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Histochemical and morphometric evidences of the curative role of aqueous zest extract of Citrus sinensis on anti-neoplastic drug-induced testicular degeneration in animal models

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SUMMARY

This study examined the curative effects of aqueous zest extract of *Citrus sinensis* and Cisplatin (CIS)-induced testicular degeneration. Sixteen male Wistar rats (10 to 12 weeks old) weighing 306-238 g were used in this study. The animals were divided as follows: Group A was treated orally with 2.5 ml/kg body weight/daily; Group B was treated with a single dose of 10 mg/kg body weight; Group C and D rats were given a single dose of 10 mg/kg body weight of cisplatin and then treated orally with 10 and 40 mg/kg body weight of aqueous zest extract of *Citrus sinensis*. The procedure lasted for 8 weeks.

Results showed a significant ($p< 0.05$) decrease in final body weight, testis weight, testis weight/ body weight ratio, normal sperm morphology ($p<0.01$) and a significant decrease in tubular diameter ($p > 0.01$), perimeter ($p > 0.01$ and) and length ($p > 0.001$), width ($p >0.05$) and increase ($p > 0.05$), germinal epithelia height, cross-sectional area, number of profiles per unit area and numerical density of seminiferous tubules. Rats that were treated with CIS alone without pre-treatment or post-treatment with extract showed marked degeneration and atrophied seminiferous tubules with absence of late stage germ cells. There was also a reduction in PAS-positive materials of the rats treated with Cisplatin. These parameters were however ameliorated in the groups that were post-treated with the aqueous zest extract of *Citrus sinensis*. This could have been as a result of its antioxidant and free radical scavenging potentials.

Key words: *Citrus sinensis* peel – Cisplatin – Testes – Oxidative stress – Immunohistochemistry – Infertility

INTRODUCTION

Cisplatin (platinum-based drug) is one of the major standard antineoplastic drugs used in cancer therapy such as testicular, head-and-neck, ovarian and cervical carcinomas (Ravindra et al., 2010; Mashhadi et al., 2013). It was the first member of a class of platinum-containing anti-cancer drugs, which now also includes carboplatin and oxaliplatin, and its discovery was a cornerstone that triggered the interest in other metal-containing compounds as potential anticancer drugs (Desoize and Madoulet, 2002; Che and Siu, 2010).

Despite its numerous benefits, much has been reported on its side effects, which include testicular toxicity (Townsend et al., 2003; Yao et al., 2007; Sqhni et al., 2009), and these have limited its usage (Giaccone, 2000). Cisplatin is restricted to a moderately narrow variety of tumor types, as some

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tumors (colorectal and non-small cell lung cancers) have intrinsic resistance to cisplatin, while others (ovarian or small cell lung cancer) increase acquired resistance after the initial treatment (Fuertes et al., 2003; Mohamad et al., 2014).

Studies have shown that free radical generation via an oxidative stress pathway was the main factor for cisplatin-induced testicular toxicity. The mechanisms of cisplatin toxicity involve the binding to DNA and non-DNA targets, and induction of apoptosis, necrosis or both (Cvitkovic, 1998). The final cellular outcome is generally apoptotic cell death (Keland, 2007), although the pathway(s) from platinum–DNA binding to apoptosis remains incompletely elucidated (Keland, 2007).

Indeed, sperm DNA damage has been reported in post-cancer treatment in both fertile and infertile men (Morris, 2002). In animal models, such DNA damage after genotoxic treatment has been associated with an increased number of pathological pregnancies.

Citrus is one of the most important fruit crops grown in the world (Tao et al., 2007). The citrus family boasts of rich phytochemicals such as flavanones, polyphenols, anthocyanins and hydroxycinnamic acids. *Citrus sinensis* peel is waste material, obtained after extraction of juice from citrus fruit. It has been reported to contain higher amounts of total phenolics such as limonene, hesperidin, narirutin, naringin and eriocitrin compared to the edible portions (Bok et al., 1999; Ziaur, 2006; Xu et al., 2008; