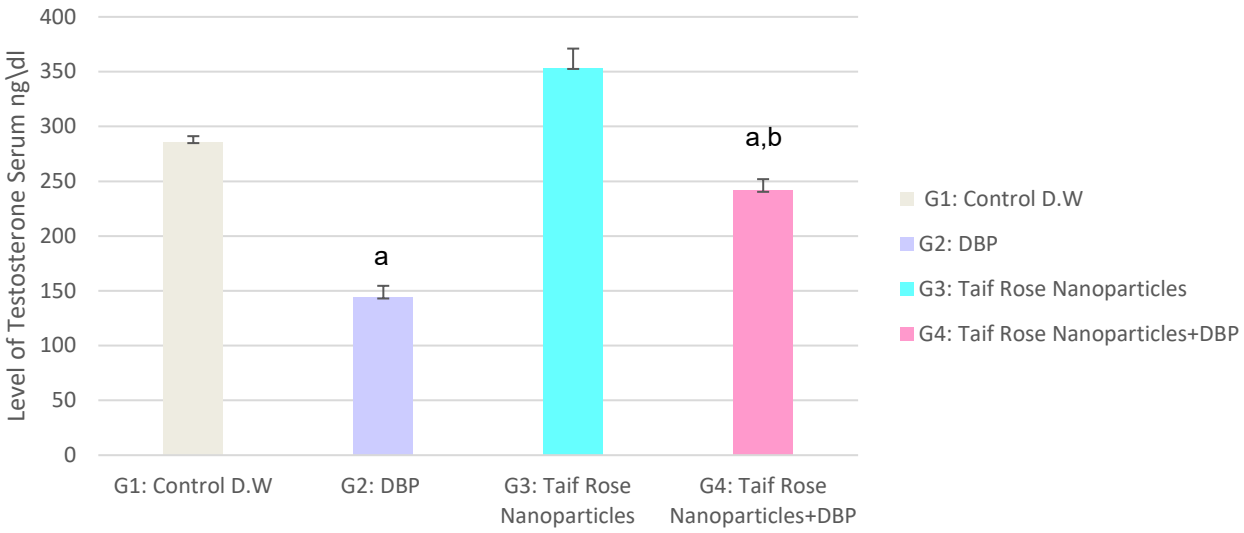

Figure 6: *t*-test of the effect of daily administration of Taif rose Nanoparticles (250mg/kg) and Taif Rose Nanoparticles (2000mg/kg, oral) + DBP (500mg/kg, oral) on Testosterone Hormone levels in serum of male albino rats.

Data expressed as Mean ± S.E for six animals. Statistical analysis *t*-test P<0.05. *: statistically significant at (P <0.05) compared to the Control G1. a: statistically significant compared to control G1. b: statistically significant compared to DBP G2.

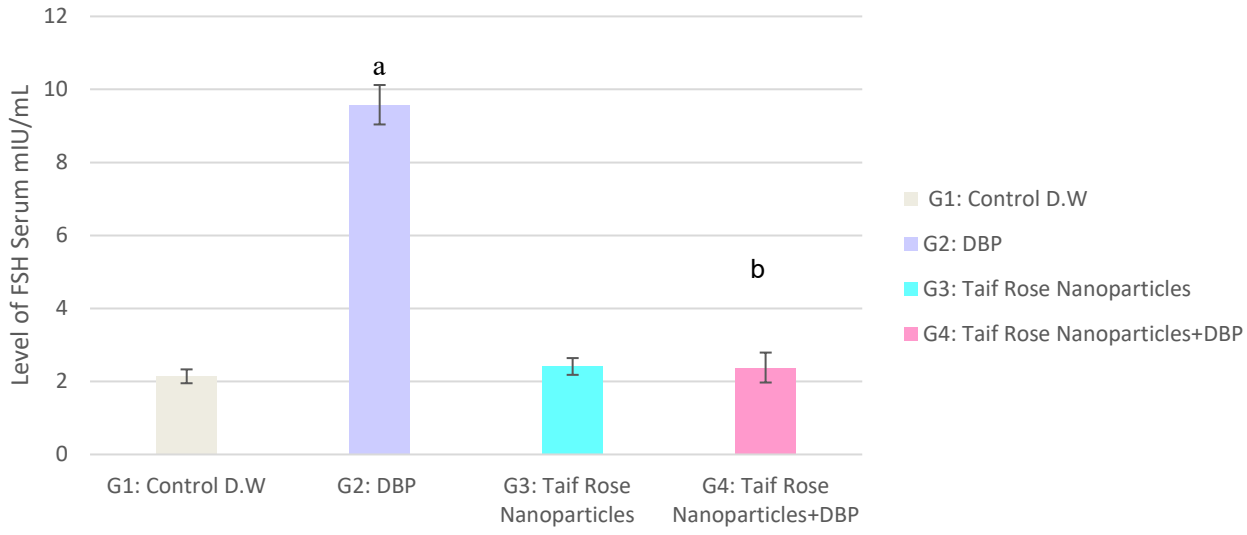

Figure 7: *t*-test of the effect of daily administration of Taif rose Nanoparticles (250mg/kg) and Taif Rose Nanoparticles (2000mg/kg, oral) + DBP (500mg/kg, oral) on FSH Hormone levels in serum of male albino rats.

Data expressed as Mean ± S.E for six animals. Statistical analysis *t*-test P<0.05. *: statistically significant at (P <0.05) compared to the Control G1. a: statistically significant compared to control G1. b: statistically significant compared to DBP G2.

