

Comparative study of the protective role of ashwagandha nanoparticles for female albino rats treated with paraben preservative

Aya Hussein Athab*, Alaa Hussein Al-Safy, Kiaser Abdulsajjad M. Hussain

Department of Biology, College of Education for Pure Sciences, Kerbala University, Karbala, Iraq

SUMMARY

AUTHORS' CONTRIBUTION: (A) Study Design · (B) Data Collection · (C) Statistical Analysis · (D) Data Interpretation · (E) Manuscript Preparation · (F) Literature Search · (G) No Fund Collection

Background: Introduction: Ashwagandha has anti-inflammatory, immunomodulatory and antibacterial properties. It has been demonstrated that parabens, which are used as preservatives in many food products, have negative effects. In addition, plant-based phytoparticles have been shown to exhibit nanomaterials with non-toxic, clean and environmentally friendly methods. This has attracted interest from researchers due to the outstanding properties of nanotechnology.

Method: A total of 24 female albino rats were divided into four groups: The control group, the paraben group, the Ag-Nano group and the combination group. Blood samples were taken from each animal for WBC, Hb and hormonal tests at the end of the experiment.

Results: Paraben preservatives significantly increased haemoglobin levels, but showed a nonsignificant decrease in white blood cells. There was a significant decrease in both estrogen and progesterone levels compared to the control. The nano-particles treated group showed no significant difference in haemoglobin and estrogen, while there was a significant increase ($P < 0.05$) in WBC and PR levels compared to the control. The combination group showed No significant difference in haemoglobin, white blood cells, but there was a significant increase ($P < 0.05$) in both estrogen and progesterone levels compared to the control.

Conclusion: The nano-particles treated group had no significant difference in levels of haemoglobin and estrogen compared to the control, but it showed a significant increase in WBC and PR levels ($P < 0.05$). The combination group had no significant difference in haemoglobin and white blood cells, but there was a significant increase in both estrogen and progesterone levels ($P < 0.05$) compared to the control.

Keywords: Paraben; Ashwagandha; Estrogen; Progesterone; WBC; HB

Address for correspondence:

Aya Hussein Athab,
Department of Biology, College of Education for Pure Sciences,
Kerbala University, Karbala, Iraq

Word count: 1795 **Tables:** 01 **Figures:** 01 **References:** 13

Received: 02.08.2024, Manuscript No. gpmp-24-146674; **Editor assigned:** 03.08.2024, PreQC No. P-146674; **Reviewed:** 15.08.2024, QC No. Q-146674; **Revised:** 29.08.2024, Manuscript No. R-146674; **Published:** 30.09.2024

INTRODUCTION

Ashwagandha, or *Withania somnifera*. The raw material used in medicine is the root, and the name 'Ashwagandha' is derived from the word 'ashwa', which means horse. It is believed that after consuming the root, one gains powers similar to those of a horse. The second part of the name, 'gandha', means fragrance and refers to the characteristic smell of the fresh root of the plant [1]. Ashwagandha's potential health benefits because it have anti-inflammatory, immunomodulatory, and antibacterial properties [2]. Parabens have no noticeable taste or smell and are soluble in oils and water. Humans are exposed to parabens orally through the consumption of processed foods such as jellies, beverages, canned foods, canned fruits and jams [3]. Parabens that are used on a daily basis due to their presence in various consumables have negative effects [4].

The biosynthesis of phytoparticles by plants exhibits nanomaterials, as they provide non-toxic, clean and environmentally friendly methods with different physical and chemical properties, and over the past few years, nanotechnology has attracted considerable research interest due to its outstanding mechanical, electromagnetic and optical properties [5]. Nanotechnology is a multidisciplinary field that includes nanomaterials, nanoelectronics, and nanobiotechnology as three widely overlapping fields, which have been used to improve the poor solubility of drugs and maintain the therapeutic efficiency of drugs and their effectiveness, the application of nanoparticles has attracted much attention especially in medicine by increasing the therapeutic index of drugs and is one of the most promising technologies that scientists rely on to create pharmaceutical inventions that change the concept of medicine and treatment for many diseases.

