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Original Article

# Antioxidant evaluation study of black rice anthocyanins nano-composite as prospective against infertility induced by $AlCl_3$ in rats

## Estudo de avaliação antioxidante do nanocompósito de antocianinas de arroz-preto como perspectiva contra infertilidade induzida por $AlCl_3$ em ratos

S. M. AlGhannam[a] and S. N. A. El-Rahman[b*]

[a]Alghad College for Applied Medical Sciences, Dammam, Saudi Arabia

[b]Agricultural Research Center, Food Technology Research Institute, Crops Technology Research Department, Giza, Egypt

**Abstract**

Anthocyanins are known as an antioxidant, and their water-soluble purple-colored pigments are very nutritive. Therefore, the present study investigated the antioxidant activity of black rice anthocyanins nano-composite against infertility induced by $AlCl_3$ in rats. Anthocyanin silver nanoparticles (An-AgNPs) were prepared by reducing black rice anthocyanin with the metallic ions. Antioxidant activity (DPPH %) of anthocyanin was determined. Also, the morphology of (An-AgNPs) was examined by scanning electron microscopy (SEM). Albino rats were divided into five groups (negative control (NC): fed on basel diet, positive control (PC): treated with $AlCl_3$ (34 mg/kg bw) for seventy days, and three other groups treated with $AlCl_3$ (34 mg/kg bw) + An-AgNPs at 10, 15, and 20 mg/kg, b.w/ day, respectively for seventy days. Serum testosterone, LH, FSH, and estradiol were measured. Additionally, Sperm motility, Sperm count (Testicular and Epididymal), fructose in semen, and semen quality were determined. The values of the anthocyanin component and DPPH radical scavenging activity obtained were 3603.82±6.11 mg CCE/g and 84.62±1.98, respectively. An-AgNPs shows tend to agglomerate, particles are uniform in size and shape, and the diameter of the particles ranges between 70nm to 130nm. LH, estradiol and testosterone levels increased significantly in rats treated with An-AgNPs 10, 15, 20 mg/kg b.w+ $AlCl_3$ (34 mg/kg bw) also exhibited significantly higher sperm motility, sperm count, and daily sperm production, and decreased sperm transit rate than G2. In comparison to G2, animals treated with $AlCl_3$ (34 mg/kg bw) + An-AgNPs 10, 15, 20 mg/kg b.w(G3 to G5) had significantly higher semen and semen quality (P 0.05). We can conclude that the An-AgNPs showed a strong effect against infertility induced by $AlCl_3$; this represents a suitable natural supply of biological substances for medicine and anthocyanins could be considered the ideal ingredients against oxidative stress-induced infertility.

