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Research Article
Examining the Influence of Amniotic Fluid on Experimentally Induced Testicular Injury Due to Cisplatin Chemotherapy in a Rodent Model

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Abstract

Background and Objective: Cisplatin (CP), also known as cis-diamminedichloroplatinum (II), is a highly effective chemotherapeutic agent utilized in treating various malignancies, including those of the lung, uterus, rectum and testis. Due to its severe side effects associated with clinical use, CP's administration requires cautious evaluation. Testicular toxicity and injury are among the adverse consequences of CP treatment. The primary objective of this research was to explore the potential protective capabilities of AmnioMax (AMX) against CP-induced testicular damage by examining sperm parameters, testicular tissue alterations and oxidative/antioxidative markers within the rat testes. **Materials and Methods:** With 11 rats in each of the five groups (control, AMX, CP, CP+AMX and AMX+CP), there were 55 male Wistar albino rats utilized in the study. To assess oxidative stress, oxidative stress index (OSI) and total oxidant status (TOS) were determined, while total antioxidant status (TAS) was used to calculate for the antioxidant defense system in both tissue and blood specimens. The histology of the testicular tubules was evaluated using the Johnsen testicular biopsy score. A p-value of 0.05 or below was considered statistically significant. **Results:** This study investigated the potential protective effect of amniotic fluid on testicular injury induced by cisplatin chemotherapy in a rodent model. Biochemical assessment revealed a significant increase in total testis protein levels and mitochondrial IDH in the CP+AMX group compared to the CP group. Sperm analysis showed a significant improvement in sperm motility and viability in the CP+AMX group compared to the CP group. Histopathological examination demonstrated a significant reduction in fibrosis and a significant increase in JTBS in the CP+AMX and AMX+CP groups compared to the CP group. Overall, these findings suggest that amniotic fluid may have a protective effect against cisplatin-induced testicular injury. **Conclusion:** When compared in terms of whether the AMX injection should be administered before or after CP, the CP+AMX group had higher TAS levels and higher IDH levels, sperm viability and motility and Johnsen testis biopsy score compared to the AMX+CP group. Considering this, it can be concluded that the administration of AMX after CP is more appropriate.

Key words: Cisplatin-induced testicular injury, amniotic fluid protection, oxidative stress markers, sperm analysis and histopathology, antioxidant defense system, rat model

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

An extremely powerful anti-cancer drug called cisplatin, also known as cis-diamminedichloroplatinum (II), is used to treat a number of malignancies including lung, uterine, rectum and testis cancer[1]. A DNA-alkylator, CP acts by creating crosslinks within DNA and causing double helix breakage with antitumor effects[2]. The CP should be carefully assessed due to serious side effects that are caused by clinical use. Testis toxicity and testis injury are some of the side effects of CP[3]. Though the basic effect of CP on the testis has not been clarified, most studies generally defined the main mechanism of toxicity induced in the testis by CP as oxidative stress mediated by the consumption of antioxidants with increasing oxygen radicals[4]. Results show that CP's reproductive toxicity is inescapable, necessitating the development of fresh preventative and treatment methods[5]. In light of this information, protective agents to minimize the reproductive toxicity linked to CP they researched and most studies focused on antioxidants[6].

Amniotic fluid cells must be grown in a culture medium that is nutrient-rich for minimal proliferation to take place. For the short-term cultivation of human amniotic fluid cells for cytogenetic research and *in vitro* diagnostic procedures, Gibco® AmnioMax™-II Complete Media is a full-support medium. Chorionic villus samples and primary cultures of human amniotic fluid cells have been created and given the all-clear for *in vitro* proliferation for use in prenatal diagnostic tests. Fetal bovine serum (FBS), gentamicin and L-glutamine are ingredients in AmnioMax™, which ensures that cell adhesion and proliferation are maximized. To guarantee improved pH stability during culture manipulations, this optimized medium contains a buffering mechanism developed[7]. A possible protective effect of human amniotic fluid-derived cell culture medium (Gibco AmnioMax-II) (AMX) against CP-derived testis toxicity has not been investigated to this point.

The objective of this research was to explore the potential protective role of AMX against CP-induced testicular damage by examining sperm characteristics, testicular tissue alterations and oxidative/antioxidative indicators within the rat testes.

MATERIALS AND METHODS

Study area: This examination was carried out in Istanbul, Turkey from September, 2019 to March, 2020 at the Experimental Application and Research Center of Bezmialem Vakif University.