METHOD

The Ashwagandha plant (*Withania somnifera*) was collected in September 2023 from various geographical locations within the Holy Karbala Governorate. The roots were subsequently cleaned and ground to produce a fine powder. The preservative propyl 4-hydroxybenzoate, also known as paraben, was employed. The dosage was 166 mg/kg administered *via* a Gavage tube. The synthesis of silver nanoparticles using *Withania somnifera*. The plant extract (7 g) was distilled into 100 ml of non-ionic distilled water over an hour with a ratio of 80:20 (silver nitrate solution: plant extract) and a pH of 7. The colour change was

observed and the solution was kept in the dark for five days until the colour was stable.

Animals

The animals used in the experiment were 24 female albino rats (*Rattus norvegicus*), with a weight range of 250-265 g, purchased from the animal house of the Faculty of Veterinary Medicine at Kufa University. The animals were fed and watered in accordance with a 12/12-hour light/dark cycle and were permitted to acclimatize for a period of two weeks. The animals were divided into four groups:

- 1. The control group:** (6) female rats which received the phosgene salt solution for 14 days.
- 2. The paraben group:** (6) female rats were given the preservative (paraben) at a dose of 4.6 mg/kg per day by oral gavage for 20 days.
- 3. The Ag-Nano group:** (6) female rats were given the nanoparticles at a dose of 150 mg/kg per day by oral gavage for 20 days.
- 4. The Ag-Nano -Paraben group:** It included (6) female rats administered the nanoparticles at a dose of 150 mg/kg after two hours, administration the preservative at a dose of 4.6 mg/kg per day by oral gavage for 20 days.

At the end of the experiment, the animals were subjected to a general anesthesia and a blood sample collected immediately and then killed by decapitation.

Collection of blood samples

For each animal, 5 ml was collected directly from the heart and 2 ml was transferred to EDTA tubes for WBC and Hb testing. The remaining 3 ml was placed in tubes without anticoagulant (clot activator with gel) for serum separation and stored in the refrigerator at (20°C) for other hormonal tests.

Statistical analysis

The results were analyzed according to a randomized complete block design to study the effect of treatment with nanoparticles and time duration on the studied parameters, and the analysis of variance was used according to the randomized complete block design to study the effect of treatment and nanoparticles on immunological and serological parameters. The significance of the differences between the means was tested using the Revised Least Significant Differences (LSD) test at a significance level of $P < 0.05$.

RESULTS

Effect of treatments on haemoglobin and WBCs levels in the blood of female albino rats

The results of Haemoglobin (HGB) level analysis, as shown in the **Tab. 1.**, showed that dosing female rats with 4.6 mg/kg paraben preservative resulted in a significant increase ($P < 0.05$) in haemoglobin level compared to control group, on another hand the 150 mg/kg nanoparticles treated group showed no significant difference in haemoglobin level compared to control group. The group treated with preservative paraben 4.6 mg/kg and nanomaterial loaded extract showed no significant difference ($P < 0.05$) in haemoglobin level compared to the control and nanomaterial treated groups **Fig. 1.**

The results of the leukocyte analysis, as shown in **Tab. 1.** and **Fig. 1.** showed that administration of the preservative paraben at a concentration of 4.6 mg/kg to female rats resulted in a non-significant decrease in the number of white blood cells (WBC) compared to the control group, and a significant increase ($P < 0.05$) was found in the nanoparticles treated group at 150 mg/kg compared to the control group. There was no significant difference ($P < 0.05$) in the group treated with paraben preservative and nanomaterial-loaded extract compared to the control group ($P < 0.05$).