**Keywords:** antioxidant activity, $AlCl_3$, black rice anthocyanin, nano-composite, infertility.

**Resumo**

As antocianinas são conhecidas como antioxidantes e seus pigmentos roxos solúveis em água são muito nutritivos. A partir dessa importante propriedade, o presente estudo investigou a atividade antioxidante do nanocompósito de antocianinas de arroz-preto contra a infertilidade induzida por $AlCl_3$ em ratos. Nanopartículas de prata antocianina (An-AgNPs) foram preparadas reduzindo a antocianina do arroz-preto com os íons metálicos. A atividade antioxidante (DPPH%) da antocianina foi determinada. Além disso, a morfologia de (An-AgNPs) foi examinada por microscopia eletrônica de varredura (MEV). Ratos albinos foram divididos em cinco grupos: controle negativo (NC) - alimentados com dieta basal (G1); controle positivo (PC) - tratados com $AlCl_3$ (34 mg/kg peso corporal) por setenta dias (G2), e outros três grupos (G3, G4 e G5) - tratados com $AlCl_3$ (34 mg/kg pc) por kg de peso corporal) + An-AgNPs a 10, 15 e 20 mg/kg de peso corporal/dia, respectivamente, durante setenta dias, de testosterona sérica, LH, FSH e estradiol. Além disso, foram medidas a motilidade espermática, a contagem de espermatozoides (testicular e epididimal), a frutose no sêmen e a qualidade do sêmen, sendo todas determinadas. Os valores do componente antocianina e da atividade eliminadora de radicais DPPH obtidos foram 3.603,82±6,11 mg CCE/g e 84,62±1,98, respectivamente, em tamanho e forma, e o diâmetro das partículas variou entre 70 nm e 130 nm. Os níveis de LH, estradiol e testosterona aumentaram significativamente em ratos tratados com An-AgNPs 10, 15, 20 mg/kg de peso corporal + $AlCl_3$ (34 mg/kg de peso corporal), tendo também exibido motilidade espermática, contagem de espermatozoides e produção diária de espermatozoides significativamente maiores, além de diminuição da taxa de trânsito espermático, do que o G2. Em comparação ao G2, os animais tratados com $AlCl_3$ (34 mg/kg pc) + An-AgNPs 5, 10, 20 mg/kg pc (G3 a G5) apresentaram sêmen e qualidade do sêmen significativamente maiores (P 0,05). Podemos concluir que os An-AgNPs apresentaram forte efeito contra a infertilidade induzida por $AlCl_3$, o que representa um fornecimento natural adequado de substâncias biológicas para uso na medicina. Ademais, as antocianinas podem ser consideradas os ingredientes ideais contra a infertilidade induzida pelo estresse oxidativo.

**Palavras-chave:** atividade antioxidante, $AlCl_3$, antocianina de arroz-preto, nanocompósito, infertilidade.

*e-mail: soheirkenawy@yahoo.com
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## 1. Introduction

Male infertility is a hotly contested topic that affects men all around the world (Hussain et al., 2023). About 40% of all cases of infertility are caused by males, and it is known that hereditary factors, varicocele, hypogonadism, cryptorchidism, and other diseases can contribute to infertility (Alahmar, 2017). There are numerous systems at work. It is acknowledged that oxidative stress is the primary cause, with the excess generation of free radicals having an impact on both the quantity and quality of sperm (Hussain et al., 2023). Free radicals that are highly reactive oxidizing agents are known as reactive oxygen species (ROS). Production of reactive oxygen species (ROS) is a normal physiological process in many organs, including the testicle; nevertheless, abnormalities in their synthesis induce cell oxidation and DNA damage (Sikka, 1996). Unsaturated fatty acids are abundant in sperm plasma membranes. As a result, it is particularly vulnerable to peroxidative damage. Lipid peroxidation damages the lipid matrix's structural integrity in spermatozoa membranes and has been associated with motility loss and abnormalities in membrane integrity (Henkel, 2005). Excess reactive oxygen species (ROS) can potentially affect male fertility and degrade sperm quality metrics because the antioxidant system is unable to manage them. Additionally, seminal plasma proteomes that affect male fertility interact with oxidative stress. Increased ROS production disrupts the DNA and other biological components, making it impossible for sperm to fertilise an ovum (Hussain et al., 2023). Aluminum (Al) is a common element in the earth's crust. It can be found in over 300 different minerals. Some of the most common foods containing trace amounts of aluminum-containing additives are processed cheese, frozen droughts, and cake mixes. Aluminum cooking utensils; are thought to account for 20% of daily aluminum intake, which ranges from 3.5 to 51.6 mg (Mayyas et al., 2005). According to several reports, administering AlCl3 causes oxidative stress, which triggers cell death and lipid membrane peroxidation (Jadhav and Kulkarni, 2023), also Al is harmful to the male reproductive system (Moselhy et al., 2012). Guo et al. (2009) discovered that after a long period of exposure, Al was accumulated in the testis, and through histopathologic study, Yousef and Salama (2009) observed that produced marked lesions in seminiferous tubules. Aluminum has been demonstrated to reduce testosterone levels in mouse testes and plasma depending on the amount and length of exposure: at 175mg AlCl3/kg/day, the reduction was substantially more significant than at 66mg AlCl3/kg/day (Guo et al., 2001). Aluminum accumulation in the testes has been linked to spermatid and spermatocyte necrosis, as well as lower fertility in mice (Llobet et al., 1995).