Ethical approval: The Bezmialem Vakif University's local ethics committee for animal studies gave its clearance (2019/98) before the procedures got started and the whole study complied with the Research Council's Handbook for the Care and Use of Laboratory Animals.

Animals: A total of 55 adult Wistar Albino rats weighing between 250 and 300 g were used in this experiment. These animals were kept in clean, well-ventilated plastic cages with a 12 hrs light-dark cycle and regulated climatic conditions, such as a room temperature of 25°C. The aforementioned living arrangements were arranged a week before the trial began. The rats were provided with unlimited access to food and water during the experiment to ensure that they received the correct nutrition.

Experimental protocol: Five groups of 11 rats each were created: Control (C), AMX, CP, CP+AMX and AMX+CP. All medications were given intraperitoneal. Day 1 was considered to be the day when therapy started. During the first four days, the control group received 1 mL kg^{-1} of physiological saline (PS). During the first day, the AMX group received 1 mg kg^{-1} PS and for the next three days, just 0.3 cc of AMX was given. On the first day, the CP group received a single dosage of 7 mg kg^{-1} cisplatin and for the next three days, they received 1 mL kg^{-1} PS. On the first day, the CP+AMX group received a single dosage of 7 mg kg^{-1} cisplatin and over the next three days, they received 0.3 cc AMX. Finally, the AMX+CP group received 0.3 cc AMX throughout the first three days and 7 mg kg^{-1} CP on the fourth. The choice of these dosages in related studies was the primary justification for utilizing them[8].

Tissue collection: All rats received bilateral orchiectomy on day 28. Intraperitoneal anesthesia was administered prior to the surgery using a mixture of ketamine (50 mg kg^{-1}) and xylazine (5 mg kg^{-1}). The rats were put to death after undergoing bilateral orchiectomy using a strong ether solution. Blood samples were taken through the cardiac puncture and semen samples were taken from the epididymis. The testis on the left side underwent two cold PS washes and was subsequently stored at a temperature of -80°C until the day of examination for the purpose of conducting biochemical analysis. Meanwhile, the testes located on the right side was immersed in a formalin solution to undergo histological investigation.

Sperm analysis: Using a modified method and a hemocytometer, the number of sperm was calculated[9]. The epididymis was first painstakingly cut into pieces and placed in 5 mL of PS. It was then allowed to rock for a total of 10 min. It then underwent a 2 min incubation at ambient temperature. Using a mixture of 100 mL of distilled water, 1 mL of formalin (35%), 25 mg of eosin and 5 mg of $NaHCO_3$, the resultant supernatant fluid was diluted 1:100. The diluted fluid was then aliquotted into a 10 µL container and allowed to settle for 5 min in the sperm count chamber (Makler Counting Chamber, TS Scientific, Perkasie, Pennsylvania, USA). The material was then inspected under a light microscope (Olympus BX51, Tokyo, Japan) with 400x magnification.

Biochemical analysis: The testicular tissue was divided into minute pieces and diluted ten times in a 50 mM phosphate-buffered saline solution. Subsequently, the tissue was subjected to homogenization utilizing an automatic homogenizer (TissueLyser LT, Qiagen, Dublin, Ireland). Following the homogenization process the mixture was subjected to centrifugation at 10,000 rpm for 15 min at 4°C. The assessment of the total antioxidant status (TAS) in the tissue of the testicle was conducted through the utilization of a Rel Assay kit (Rel Assay Diagnostics, Gaziantep, Turkey) through an autoanalyzer (AU5800, Beckman Coulter Inc., Brea, California, USA)[10]. The underlying assumption of the tool is that the ABTS radicals, specifically the blue-green 2,2'-azino-bis (3 ethylbenzothiazoline-6-sulfonic acids), undergo reduction by antioxidant molecules, resulting in a loss of coloration). The overall quantity of antioxidants in the sample was correlated with variations in absorbance at 660 nm. Trolox, a water-soluble vitamin E substitute, served as the calibrator. The information is provided in terms of mmol Trolox Eq/L/mg protein.

The testicular tissue's total oxidant status (TOS) was determined using an AU5800 autoanalyzer and a Rel Assay kit[11]. This kit's fundamental idea is that oxidant molecules change iron ions from their ferrous state to their ferric one. Iron ions create a chromogenic-colored complex in an acidic environment. The total number of oxidant molecules present in the sample is related to the color intensity, which is determined using a spectrophotometer (Thermo Scientific, Multiskan FC, USA). The data were measured using hydrogen peroxide as a calibrator and they are given in units of mol H_2O_2 Eq/L/mg protein. The TOS to TAS ratio is represented by the oxidative stress index (OSI) and the findings are communicated as an arbitrary unit (AU).