Effect of PHB treatment on Estrogen ER levels in female rats treated with Ashwagandha nanoparticles Ag-Nano

The results presented in **Tab. 1.** showed that treating female rats with PHB resulted in a significant decrease ($P < 0.05$) in estrogen level (39.15) compared to the control group (51.24). In addition, the results showed no significant difference ($P < 0.05$) in ES levels (51.24) in female rats treated with Ag-Nano compared to the control group (51.24). However, there was a significant increase ($P < 0.05$) in the nanoparticles preservative group compared to the paraben group **Fig. 1.**

Effect of PHB treatment on estrogen and progesterone PR levels in female rats treated with aqueous extract of Ashwagandha Root (AR) and Ashwagandha nanoparticles (Ag-Nano)

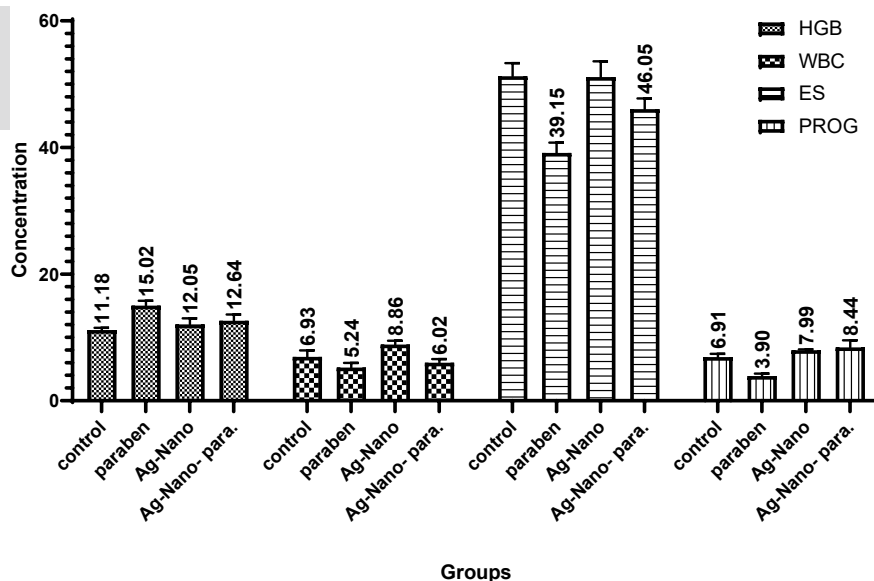
The results of the **Tab. 1.** showed that the treatment of

Tab. 1. Haemoglobin, WBC, estrogen and progesterone levels compared to the control and nanomaterial treated groups.

Group/Parameters	HGB	WBC	ES	PROG
Control	11.18 ± 0.24	6.93 ± 0.72	51.24 ± 1.48	6.91 ± 0.35
Paraben	15.02* ± 0.54	5.24* ± 0.51	39.15* ± 1.15	3.90* ± 0.28
Ag-Nano	12.05 ± 0.68	8.86* ± 0.43	51.12 ± 1.77	7.99* ± 0.08
The Ag-Nano-paraben	12.64 ± 0.69	6.02 ± 0.40	46.05 ± 1.21	8.44* ± 0.79

*: Significant, $p < 0.05$. mean ± SD.

Fig. 1. Haemoglobin, WBC, estrogen and progesterone levels compared to the control and nanomaterial treated groups.



female rats with paraben preservative resulted in a significant decrease ($P < 0.05$) in the level of progesterone PR (3.90) as compared to the control group (6.91). The table results also showed a significant increase ($P < 0.05$) in PR level of female rat group treated with ashwagandha nanoparticles (Ag-Nano) as well as nanoparticles preservative group compared to paraben preservative group **Fig. 1**.

The endocrine system is a complex structure of internal organs which are capable of hormone production. Hormones are signals that are transmitted through the circulatory system to their target sites. The proper functioning of the endocrine system is essential for maintaining homeostasis in the human body. Dysfunctions of hormonal function can be caused by both internal and external factors [6]. Feedback systems for reproductive hormones, including FSH and LH, may be impaired through exposure to parabens. There is some controversy about the effect of parabens on the reproductive system - several studies suggest that paraben exposure is a possible factor that worsens sexual reproductive function, both through estrogenic activity and through its anti-androgenic properties [7,8].

Our study agrees with that of Nishihama and colleagues, who showed that one of the environmental factors that can lead to infertility is an increased level of parabens in the body. They also showed that this increase in parabens in the human body can shorten the length of the menstrual cycle and reduce fertility because parabens contribute to reducing the activity of CYP19a1, which encodes an enzyme called aromatase. Aromatase converts androgens to Oestrogens, leading to a decrease in oestrogen levels [7].