Lack of testosterone may increase the chance of spermatogenesis failure and sexual dysfunction. The steroidogenicity process in Leydig cells can be harmed by oxidative stress, which is caused by disease, the breakdown of homeostasis, and exposure to pollutants. This decreases testosterone synthesis. Anthocyanins are a class of poisonous antioxidants that are frequently found in dietary sources. They are a prime candidate to treat the steroidogenesis problem caused by oxidative stress (Hu et al., 2023). One source of dietary staples with significant health advantages is rice, particularly black rice due to the anthocyanin content. One of the phenolic molecules known as anthocyanin enters flavonoid compounds and serves as an antioxidant for both the plant and humans who consume black rice (Sholikhah et al., 2021). In order to increase testosterone levels in rats, according to Esomonu et al. (2005), flavonoids extract increased erythropoiesis in rat models. As a result, flavonoids were among the first antioxidant found to modulate testosterone. Then, Oluyemi et al. (2007) discovered that Garcinia flavonoids extract increased sperm count in animals. It is a flavonoid parent molecule that forms naturally when anthocyanidin and monosaccharide combine. Through the inactivation or stabilisation of free radicals and the reduction of cellular oxidative stress, it has been shown to mediate antioxidant reactions (Harborne and Wiliam, 2000). Okasha et al. (2008) found that anthocyanin increased serum prolactin levels in lactating albino rats. These findings point to a link between flavonoids (specific anthocyanins) and changes in reproductive hormone levels. However, there is still a major gap in our knowledge of the structure–function relationship of anthocyanin on the activity mentioned above (Hu et al., 2023). Also, no previous experiments have studied the effects of black rice anthocyanin silver nanoparticles against $AlCl_3$ induced-infertility. Therefore, this work aimed to extract the black rice anthocyanin, and study the effect of reproductive toxicity of aluminum chloride on fertility biomarkers in male rats, as well as the protective effect of black rice anthocyanin silver nanoparticles against potential testicular dysfunction caused by aluminum chloride.

## 2. Materials and Methods

### *2.1. Extraction of anthocyanin from black rice*

The anthocyanin extracted from 100g of black rice with 150 ml methanol (purity e"99.0% was purchased from Sigma-Aldrich, St. Louis, MO, USA) and stirred for 24 hours. Black rice anthocyanin extract was obtained by centrifuging the final extract (Septiani et al., 2017).

### *2.2. Determination of anthocyanin and its antioxidants Activity (DPPH %)*

Kim et al. (2008) calculated anthocyanin as mg cyanidin chloride Equiv./g dry weight. According to Aromatic et al. (2013), the discoloration ethanol solution of DPPH radical 0.2 aromatics in ethanol was used to evaluate the free radical scavenging ability of anthocyanin extract.

### *2.3. Preparation of anthocyanin silver nanoparticles*

Anthocyanin silver nanoparticles were prepared by reducing black rice anthocyanin with metallic ions. To synthesize anthocyanin nanoparticles, 0.06 M $AgNO_3$ concentrations were used: 6.6 ml methanol and 16.6 ml anthocyanin extract from black rice were added to 200 ml boiling distilled water. Notice immediate color change, which indicates the presence of anthocyanin silver nanoparticles. At room temperature, continue stirring until

the solution has cooled (Olenic and Chiorean, 2015). Then, the solution was centrifuged at 6000rpm for 20min and was placed the sample in the refrigerator.

### *2.4. Scanning electron microscopy (SEM)*

The microstructure of anthocyanin silver nanoparticles was examined by a scanning electron microscope JEOL, JSM-5200, Tokyo, Japan. The samples were sputter-coated with gold at a vacuum evaporator from 5 to 15 kV with accelerating voltage and magnification power of 750-6,000×.