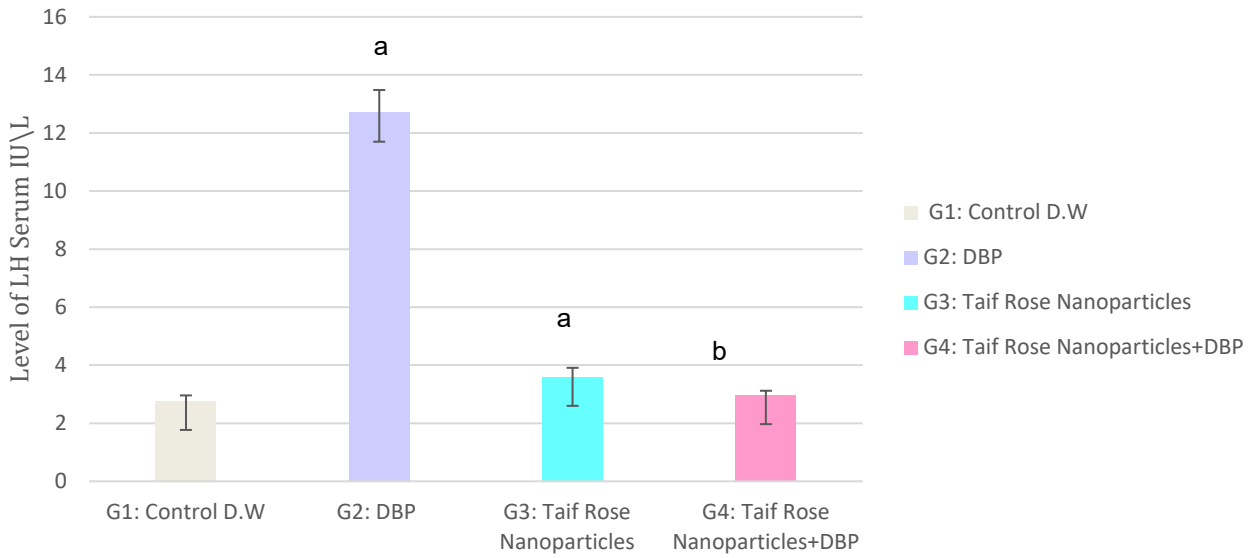

Figure 8: *t*-test of the effect of daily administration of Taif rose Nanoparticles (250mg/kg) and Taif Rose Nanoparticles (2000mg/kg, oral) + DBP (500mg/kg, oral) on LH Hormone levels in serum of male albino rats

Data expressed as Mean ± S.E for six animals. Statistical analysis *t*-test P<0.05. *: statistically significant at (P<0.05) compared to the Control G1. a: statistically significant compared to control G1. b: statistically significant compared to DBP G2.

There is a significant decrease (P<0.05) in testosterone level in treated (G2) group (144.00 ± 10.56) compared to control group (285.80 ± 5.34). On the other hand, there is a significant (P<0.05) increase in FSH and LH levels in the treated group G2 (348%-358 %) compared to the control group G1.

The Testosterone and LH levels significantly (P<0.05) increased in Taif rose nanoparticles G5 (Testosterone 24% and LH 30%) compared to the control group G1. The activities of FSH level in rats received Taif rose nanoparticles G5 showed an insignificant change compared to the control group G1.

The result showed that the level of testosterone in the serum was significantly (P<0.05) increased in treated group G6 by (86%) compared to the positive control group G2. In addition, there is a significant decrease (P<0.05) in the treated group G6 of FSH (-75%) and LH (-77%) compared to the Positive control group G2.

Oxidative stress result:

Data in Table 2 and (Fig. 9, 10, 11 and 12) showed *t*-test of the effect of daily administration of Taif rose Nanoparticles (250mg/kg) and Taif Rose Nanoparticles (2000mg/kg, oral) + DBP (500mg/kg, oral) on male reproductive hormones: Testosterone, FSH and LH.

Data showed that there is a significant decrease (P<0.05) in GSH, SOD and catalase level in the control DBP G2 group when compared to the control group G1 GSH level (5.40 ± 1.85), SOD level (106.33 ± 13.77) and Catalase level (87.66 ± 3.32) and there is a significant increase in LPO in control DBP group G2 (390%) compared to negative control group G1.

There is a significant (P<0.05) difference between the control group G1 and the Taif rose nanoparticles group G5 in GSH level (-26%) and LPO level (56%). There is insignificant change between (G5) and (G1) in SOD and Catalase level.

The administration Taif rose nanoparticles and DBP combination showed maintenance of enzymes level by significant (P<0.05) increase on GSH level (161%), SOD level (63%) and Catalase level (35%) in positive control DBP G2.

Also, there is a significant (P<0.05) decrease in the level of LPO by (-77%) in Taif rose nanoparticles + DBP group G6 compared to the positive control group G2.

Table 2: *t*-test of the effect of daily administration of Taif rose Nanoparticles (250mg/kg) and Taif Rose Nanoparticles (2000mg/kg, oral) + DBP (500mg/kg, oral) on oxidative stress of GSH, SOD, LPO and catalase levels in serum of male albino rats.

Parameter / Groups	Level of GSH Mean ± S.E. ng\ml	Level of SOD Mean ± S.E. u\ml	Level of Catalase Mean ± S.E. Mu\L	Level of LPO Mean ± S.E. nmol/ml
G1	16.10 ± 1.44	174.80 ± 4.97	117.80 ± 1.06	1.43 ± 0.17

Control				
G2 Control DBP	5.40 ± 1.85 [a]	106.33 ± 13.77 [a]	87.66 ± 3.32 [a]	7.02 ± 0.57 [a]
	-66%	-39%	-26%	390%
G3 Taif Rose Nanoparticles	11.96 ± 1.95	177.50 ± 5.59	118.50 ± 2.64	2.23± 0.45
	-26%	2%	1%	56%
G4 Taif Rose Nanoparticles + DBP	14.08 ± 1.12 [b]	173.66 ± 5.64 [b]	118.16 ± 1.77 [b]	2.12 ± 0.28 [a,b]
	161%	63%	35%	-77%

Data expressed as Mean ± S.E for six animals. Statistical analysis *t*-test P<0.05. *: statistically significant at (P ⩽0.05) compared to the Control G1. a: statistically significant compared to the Control G1. b: statistically significant compared to the Control G2.