Using a total protein kit (AMP Diagnostics, Austria) and bovine serum albumin as the reference, the homogenate's total protein concentration was determined.

An established procedure was used to obtain the testicular mitochondrial fraction[12]. In a nutshell, a 0.25 M sucrose homogenate containing 20% of the testis was created. The mitochondrial pellet was then extracted from the post-nuclear supernatant by centrifuging it at 10,000 g for 20 min. The mitochondrial pellet was then cleaned three times with a solution of potassium chloride at 1.15% before being suspended in a 0.25 M sucrose solution containing 10 mg of protein per mL. At 4°C, all processes were completed. Further biochemical experiments used the isolated mitochondrial fraction.

Isocitrate dehydrogenase (IDH) was measured using a recognized technique[13]. Fifty milliliters of the mitochondrial suspension was combined with 2.5 mL of the buffer substrate solution (0.2 M Tris buffer pH 8.5, 4.6 mM isocitrate solution, 52 mM NaCl and 2.88 mM NADP). At 340 nm, variations in absorbance were seen for 3 min in a row. Using a molar extinction value of 6.22×10^3 M cm^{-1}, the amount of NADH that was oxidized in nano moles per minute per milligram of protein was used to calculate enzyme activity.

Histopathological examination: A 10% formalin solution was used to saturate the testicular tissue. The sample was then cut into 4 µm pieces, which were then embedded in paraffin blocks and stained with hematoxylin and eosin. Afterward, these slices were inspected at a magnification of ×400 using a light microscope. Scores were given according to the degree of fibrosis, with 0 denoting no fibrosis, 1 indicating fibrosis in less than 25% of the sample, 2 denoting fibrosis in 25-50% of the sample and 3 denoting fibrosis in more than 50% of the sample. The Johnsen testis biopsy score (JTBS)[14] was used to evaluate spermatogenesis in the testicular tissue. During each test, 100 seminiferous tubules in total were inspected (Table 1).

Table 1: Johnsen testicular biopsy score

Scores	Histopathological observations	Scores	Histopathological observations
1	Absence of cells in the tubular section	6	Limited spermatids (5/tubule) present
2	Presence of Sertoli cells only	7	Abundant spermatids with no discernible distinctions
3	Germ cells limited to spermatogonia	8	Late spermatids lacking mature spermatozoa
4	Sparse spermatocytes (5/tubule)	9	Sparse spermatozoa (5/tubule) observed
5	Abundance of spermatocytes	10	Complete spermatogenesis occurring with numerous spermatozoa present

Statistical analysis: In order to interpret the data with 86% real power at a 95% confidence interval, power assessments were conducted prior to the investigation. Based on $\eta^2 = 0.35$ and effect size f = 0.73, it was decided that a sample size of 11 rats per group would be enough. According to how well the normalcy criteria were met, numerical data were either displayed as a mean and standard deviation or median (range). Count (n) and Percentage (%) were used to display categorical variables. The Shapiro-Wilk Test was used to examine the normality of numerical quantities. For non-parametric test assumptions, the Dunn-Bonferroni Test and the Kruskal-Wallis Test were used, while for parametric test assumptions, the one-way evaluation of variation and the Tukey's Test were used. To investigate the relationship between categorical parameters within the group, the Fisher-Freeman-Halton exact analysis was used. For each of the analyses performed using the Statistical Package for the Social Science (SPSS Inc., Chicago, Illinois, USA) version 22.0, a level of significance of less than or equal to 0.05 was deemed to be statistically meaningful.

RESULTS

Biochemical assessment: Five groups included in this study were compared and significant differences were not identified for TAS (p = 0.095, Kruskal Wallis), TOS (p = 0.67, Kruskal Wallis) and OSI (p = 0.23, ANOVA) values. When comparing the TAS levels between the CP and AMX+CP groups, there was no discernible change (p = 0.49) after doing two-way analyses. Comparing the CP+AMX group to the CP group, however, revealed a significant increase (p = 0.039, Mann-Whitney U) (Table 2).

When the testicular protein amount is examined, differences were not identified when the 5 groups were compared with each other (p = 0.146, Kruskal Wallis). When testicular levels were examined using two-way analyses, the CP+AMX group was shown to have a substantial decrease when compared to the CP group (p = 0.045, Mann-Whitney U), whereas, the CP and AMX+CP groups showed no change (p = 0.53, Mann-Whitney U) (Table 2).