### *2.5. Biological methods*

The Vaccination Centre in Helwan, Giza, Egypt, provided forty male wistar albino rats (150±20g). They were housed in the Ophthalmology Research Institute's animal house, Giza, Egypt, under conventional settings, with a relative humidity of 55%. For ten days, all rats were administrated a nutrition diet that included (a salt mixture of 4%, corn oil of 10%, vitamin mixture of 1%, starch of 70%, casein of 10%, and cellulose of 5%), after that, rats were weighed and divided into five groups (eight rats each) as follows:

G1; Negative control (NC) group for seventy days.

G2; Positive control (PC) group was treated with AlCl3 (34 mg/kg bw) for seventy days.

G3; Treated with AlCl3 (34 mg/kg bw) + An-AgNPs (10 mg/kg.b.w) for seventy days.

G4; Treated with AlCl3 (34 mg/kg bw) + An-AgNPs (15 mg/kg.b.w) for seventy days.

G5; Treated with AlCl3 (34 mg/kg bw) + An-AgNPs (20 mg/kg.b.w) for seventy days.

Feed consumption and total body weight were recorded every three days during the experimental period. At the end of the experiment, blood was collected from the orbital plexus and centrifuged for 30 minutes at 4°C and 1500 xg to separate the serum, then, stored in a freezer at – 4°C for analysis. Institutional review board (IRB): 2015-10-239.

### *2.6. Biochemical assays in serum*

#### *2.6.1. Hormonal assay*

Serum testosterone, LH, FSH, and estradiol were measured according to standard methods (Exley, 1998; Uotila et al., 1981; Tietz, 1995), respectively.

### *2.7. Sperm parameters*

#### *2.7.1. Sperm motility*

After tissue isolation, sperm motility was assessed, and cauda epididymis was sliced into little pieces and placed in Petri dishes with a nutrition medium that had been pre-warmed (RPMI). At 37°C, sperm were allowed to swim out for 5 minutes. A 400-fold magnification light microscope was used for the examination. The calculation of sperm motility was estimated by dividing the total number of sperm cells (motile and nonmotile) by the total number of live sperm cells. According to the Akdag et al. (1999) method, categorized the sperm cells that were not moving as non-motile, whereas those that moved were classified as motile.

#### *2.7.2. Sperm count*

Testicular sperm count: Each rat's testis was dissected and deposited in 1 ml of phosphate buffer saline. Surgical blades were used to cut and remove the tunica albuginea, and surgical blades were used to manually mince the residual seminiferous tubules in 1 ml of phosphate buffer saline. The testicular cell suspension was pipette numerous times to achieve a homogenous cell suspension. Utilizing the Fatma et al. (2009) method, measured the sperm concentration in the testis and quantified it as million sperm cells per ml of solution with one drop of the suspension placed on a "Makler Counting Chamber". The left testis was removed, and the left epididymis was split in half to count the sperm (head and body plus tail). Each fraction was homogenized in Triton Merthiolate buffer solution using a mixer. Then, homogenization-resistant spermatids were counted using an erythrocytometer according to the Omura et al. (1996) method.

After that, tunica albuginea was removed, and both testes were chopped and homogenized for 1 minute in 10 ml of 0.9 percent NaCl containing 0.5 percent Triton X-100 in a POTTERS® tissuemizer at medium speed. Robb et al. (1978) divided the total number of homogenization-resistant sperms by 6.1, the number of days these sperms were present in the seminiferous epithelium. The transit rate of epididymal sperms for each male rat was calculated by dividing the number of epididymal sperms by the daily sperm output.

### *2.8. Determination of fructose in semen and semen quality*

To determine the level of fructose in semen and the quality of the semen, the methods of Foreman et al. (1973) and Reddy and Bordekar (1999) were used, respectively.

### *2.9. Statistical analysis*

A one-way ANOVA was used to analyze the biological evaluation data. The significance level was set at P 0.05 is presented as means with standard deviations for all variables. To compare the significant differences in treatment methods, LSD was used.