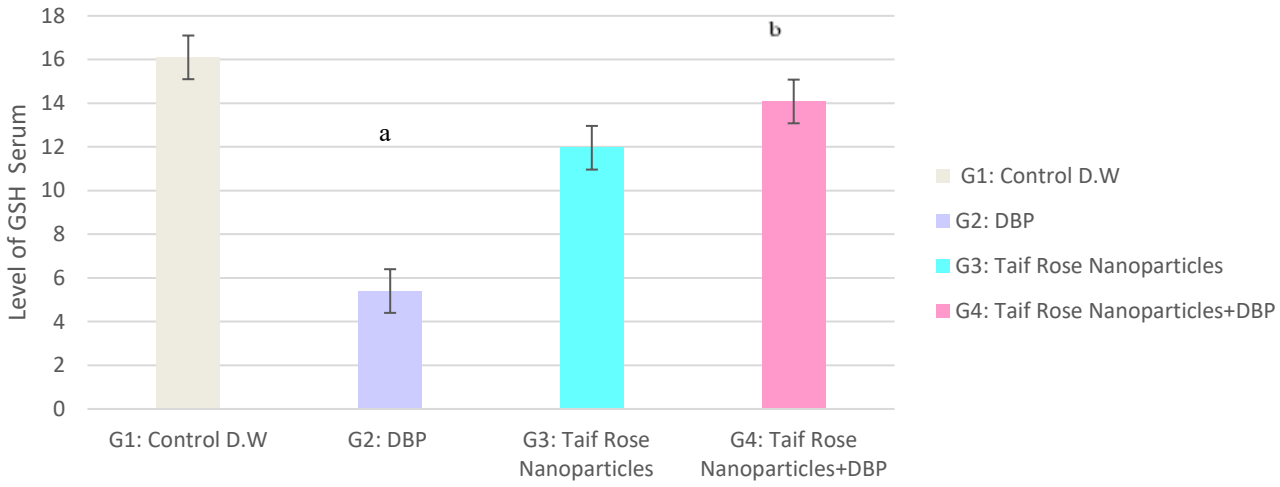

Figure 9: *t*-test of the effect of daily administration of Taif rose Nanoparticles (250mg/kg) and Taif Rose Nanoparticles (2000mg/kg, oral) + DBP (500mg/kg, oral) on oxidative stress of GSH levels in serum of male albino rats.

Data expressed as Mean ± S.E for six animals. Statistical analysis *t*-test P<0.05. *: statistically significant at (P ⩽0.05) compared to the Control G1. a: statistically significant compared to control G1. b: statistically significant compared to DBP G2.

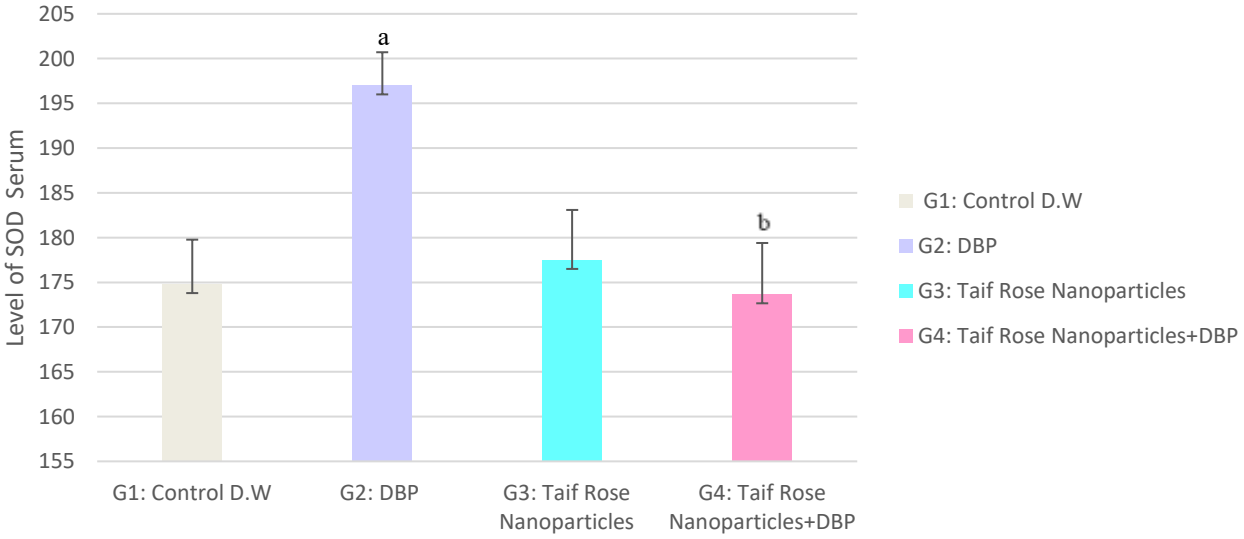

Figure 10: Effect of daily administration of Taif rose Nanoparticles (250mg/kg) and Taif Rose Nanoparticles (2000mg/kg, oral) + DBP (500mg/kg, oral) on oxidative stress of SOD levels in serum of male albino rats.

Data expressed as Mean ± S.E for six animals. Statistical analysis *t*-test P<0.05. *: statistically significant at (P ⩽0.05) compared to the Control G1. a: statistically significant compared to control G1. b: statistically significant compared to DBP G2.