When mitochondrial IDH levels are assessed, significant differences were identified in the analysis of the 5 groups (p = 0.003, ANOVA). When CP and AMX+CP groups were examined, two-way analyses indicated no changes in the levels of mitochondrial IDH (p = 0.392, independent samples t-test), while the CP+AMX group exhibited a significant increase relative to the CP group (p = 0.004, independent samples t-test) (Table 2).

Sperm analysis: There were no discernible changes between the groups when sperm concentrations were compared (p = 0.797, Kruskal Wallis). Sperm motility (p = 0.003) and viability (p = 0.009, Kruskal Wallis) were shown to vary significantly with concentration, in contrast. As compared to the control group, two-way analyses following CP delivery showed substantial drops in both sperm motility (p = 0.002) and sperm viability (p = 0.002) (Mann-Whitney U). Both sperm motility (p = 0.011) and sperm viability (p = 0.025, Mann-Whitney U) significantly increased between rats administered CP and the CP+AMX group. Nonetheless, sperm motility (p = 0.197) and viability (p = 0.113) did not differ significantly between the AMX+CP group and the CP group as compared to the CP group (Table 3).

Table 2: Biochemical investigation

Biochemical variables	Control (n = 11)	AMX (n = 11)	CP (n = 11)	CP+AMX (n = 11)	AMX+CP (n = 11)
TAS (mmol Trolox equivalent L^{-1})	0.169	0.177	0.168	0.182[b]	0.158
TOS (μmol H_2O_2 equivalent L^{-1})	4.61	4.18	4.65	3.80	4.39
OSI (AU)	26.57	23.77	27.19	20.72	27.72
Total testis protein	569.4	550.4	580.1	533.5[b]	628.9
Isocitrate dehydrogenase	0.163	0.150	0.164	0.21 [b]	0.148

Presented values are expressed as the mean, AMX: AmnioMax™, CP: Cisplatin, TAS: Total antioxidant status, TOS: Total oxidant status, OSI: Oxidative stress index and [b]p<0.05 when compared to the CP group

Table 3: Sperm analysis

Sperm parameter	Control (n = 11)	AMX (n = 11)	CP (n = 11)	CP+AMX (n = 11)	AMX+CP (n = 11)
Concentration (10^6 mL^{-1})	101.0±44.2	95.0±42	85.1±33	118.2±106.4	107.7±44.9
Motility (%)	47.0±10.9	48.7±12.9	23.9±15.9[a]	44.7±17.7[b]	33.1±16.1
Viability (%)	68.2±6.1	68.7±8.2	45.7±23.4[a]	64.6±12.5[b]	60.4±13.3

Displayed values represent the Mean±Standard deviation, AMX: AmnioMax™ and CP: Cisplatin, [a]p<0.05 when compared to the control group and [b]p<0.05 in comparison to the CP group

Table 4: Histopathological investigation

Histopathological variables	Control (n = 11)	AMX (n = 11)	CP (n = 11)	CP+AMX (n = 11)	AMX+CP (n = 11)
Johnsen testis biopsy score	9.56±.0.26	9.03±0.26	7.23±0.29[a]	9.04±0.18[b]	8.93±0.14[b]
Fibrosis	0 (0-0)	0 (0-0)	1 (1-2)[a]	0 (0-1)[b]	0 (0-1)[b]

Displayed values represent the Mean±Standard deviation or median (range), AMX: AmnioMax™, CP: Cisplatin, [a] $p<0.05$ when compared to the control group and [b] $p<0.05$ in comparison to the CP group

Histopathological investigation: In the testicular tissue, there were significant differences between the groups during the histological examination in terms of fibrosis ($p<0.001$) and JTBS for measuring spermatogenesis ($p<0.001$, Kruskal Wallis). The two-way analyses showed a substantial rise in fibrosis ($p<0.001$, Mann-Whitney U) and a significant reduction in JTBS in the CP group compared to the control group ($p<0.001$, Mann-Whitney U). Comparing the CP group to the CP+AMX group, there was a substantial rise in JTBS ($p<0.001$) and a significant reduction in fibrosis ($p = 0.001$, Mann-Whitney U). Likewise, as compared to the CP group, the AMX+CP group had a significantly higher level of JTBS ($p<0.001$) and a significantly lower level of fibrosis ($p = 0.002$) (Table 4).