## 3. Results and Discussion

### *3.1. Anthocyanin content and its antioxidant activity DPPH %*

Anthocyanin content and the antioxidant activity of anthocyanin extract radical scavenging activity (expressed as absorbance percentage) were determined, and the data are tabulated in Table 1. The value of the anthocyanin component obtained was 3603.82±6.11 mg CCE/g. Recently,

**Table 1.** Anthocyanin content and lipid peroxidation inhibitory.

| Anthocyanin mg CCE/g dry weight | Anthocyanin DPPH radical scavenging activity (%) |
|---|---|
| 3603.82±6.11 | 84.62±1.98 |

Values are means ± SD (n = 3).

it was discovered that anthocyanins are in charge of guarding the testicles and spermatogenesis from the threat of oxidative stress (Li et al., 2020). Additionally, we have noted that anthocyanins can significantly speed up the process of steroidogenesis and alleviate the oxidative stress caused by exogenous pollutants on Leydig cells in vitro (Li et al., 2019).

The DPPH radical scavenging activity value demonstrates the good antioxidant capacity of the anthocyanin extract; this result can occur because by contributing hydrogen atoms to create the DPPH-H complex, anthocyanins can trap DPPH radical complexes (Rufino et al., 2010). Also, because the majority of anthocyanins have a hydroxyl group in position 3 that is very simple to escape due to enthalpy and the lowest energy hydroxyl group link on ring B, anthocyanin extract has the ability to donate hydrogen atoms, which can lead to this scenario (Vaya et al., 2003). Alnamshana (2022) reported that phenolic acid was present in BREE (Black rice ethanol extract) in high concentrations (250.30 mg GA/g). 12.57 mg Q/g of flavonoid chemicals, and 395.76 mg of cyanidin chloride were found in anthocyanin.

### *3.2. Scan electron microscope*

Figure 1 shows SEM images of An-AgNPs made from silver nitrate and black rice anthocyanin doping. As seen from Figure 1, the An-AgNPs sample tends to agglomerate and particles uniform in size and shape, and the diameter of the particles ranged between 70nm to 130nm. Dupeyrón et al. (2017) prepared anthocyanin nanoparticles and showed that the sample image is visible as an irregular cluster of large size (~10µm). Similarly, other images show that NPs of the sample tends to agglomerate too. The agglomerates seem to be the results of smaller NPs agglomerated. NPs are agglomerated mostly because of intermolecular hydrogen bonds.