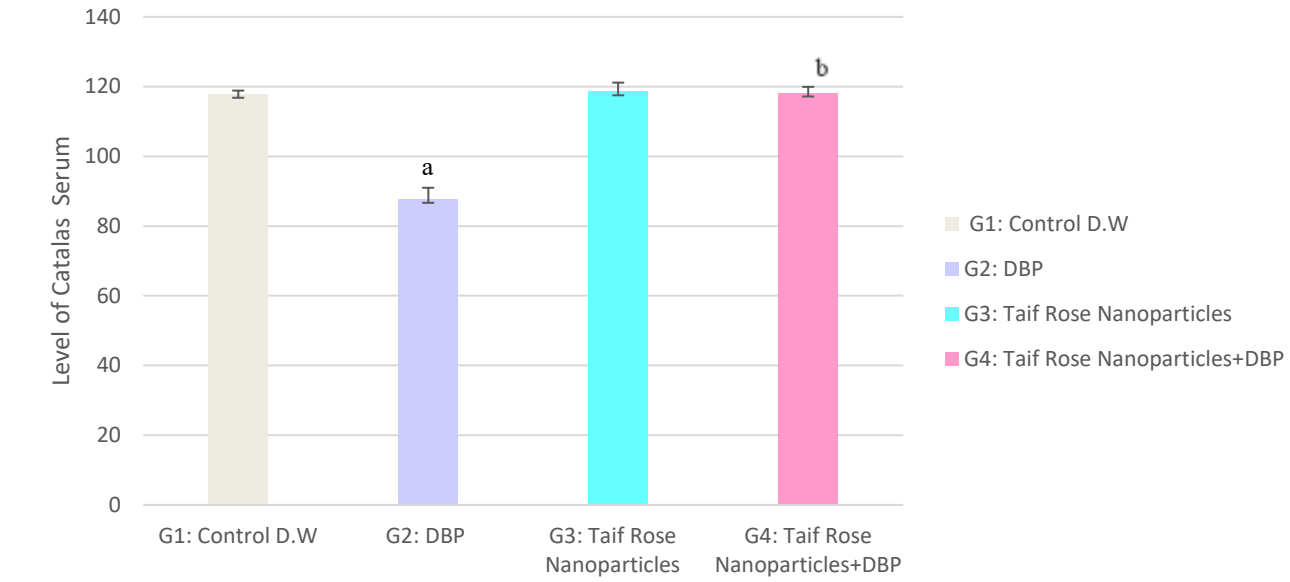

Figure 11: *t*-test of the effect of daily administration of Taif rose Nanoparticles (250mg/kg) and Taif Rose Nanoparticles (2000mg/kg, oral) + DBP (500mg/kg, oral) on oxidative stress of Catalase levels in serum of male albino rats.

Data expressed as Mean ± S.E for six animals. Statistical analysis *t*-test P<0.05. *: statistically significant at (P ⩽0.05) compared to the Control G1. a: statistically significant compared to control G1. b: statistically significant compared to DBP G2.

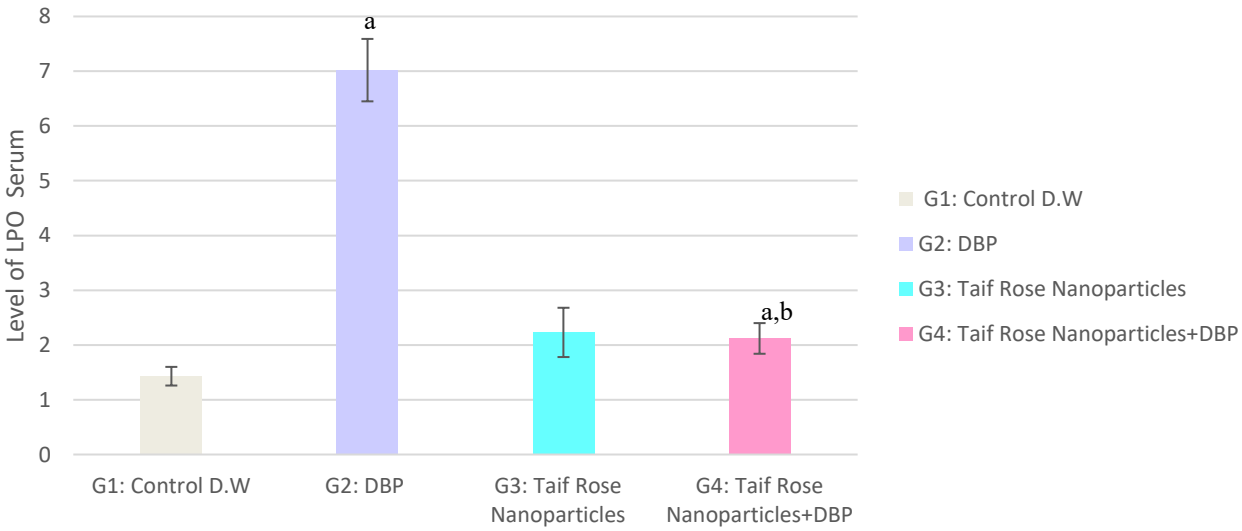

Figure 12: *t*-test of the effect of daily administration of Taif rose Nanoparticles (250mg/kg) and Taif Rose Nanoparticles (200mg/kg, oral) + DBP (500mg/kg, oral) on oxidative stress of LPO levels in serum of male albino rats.

Data expressed as Mean ± S.E for six animals. Statistical analysis *t*-test P<0.05 . *: statistically significant at (P⩽0.05) compared to the Control G1. a: statistically significant compared to the Control G1. b: statistically significant compared to the Control G2.

Genetic result:

Data in Table 3 and (Fig.13, 14, 15, 16, 17 and 18) showed *t*-test of the effect of daily administration of Taif rose Nanoparticles (250mg/kg) and Taif Rose Nanoparticles (2000mg/kg, oral) + DBP (500mg/kg, oral) on head and tail of comet assay.

Comet Assay Picture Results:

Fig.13 represents control group G1 showed normal head morphology of comet assy. In contrast, Fig.14 represents DBP group G2 showed clearly an increase in

the tail of comet assay. Fig.15 represents Taif rose nanoparticles group G3 showed normal head morphology of comet assy. Fig.16 represents Taif rose nanoparticles + DBP showed little increase in the tail of comet assay.