DISCUSSION

The current study aimed to evaluate the potential protective effects of AmnioMax™ (AMX) against cisplatin (CP)-induced testicular damage in rats. The biochemical assessment revealed that the administration of AMX along with CP significantly increased the total antioxidant status and mitochondrial isocitrate dehydrogenase levels and decreased testicular protein amount compared to CP treatment alone. However, no significant differences were observed in the total oxidant status and oxidative stress index levels. In the sperm analysis, CP administration significantly reduced sperm motility and viability, which were ameliorated by the co-administration of AMX. Furthermore, the histopathological investigation showed that AMX administration significantly improved the Johnsen testis biopsy score and reduced fibrosis in the testicular tissue of rats treated with CP. These findings suggest that AMX has protective effects against CP-induced testicular damage in rats.

In the last 20 years, there has been a 75% rise in the survival of younger male patients with cancer due to early detection and more precise evidence-based interventions[15]. Unfortunately, some of these patients require chemotherapy and radiotherapy for cancer treatment. The concept of oncofertility emerged due to degradations occurring in testes linked to chemotherapy and radiotherapy performed for cancer. The concept of oncofertility aims to sustain fertility in patients receiving chemotherapy or radiotherapy. Currently, cryopreservation of sperm is commonly used to sustain fertility in cancer patients. Additionally, a search for alternative solutions has begun to problems like sperm cryopreservation, low fertility rates and genetic anomalies[16].

The adverse effects of antineoplastic drugs often include testis toxicity, which can significantly restrict their clinical utility[17]. One of these antineoplastic medications is CP. The most important factor in using CP for cancer chemotherapy is protection against the side effects that CP may cause[18].

The CP alkylates DNA by creating intra-strand DNA crosslinks. In this way, interventions occur to DNA repair mechanisms and thus, p53 activation and cessation of the cell cycle induce apoptosis. Reactive oxygen species (ROS) include oxygen and nitrogen-reactive species created in mitochondria during regular cellular activity[19]. When ROS levels are too high, the mitochondrial membrane may alter, which then results in the creation of additional ROS[20]. By creating large levels of ROS, CP decreases the action of antioxidants. It also enhances lipid peroxidation, denatures structural proteins and leads to cell apoptosis[21]. Due to its high metabolic activity and substantial concentrations of highly unsaturated lipid acids, testis tissue is particularly vulnerable to oxidative stress[22]. In light of this information, the idea that antioxidants may reduce testicular toxicity arose and research about the role of antioxidants gained speed.

Gibco® AmnioMax™-II Complete Media has been specifically designed and authorized for the *in vitro* expansion of primary cultures of human amniotic fluid cells and chorionic villus samples, which are commonly utilized for prenatal diagnostic tests. The effectiveness of this medium has been demonstrated in various research studies conducted previously[23-26]. AmnioMax™ is comprised of key components, such as fetal bovine serum (FBS), gentamicin and L-glutamine, which facilitate the adherence and optimum growth of cells. The glutamine in the structure was shown to have protective effects against cellular toxicity and testis toxicity[27]. Another study showed that AMX suppressed oxidative stress and reduced stimulation of the antioxidant system in ischemia-reperfusion injury in rat testes and prevented morphological injury in this way[28].

Prooxidants producing ROS and antioxidants scavenging ROS are balanced in normal cells[29]. However, the toxicity induced by CP disrupts this balance toward the oxidative side[30]. In our study, TOS was used as a marker for prooxidant molecules and TAS was used as a marker for antioxidant molecules. At the same time, OSI (TOS/TAS) was assessed. Mirapoglu and colleagues previously investigated the impact of AMX administration on tracheal healing in rats and reported significant differences in TAS, TOS and OSI values compared to a control group[8]. In this study, the potential protective effects of AmnioMax™ (AMX) on cisplatin (CP)-induced testicular toxicity in rats were investigated. The results showed that there were no significant variations in total antioxidant status (TAS), total oxidant status (TOS) and oxidative stress index (OSI) values after CP administration. However, a significant increase in TAS was identified with the administration of AMX after CP. This result shows that AMX created a barrier to toxicity induced by CP by increasing antioxidant production.

Sperm concentration, viability and motility are important markers of sperm quality. Disrupted sperm quality is one of the most important markers of infertility. The ROS reduces sperm motility by reducing mitochondrial membrane potential in sperm cells[31]. Additionally, damage to the sperm cell membrane by ROS due to CP may cause a reduction in sperm viability[32]. Current results showed that CP significantly reduced sperm viability and motility. This result was parallel to the studies showing reduced sperm motility in animals following spermatogenic injury and germ cell apoptosis linked to CP treatment[33-35]. The present study suggested that the administration of AMX following CP treatment might have a beneficial impact on male fertility, as it appears to increase sperm viability and motility.