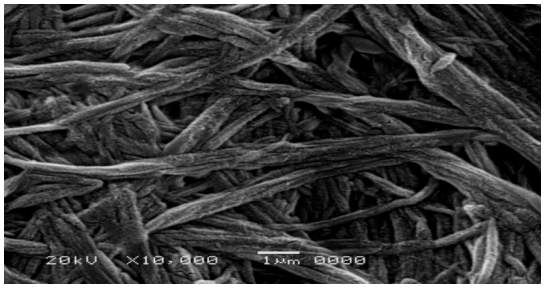


**Figure 1.** SEM of silver Nano-particles/ anthocyanin Nano-composite.

### *3.3. Biochemical assays*

Table 2 shows that serum LH, estradiol, and testosterone concentrations are significantly lower ($P \leq 0.05$), and serum FSH concentrations are considerably greater in rats treated with $AlCl_3$ (G2) than in NC rats (G1). LH, estradiol, and testosterone levels increased significantly ($P \leq 0.05$) from G3 to G5 (5.722, 7.897, 8.868 IU/L), (28.065, 38.083, 44.383 pg/ml), and (280.910, 398.300, 447.802 ng/dl), respectively, compared to G2 (0.372 IU/L, 9.435pg/ml, and 143.473ng/dl, respectively). In addition, FSH serum concentrations decreased considerably from G3 to G5 treated rats (11.825, 8.900, and 7.190 IU/L, respectively) than with G2 (15.318 IU/L). These results were reported in rats by Soheir and Haya (2013), Soheir et al. (2018); and in mice by Guo et al. (2005). The significant decrease in male libido and fertility after aluminum treatment may be due to excessive aluminum deposition in the testicles and low levels of testosterone. A distinction was explained by the fact that aluminum accumulation did not immediately influence androgen biosynthesis enzymes or disrupt hypothalamic-pituitary-gonadalxis. However, after 70 days of treatment, $AlCl_3$ reduced the activity of 17-ketosteroid reductase significantly ($P \leq 0.05$). As evidenced by the suppression of LH-stimulated steroidogenesis in Leydig cells (Guo et al., 2005), Al-induced NO may be a testosterone suppressor. Stress-induced decreases in testicular NO resulted in a reduction in steroidogenic enzyme activity (Kostic et al., 2000). Excess NO molecules caused by Al, according to (Guo et al., 2005), might directly block the principal second messenger, cAMP, which mediates. Less testosterone was produced in Leydig cell steroidogenesis due to gonadotropin activity through transforming cholesterol to pregnenolone.

Due to damage to the Leydig cells or other endocrine tissues, like the anterior pituitary, the presence of OS in the testicles causes a decline in testosterone production

**Table 2.** Influence An-AgNPs on serum LH, FSH, Estradiol and Testosterone concentrations in rats treated with $AlCl_3$ for 70 days.

| Treatments | LH (IU/L) | FSH (IU/L) | Estradiol (pg/ml) | Testosterone (ng/dl) |
|---|---|---|---|---|
| G1 (NC) | $8.877^{a}$±0.019 | $7.280^{d}$±0.010 | $45.497^{a}$±0.055 | $450.640^{a}$±0.145 |
| G2 (PC) | $0.372^{d}$±0.178 | $15.318^{a}$±0.019 | $9.435^{e}$±0.023 | $143.473^{e}$±0.191 |
| $AlCl_3$ (34 mg/kg b.w) | | | | |
| G3 (An-AgNPs 10 mg/kg b.w+ $AlCl_3$ 34 mg/kg b.w) | $5.722^{c}$±0.017 | $11.825^{b}$±0.016 | $28.065^{d}$±0.077 | $280.910^{d}$±0.054 |
| G4 (An-AgNPs 15 mg/kg b.w+ $AlCl_3$ 34 mg/kg b.w) | $7.897^{b}$±0.011 | $8.900^{c}$±0.008 | $38.083^{c}$±0.068 | $398.300^{c}$±0.114 |
| G5 (An-AgNPs 20 mg/kg b.w+ $AlCl_3$ 34 mg/kg b.w) | $8.868^{a}$±0.017 | $7.190^{e}$±0.010 | $44.383^{b}$±0.134 | $447.802^{b}$±0.138 |
| LSD | 0.236 | 0.039 | 0.233 | 0.396 |

Each statistic represents the average of ten rats in each group. ANOVA multiple fury tests revealed a significant difference from controls ($p \leq 0.05$).

(Turner et al., 2005). Notably, the physiological synthesis of hormones also results in the formation of ROS, which are primarily produced by mitochondrial respiration and the catalytic activity of the steroidogenic cytochrome P450 enzymes (Hanukoglu, 2006). In this method, the creation of ROS inhibits the significant steroid production and damages the spermatozoa's mitochondrial membranes (Luo et al., 2006). Through an indirect impact on the generation of male hormones, which is linked to spermatogenesis, OS is linked to a larger number of immature spermatozoa (Aitken et al., 2003). Four anthocyanins, including, delphinidin-3-glucoside (Dp3-glu), cyanidin-3-glucoside (Cy-3-glu), cyanidin-3,5 diglucoside (Cy-3,5-diglu), and pelargonidin-3-glucoside (Pg-3-glu), were used in the current study after employing 2,20-Azobis(2-amidinopropane) dihydrochloride (AAPH) to induce oxidative stress in R2C cells and reverse testosterone production. The findings showed that all four types of anthocyanins can reduce ROS production, mitigate mitochondrial membrane potential damage, and support higher testosterone levels. When it came to antioxidative performance, cell dysfunction, and upregulating the expression of the steroidogenic acute regulatory protein (StAR), Cy-3,5-diglu with diglycoside outperformed the others (Hu et al., 2023). Anthocyanin in purple sweet potatoes dramatically raised female FSH levels, the number of follicles, and the number of primary, secondary, and Graafian follicular granulosa cells (Ningrum et al., 2021).