Statistical Results of Comet Assay:

The result showed that there is highly significant increase at (P<0.05) in the tail length of treated DBP group (G2) by 2467% as compared to control group (G1). In addition, the treatment group DBP (G2) showed significant decrease at (P<0.05) in head length as compared to control group (G1) (G1: 221.00 ± 0.99; G2: 105.12 ± 0.22).

The Taif rose nanoparticles group (G5) shows a decrease in head length by (-4%) as compared to the control group (G1). Also, there is a significant enhancement at (P<0.05) in head length in the group treated by Taif rose nanoparticles + BBP (G6) as compared to the control DBP group (G2) (G2: 105.12 ± 0.22; G6: 131.69 ± 1.10). In contrast, there is a significant decrease at (P<0.05) in the tail length of (G6) when compared to (G2) (G2: 317.42 ± 9.09; G6: 288.17 ± 1.60).

Table 3: *t*-test of the effect of daily administration of Taif rose Nanoparticles (250mg/kg) and Taif Rose Nanoparticles (2000mg/kg, oral) + DBP (500mg/kg, oral) head and tail length of comet assay of male albino rats.

Parameter / Groups	Comet head length Mean ± S.E. Px	Comet tail length Mean ± S.E. px
G1 control (D.W)	221.00 ± 0.99	12.36 ± 0.14
G2 DBP	105.12 ± 0.22[a]	317.42 ± 9.09[a]
	-35%	2467%
G3 control withdrawal (D.W)	213.19 ± 0.60[a]	15.7 ± 0.20[a]
	-4%	27%
G4 DBP withdrawal	131.69 ± 1.10 [a,b]	288.17 ± 1.60 [a,b]
	25%	-9%

Data expressed as Mean ± S.E n=6 . Statistical analysis was performed between Control and treated animal by using a *t*-test. a: statistically significant compared to Control G1. b: statistically significant compared to the DBP G2. %: percentage of change between control and treatment group.

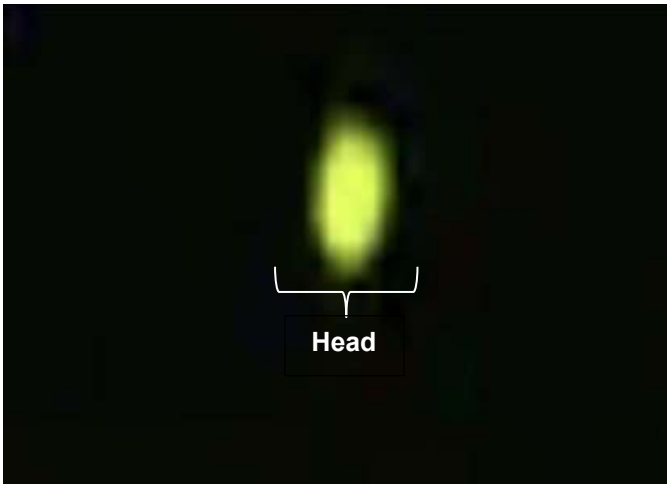

Figure13: Comet assay of testis cell from Rats received normal saline (G1) after 6 weeks.

The figure showed normal result of tail and head length. The image takes by Nikon Digital sight DS-U3.

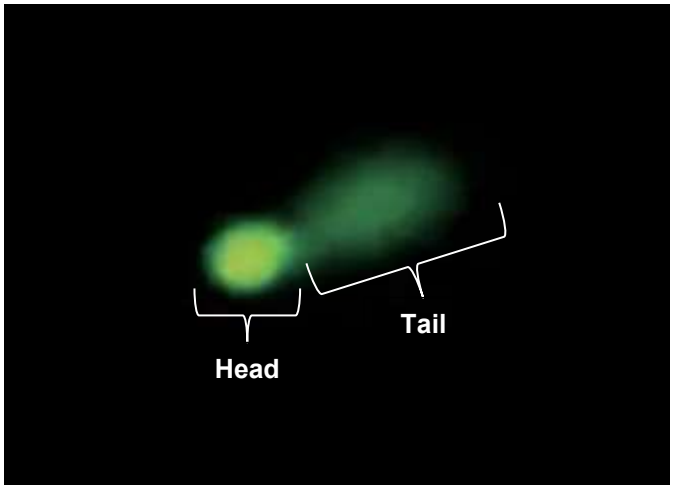

Figure 14; Comet assay of testis cell from Rats received DBP (500ml/Kg) (G2) after 6 weeks.

The figure showed clearly damaged DNA by increase in tail length. The image takes by Nikon Digital sight DS-U3

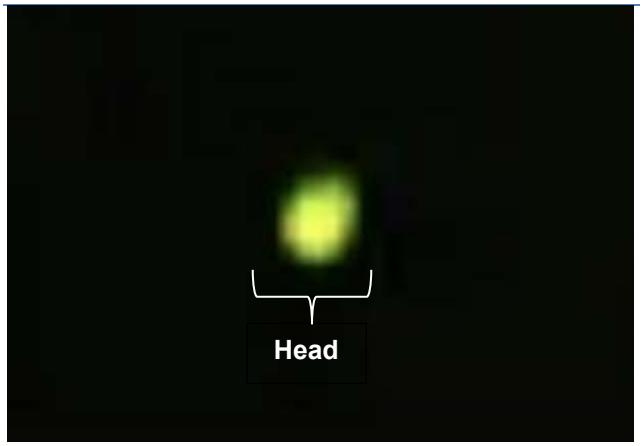

Figure 15: Comet assay of testis cell from Rats received Taif Rose Nanoparticles (200mg/Kg) (G3).