The study is also consistent with the study [9] that showed an association between increased parabens and low levels of estradiol and low levels of progesterone. This may be due to increased levels of sex hormone-binding globulin (SHBG), which is the main transporter of estrogen and progesterone, reducing the concentration of the free form of these hormones in the blood [9].

Blood counts are important indicators of toxicity in the body and preserving agents like paraben are toxic to the body and cause changes in blood counts [10]. The increase in HGB and WBC concentrations was caused by exposure to parabens to cope with oxidative stress conditions. A study also showed an increase in haematological parameters in rats exposed to parabens [11].

This increase in white blood cells is due to infections and inflammation caused by the body's exposure to ethyl paraben. During an infection, the immune system is activated and the concentration of white blood cells increases [12]. Eda Nur İnkaya and Nurhayat Barlas who thought that the reason behind the increase in WBCs was the stimulation of the immune system in response to the tissue damage induced by parabens İnkaya and Barlas [13].

CONCLUSION

The nano-particles treated group had no significant difference in levels of haemoglobin and estrogen compared to the control, but it showed a significant increase in WBC and PR levels ($P < 0.05$). The combination group had no significant difference in haemoglobin and white blood cells, but there was a significant increase in both estrogen and progesterone levels ($P < 0.05$) compared to the control.

REFERENCES

1. Singh N, Bhalla M, de Jager P, et al. An overview on ashwagandha: A Rasayana (rejuvenator) of Ayurveda. *Afr J Tradit Complement Altern Med.* 2011;8(5S).
2. Joshi VK, Joshi A. Rational use of Ashwagandha in Ayurveda (Traditional Indian Medicine) for health and healing. *J Ethnopharmacol.* 2021;276:114101.
3. Katavic S, Cehic I, Zukic N, et al. *In vitro* assessment of the antioxidative, toxicological and antimicrobial properties of battery of parabens. *Drug Chem Toxicol.* 2024;47(4):463-72.
4. Bräuner EV, Uldbjerg CS, Lim YH, et al. Presence of parabens, phenols and phthalates in paired maternal serum, urine and amniotic fluid. *Environ Int.* 2022;158:106987.
5. Khan Y, Sadia H, Ali Shah SZ, et al. Classification, synthetic, and characterization approaches to nanoparticles, and their applications in various fields of nanotechnology: A review. *Catalysts.* 2022;12(11):1386.
6. Nowak K, Ratajczak-Wrona W, Górska M, et al. Parabens and their effects on the endocrine system. *Mol Cell Endocrinol.* 2018;474:238-251.
7. Ghaemi M, Jamali M, Hadizadeh A, et al. Urinary paraben derivatives in infertile women; Is it a cause of concern?. *Fertil Gyn Andrology.* 2023;3(1).
8. Malakootian M, Chavoshani A, Hashemi M, et al. Concentrations of urinary parabens and reproductive hormones in Iranian women: Exposure and risk assessment. *Toxicol Rep.* 2022;9:1894-1900.
9. Pollock T, Weaver RE, Ghasemi R, et al. Butyl paraben and propyl paraben modulate bisphenol A and estradiol concentrations in female and male mice. *Toxicol Appl Pharmacol.* 2017;325:18-24.
10. Moussa SA, Bashandy SA. Biophysical and biochemical changes in the blood of rats exposed to lead toxicity. *Rom J Biophys.* 2008;18(2):123-133.
11. Park J, Lee H, Choi DW, et al. The mixture effect of propyl paraben and bisphenol A on the uterotrophic response in the ovariectomized rats after oral administration. *Environ Anal Health Toxicol.* 2023;38(1).
12. Akmal H, Ahmad S, Abbasi MH, et al. A study on assessing the toxic effects of ethyl paraben on rohu (*Labeo rohita*) using different biomarkers; hemato-biochemical assays, histology, oxidant and antioxidant activity and genotoxicity. *Plos One.* 2024;19(5):e0302691.
13. İnkaya EN, Barlas N. Investigation of combined effects of propyl paraben and methyl paraben on the hypothalamic-pituitary-adrenal axis in male rats. *Toxicol Ind Health.* 2022;38(10):687-701.