Table 3 reveals that in rats treated with $AlCl_3$ (G2), sperm motility, sperm count, and daily sperm production were considerably lower than in rats treated with G1 (NC). In comparison to G2 rats (PC), An-AgNPs 10, 15, 20 mg/kg b.w + $AlCl_3$ (34 mg/kg b.w) rats exhibited significantly higher sperm motility, sperm count, and daily sperm production, but a decreased sperm transit rate (day). Yousef et al. (2007) studied the influence of aluminum on aconitase. This protein binds citrate and catalyzes its isocitrate in the Krebs cycle and found a considerable lowering in aconitase as well as a reduced activity in the presence of aluminum, which means that aluminum may affect mitochondrial enzymes. Sperm motility and viability may be affected by changes in mitochondrial function. Aluminum chloride increased dead and abnormal sperm while decreasing sperm concentration and motility; additionally, necrosis of spermatocytes/spermatids was noticed in mouse testes (Guo et al., 2001). According to Dawson et al. (1998), increased levels of aluminum in human spermatozoa and seminal plasma have been linked to decreased sperm motility and viability. As a result, the observed drop in sperm motility may be attributable to a contemporaneous drop in testosterone production (Kostic et al., 2000). According to Yousef et al. (2005), rabbits given $AlCl_3$ at a dose of 34 mg/kg bw every other day for 16 weeks had a considerable drop in sperm level and motility. As a result, $AlCl_3$'s sperm toxic properties might be attributed to the compound's free radicals. According to Abd El-Rahman and Al-Ahmary (2013), sperm motility, sperm count, and daily sperm production were substantially lower in rats treated with $AlCl_3$ (34 mg/kg bw) than with NC. In contrast, the sperm transit rate (day) was significantly higher. Redox imbalance, decreased sperm DNA damage, and sperm motility are caused by an increase in ROS and a decrease in antioxidant defence. Due to the significant amounts of unsaturated fatty acids present in their cell membranes, spermatozoa are particularly vulnerable to the harmful effects of ROS. Lipid peroxidation is facilitated by reactive oxygen species, which increases intracellular oxidative burden. Lipid peroxidation, loss of membrane integrity with increased permeability, decreased sperm motility, structural DNA damage, and apoptosis are the order of events (Schuppe et al., 2008). Our findings supported anthocyanin's antioxidant function in reducing the oxidative damage to testicles and sperm induced by diabetes (Ahmadi et al., 2023).

Table 4 shows the amount of fructose in the semen and the quality of the semen in rats treated with $AlCl_3$ and $AlCl_3$ (34 mg/kg b.w) + An-AgNPs at 10, 15, 20, (G3 to G5). Fructose in semen was considerably greater and semen quality was considerably lower in rats treated with

**Table 3.** Influence of $AlCl_3$ and $AlCl_3$ + An-AgNPs for 70 days on sperm motility, sperm count, daily sperm production, and sperm transit rate.