The figure showed normal result of tail and head length. The image takes by Nikon Digital sight DS-U3

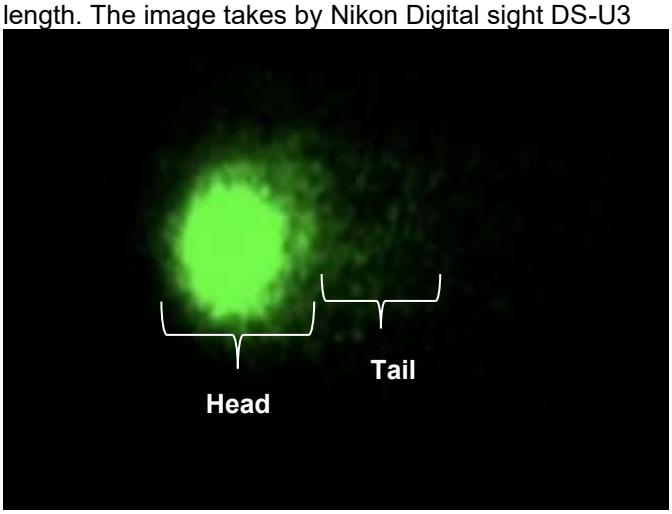

Figure16: Comet assay of testis cell from Taif Rose Nanoparticles (200mg/Kg) + DBP (500mg/kg) (G4).

The figure showed little damaged DNA by increase in tail length. The image takes by Nikon Digital sight DS-U3

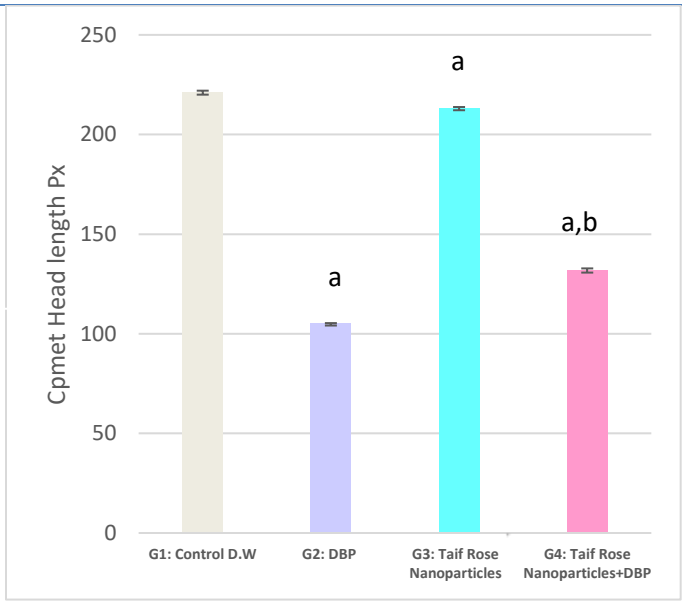

Figure17: *t*-test of the effect of daily administration of Taif rose Nanoparticles (250mg/kg) and Taif Rose Nanoparticles (2000mg/kg, oral) + DBP (500mg/kg, oral) head length of comet assay of male albino rats.

Data expressed as Mean ± S.E n=6 . Statistical analysis was performed between Control and treated animal by using a *t*-test. a: statistically significant compared to Control G1. b: statistically significant compared to the DBP G2.

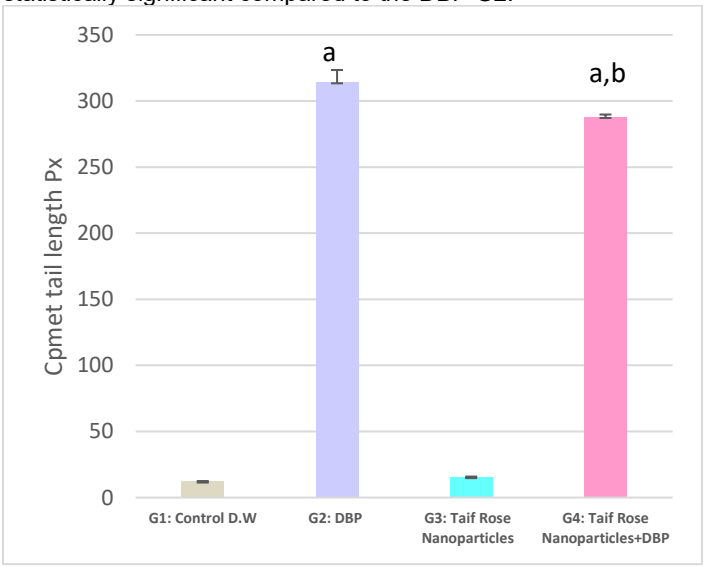

Figure18: *t*-test of the effect of daily administration of Taif rose Nanoparticles (250mg/kg) and Taif Rose Nanoparticles (2000mg/kg, oral) + DBP (500mg/kg, oral) tail length of comet assay of male albino rats.

Data expressed as Mean ± S.E n=6 . Statistical analysis was performed between Control and treated animal by using a *t*-test. a: statistically significant compared to Control G1. b: statistically significant compared to the DBP G2.

Histological result:

Testis Tissue of Negative Control Group (G1):

Testis histology of the negative control group showed normal morphology of the testis section and seminiferous tubules surrounded by Leydig cells. Also, there is evidence of spermatogenesis with orderly maturation of germ cells from the base to the center of the lumen which exhibits the Spermatogonia, Primary and Secondary Spermatocyte, spermatids, spermatozoa. **(Fig.18)**.

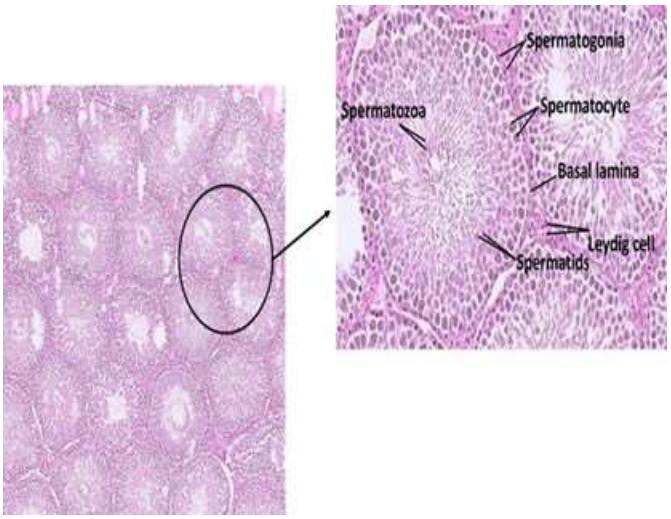

Figure 18: Light microscopy of testis section from Rats received normal saline (G1).