Following any insult or injury to the testis, the organization and arrangement of the cells in the testicular tubules may become disrupted or disturbed for a certain period of time. This disruption can interfere with the process of spermatogenesis, which ultimately affects male fertility. To assess the degree of injury or disruption to the tubules, the Johnsen testicular biopsy score (JTBS) is used as a measure. In the current study, the JTBS was used to assess testis tubules[14]. After CP, a significant reduction was observed in JTBS, consistent with other studies in the literature[36-38]. Patients administered AMX before and after CP was observed to have higher JTBS.

In this study investigation, a noticeable rise in fibrosis in the testicular tissue was observed as a result of the administration of CP. In the literature, similar studies observed that CP increased testicular fibrosis[29,30,39,40]. The AMX administration, whether before or after CP administration, was shown to partially reduce the formation of testicular fibrosis.

When the testicular protein amount is examined, significant changes were observed with CP administration, while a reduction in testicular protein amount was observed in patients receiving AMX after CP. In contrast to present research, Afsar *et al.*[41] reported a notable decline in testicular protein levels in rats treated with CP in comparison to the control group. This result was attributed to impaired spermatogenesis caused by CP according to their study.

The IDH, one of the testicular mitochondrial enzymes, is a marker of testicular mitochondrial function and oxidative stress status. It is known that mitochondrial dysfunction may affect testis functions[42]. In the current study, the administration of AMX after CP in rats significantly increased IDH levels. After AMX administration, the increase in mitochondrial IDH enzyme activity may be due to more rapid enzyme synthesis, reduced metabolite accumulation and prevention of binding of toxic agents to the active region of enzymes[43].

The lack of determination of changes occurring in the dimensions and weights of the testis between the groups may be assessed as a limitation of the study. Similarly, the lack of testicular mitochondrial enzyme analysis, apart from IDH, is a limitation of the current study.

The current study has significant implications for the field of oncofertility, particularly in the development of strategies for the protection of male reproductive health in cancer patients receiving chemotherapy. The findings of this study suggest that the administration of AMX after CP treatment may be beneficial in preventing testicular damage and improving sperm quality in male rats. These results may have implications for the development of similar therapies for human patients, which may help to reduce the risk of infertility and improve overall quality of life.

The application of AMX in this study highlights its potential use as a protective agent against testicular toxicity induced by CP. The results of this study may encourage further research to explore the potential benefits of AMX for protecting reproductive health in male cancer patients receiving chemotherapy.

Future recommendations for this study include the evaluation of the potential effects of AMX on other testicular enzymes and biomarkers, as well as the assessment of its long-term effects on reproductive health in male rats. Furthermore, the results of this study may be used as a basis for the development of clinical trials to evaluate the efficacy of AMX for the protection of reproductive health in male cancer patients receiving chemotherapy.

CONCLUSION

The CP in rats induces oxidative stress with increased oxidant production and reduced antioxidant activity. Exposure to CP can result in damage to the testis tissue and a decrease in the quantity and quality of sperm, including reductions in sperm count, motility and viability. However, in our study, AmnioMax was found to have protective effects on the testis against CP-induced toxicity, as it possesses anti-inflammatory and antioxidant properties that prevented the aforementioned side effects and resulted in an improvement in sperm quality. This is the first report in the scientific literature to demonstrate such beneficial effects of AmnioMax on testicular health. When compared in terms of whether the AMX injection should be administered before or after CP, the CP+AMX group had higher TAS levels and higher IDH levels, sperm viability and motility and Johnsen testis biopsy score compared to the AMX+CP group. Considering this, we believe that the administration of AMX after CP is more appropriate.

SIGNIFICANCE STATEMENT

This study provides critical insights into the protective effects of AmnioMax™ (AMX) against testicular toxicity induced by cisplatin (CP) in rats, highlighting its potential role as a fertility-preserving agent for cancer patients undergoing chemotherapy. The results demonstrate that AMX administration, particularly when given after CP treatment, significantly enhances antioxidant activity, improves sperm quality and mitigates the detrimental impact of CP on testicular tissue. These findings contribute to the growing body of research on fertility preservation strategies for cancer patients and emphasize the potential therapeutic value of AMX in safeguarding fertility during chemotherapy involving CP.

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