| Treatments | Sperm motility | Sperm count (×10⁶ /ml) | | Daily sperm (*) | Sperm transit rate |
|---|---|---|---|---|---|
| | (%) | Testicular | Epididymal | Production | |
| G1 (NC) | 87.427$^{a}$±0.375 | 183.100$^{a}$±0.061 | 273.218$^{a}$±0.169 | 31.740$^{a}$±00.167 | (day)7.502$^{d}$±00.101 |
| G2 (PC) | 49.132$^{e}$±0.183 | 115.988$^{e}$±0.054 | 151.245$^{e}$±0.047 | 17.278$^{e}$±0.116 | 16.257$^{a}$±0.155 |
| $AlCl_3$ (34 mg/kg b.w) | | | | | |
| G3 (An-AgNPs 10mg/kg b.w+ $AlCl_3$ 34 mg/kg b.w) | 53.773$^{d}$±0.110 | 148.040$^{d}$±0.096 | 192.872$^{d}$±0.133 | 23.023$^{d}$±0.148 | 13.095$^{b}$±0.059 |
| G4 (An-AgNPs 15 mg/kg b.w+ $AlCl_3$ 34 mg/kg b.w) | 71.913$^{c}$±0.063 | 172.403$^{c}$±0.202 | 239.452$^{c}$±0.080 | 29.028$^{c}$±0.085 | 10.718$^{c}$±0.096 |
| G5 (An-AgNPs 20 mg/kg b.w+ $AlCl_3$ 34 mg/kg b.w) | 86.055$^{b}$±0.086 | 182.108$^{b}$±0.162 | 271.500$^{b}$±0.161 | 31.030$^{b}$±0.040 | 7.998$^{d}$±0.040 |
| LSD | 0.579 | 0.375 | 0.370 | 0.350 | 0.288 |

(*) Per gram of testicular parenchyma, the count is computed. Each statistic represents the average of ten rats in each group. ANOVA multiple fury tests revealed a significant difference from controls ($p \leq 0.05$).

**Table 4.** Fructose in semen and semen quality in male rats treated with $AlCl_3$ and $AlCl_3$ + An-AgNPs for 70 days.

| Treatments | Fructose in semen (mg/dl) | Semen quality (%) |
|---|---|---|
| G1 (NC) | $1.722^{e}$±0.016 | $338.000^{a}$±4.017 |
| G2 (PC) | $8.237^{a}$±0.039 | $117.000^{e}$±2.595 |
| $AlCl_3$ (34 mg/kg b.w) | | |
| G3 (An-AgNPs 10 mg/kg b.w+ $AlCl_3$ 34 mg/kg b.w) | $3.790^{b}$±0.026 | $205.000^{d}$±2.733 |
| G4 (An-AgNPs 15 mg/kg b.w+ $AlCl_3$ 34 mg/kg b.w) | $2.098^{c}$±0.016 | $281.167^{c}$±2.548 |
| G5 (An-AgNPs 20 mg/kg b.w+ $AlCl_3$ 34 mg/kg b.w) | $1.800^{d}$±0.016 | $329.000^{b}$±2.595 |
| LSD | 0.071 | 8.597 |

Each statistic represents the average of ten rats in each group. ANOVA multiple fury tests revealed a significant difference from controls ($p \leq 0.05$).

$AlCl_3$ (PC) compared to NC. In comparison to G2, animals treated with An-AgNPs 10, 15, 20 mg/kg b.w + $AlCl_3$ (34 mg/kg b.w) (G3 to G5) had significantly ($P \leq 0.05$) higher in semen quality and significanlty lower in fructose in semen than PC. The semen quality decline in rats treated with $AlCl_3$ was comparable to the findings of Soheir and Haya (2013), Soheir et al. (2018). According to Yousef and Salama (2009), aluminum chloride caused testicular dysfunction, as well as a drop in testosterone and the quality of semen. $AlCl_3$ also damaged the quality of semen in vitro and in vivo, according to previous research (Yousef et al., 2005, 2007). Moreover, due to the spermatozoa's lack of cytoplasm and DNA compaction, which leaves little room for antioxidant enzymic translations, seminal fluid is a crucial source of antioxidants in semen. (Jeulin et al., 1989).

## 4. Conclusion

In this investigation, the black rice anthocyanins nanocomposite showed strong effects against infertility induced by $AlCl_3$ in rats. In particular, the An-AgNPs at 20 mg/kg b.w was selected as a source of fertility substances for use against infertility. It might offer a fresh tip in the search for new biological sources of new potential drug design pharmaceutical components.

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