The figure showed normal morphology. (H&E staining; magnifications X200, X360).

Testis Tissue of Positive Control Group (G2):

The histological examination of the testes in the positive control group treated with DBP (500 mg/kg) (G2) revealed significant alterations in the seminiferous tubules. Specifically, the cross-sectional analysis indicated marked atrophy of these tubules, accompanied by contracted lumens. The findings illustrated thinning and splitting of the basal lamina, atrophied and widely spaced seminiferous tubules, and areas of necrosis in some tubules, associated with inflammation. Additionally, there was evidence of congestion in blood vessels, degeneration of certain segments of the seminiferous tubules, vacuolation of the germinal epithelium, and a complete absence of the spermatogonia series, primary spermatocytes, and spermatozoa in some tubule lumen. (Fig.19).

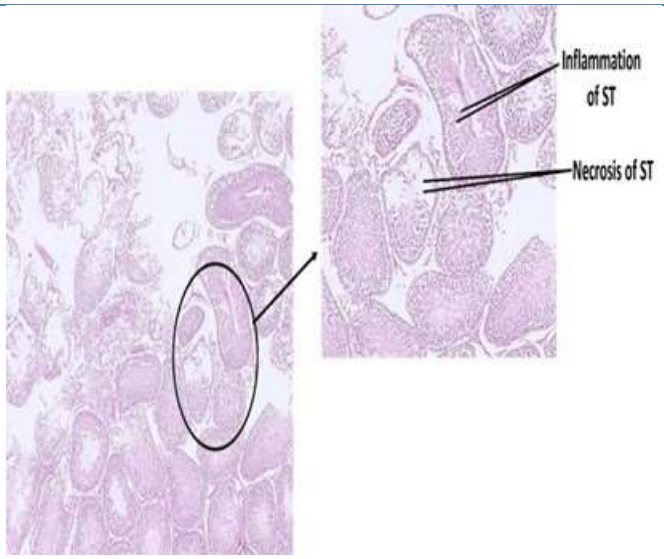

Figure 19: Light microscopy of testis section from Rats received DBP (500mg/Kg) (G2).

The figure showed inflammation and necrosis of the seminiferous tubule (ST). (H&E staining; magnifications X200, X360)

Testis Tissue of Taif Rose Nanoparticles (200mg/kg) Group (G5):

Histological examination of the testis in the Taif Rose Nanoparticles (200 mg/kg) (G5) revealed moderate atrophy of the seminiferous tubules, separated seminiferous tubules, and necrosis in some tubules, accompanied by inflammation. **(Fig.20)**.

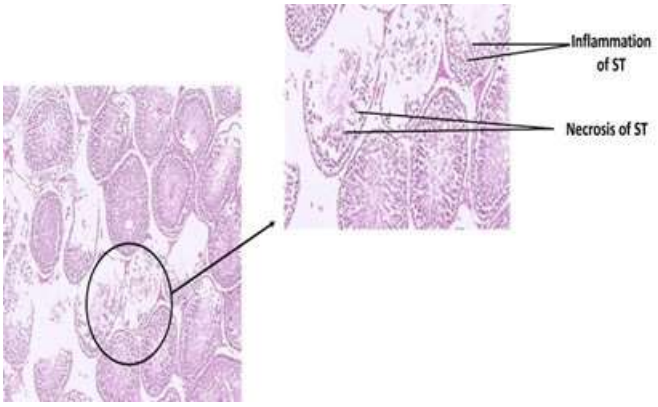

Figure 20: Light microscopy of testis section from Rats received Taif Rose Nanoparticles (200mg/Kg) (G5)

The figure showed necrosis of the seminiferous tubule. (H&E staining; magnifications X200, X360)

Testis Tissue of Taif Rose Nanoparticles (200mg/kg) + DBP (500mg/kg) Group (G6):

The testis histology of the Taif Rose Nanoparticles

(200 mg/kg) combined with DBP (500 mg/kg) (G6) demonstrated mild atrophy of the seminiferous tubules. This was characterized by contracted lumens, thinning and splitting of the basal lamina, and some separated seminiferous tubules, along with necrosis in certain tubules accompanied by inflammation. Overall, the extent of tubular damage was less severe compared to the group that was exposed solely to DBP (G2). **(Fig.21)**.

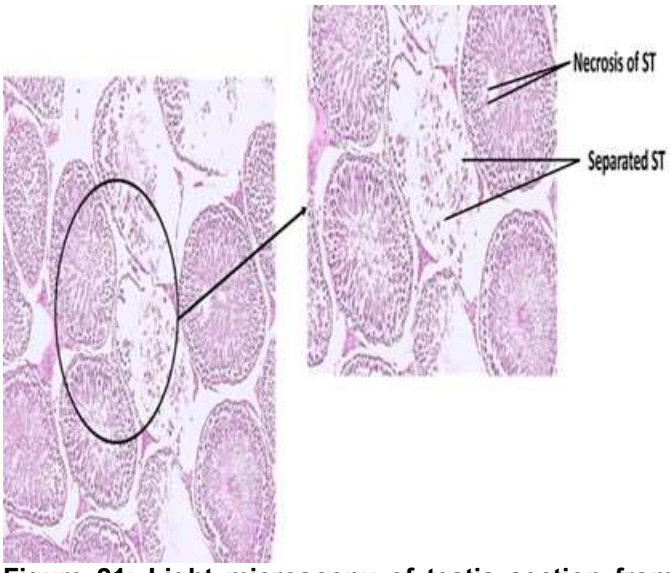

Figure 21: Light microscopy of testis section from Rats received Taif Rose Nanoparticles (200mg/Kg) + DBP (500mg/kg) (G6)

The figure showed necrosis of some seminiferous tubule. (H&E staining; magnifications X200, X360)

DISCUSSION

Dibutyl phthalate (DBP) is a common plasticizer for high molecular weight polymers that makes plastics more flexible and long-lasting (He et al. 2022). DBP is a developmental toxicant that mainly affects the liver and testis. It can cause cancer, testicular degeneration, and problems with male fertility, such as testicular atrophy and embryo death in rodents (Källsten et al. 2022). DBP also disrupts hormone homeostasis by altering gene expression and acting as an endocrine disruptor (Tang et al. 2023).

In our present study, administration of DBP at 500 mg/kg for six weeks led to a significant reduction in testosterone levels, accompanied by increases in FSH and LH ($p < 0.05$), indicating disruption of the hypothalamic–pituitary–gonadal axis. This is consistent with the findings of Källsten et al. (2022), who documented enduring effects on testosterone biosynthesis and testicular cell markers subsequent to DBP exposure, as well as with comprehensive reviews that validate DBP endocrine-disrupting properties.

Additionally, DBP exposure markedly diminished antioxidant defenses (GSH, SOD, catalase) and elevated lipid peroxidation (LPO) after six weeks, with persistent oxidative stress evident even following an eight-week withdrawal period. These results are similar to those of Aly et al. (2016), who showed that DBP (200–600 mg/kg) causes oxidative stress in adult rat testes by lowering antioxidant enzyme activities and raising LPO, which makes it harder for sperm to grow.

We also saw more damage to DNA, as shown by the comet assay, which showed a significant increase in comet tail length and a decrease in comet head size. The comet assay is a widely recognized and sensitive technique for identifying in vivo DNA damage (Li et al. 2015). Oxidative stress–induced DNA damage in the testis has been evidenced in DBP-exposed models (Tang et al. 2023).

DBP-induced reproductive toxicity seems to include endocrine disruption, oxidative stress, and subsequent DNA damage. Oxidative stress may hinder steroidogenic enzymes and enzyme systems, leading to diminished testosterone synthesis and the impairment of spermatogenic cells (Aly et al. 2016; Tang et al. 2023). This series of events damages reproductive function and genetic integrity.

Histological examination demonstrated that DBP induced significant testicular damage, including modifications in the diameter and area of seminiferous tubules. These results align with the research conducted by Källsten et al. (2022), which identified analogous testicular structural alterations in mice exposed to DBP.

The present study elucidates distinct biochemical and genotoxic effects of DBP; however, it does not investigate downstream molecular pathways (e.g., Nrf2/Keap1, miRNA regulation) that may facilitate oxidative and DNA damage responses. Future research should investigate these mechanisms to improve comprehension and therapeutic targeting.

The green synthesis of silver nanoparticles utilizing medicinal plant extracts, such as Taif Rose, has garnered interest due to its environmentally friendly methodology and economic viability in pharmaceutical applications (Egbiremhon et al. 2023). These plant-derived nanomaterials frequently possess bioactive compounds that exhibit free radical scavenging capabilities, rendering them potential natural antioxidants with negligible adverse effects (Perona et al. 2021).

This study demonstrated that Taif Rose-derived AgNPs alleviated the detrimental effects of DBP exposure by normalizing reproductive hormone levels LH, FSH, and testosterone—relative to controls treated solely with DBP. Mechanistically, these AgNPs seem to fight oxidative stress caused by DBP, as shown by the fact that oxidative stress markers returned to normal levels. This is consistent with earlier research that showed silver nanoparticles had stronger antioxidant activity than plant extracts or silver nitrate alone. This is probably because they have more reactive surfaces and are more

bioavailable (Elemike et al. 2017).

The comet assay results bolster the protective role: Taif Rose AgNPs successfully maintained DNA integrity under oxidative conditions induced by DBP, preserving both comet head and tail morphology. This finding aligns with Gur (2022), who indicated that green-synthesized AgNPs at particular concentrations can inhibit DNA damage.

The protective effects of Taif Rose AgNPs likely arise from their antioxidant properties, which involve scavenging reactive oxygen species (ROS) and stabilizing cellular structures, including DNA and tissues that regulate hormones.

Although these findings are encouraging, the precise molecular pathways implicated are not yet elucidated. Additional research is required to investigate the interactions of AgNPs with oxidative stress signaling pathways (e.g., Nrf2/Keap1) or cellular antioxidant systems. Furthermore, the specific roles of Taif Rose extract components in comparison to nanoparticle-specific characteristics necessitate further clarification.

Significance: our results show that green-synthesized AgNPs, especially those made from Taif Rose, could be useful in treating reproductive toxicity caused by environmental pollutants like DBP. This has wider effects on the development of safe, plant-based nanotherapeutics and points to a useful direction for future research in reproductive toxicology and nanomedicine.

CONCLUSIONS

This study demonstrated that DPP induces reproductive toxicity in mice, primarily through oxidative stress, hormonal imbalance, and DNA damage leading to testicular tissue defects. Treatment with Taif rose extract combined with silver nanoparticles showed a significant protective effect against DPP-induced toxicity. This treatment helped maintain normal levels of reproductive hormones such as FSH, LH, and testosterone. Moreover, results from the DNA comet assay indicated that the green biosynthesized Taif rose–silver nanoparticles had a remarkable positive impact on DNA integrity. Additionally, the study confirmed the role of Taif rose in enhancing the body's antioxidant levels, thereby mitigating oxidative stress–related damage.

Supplementary materials

Not applicable.

Author contributions

N.M.A.: Project Administration, N.M.A.; R.A.H.: Methodology, R.A.H.: Original draft writing, N.M.A.: Editing, supervision, visualization: R.A.H.: Software, Data collection and analysis.

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All of the data is included in the article/Supplementary Material.

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Conflict of interest

The authors declare no conflict of interest.

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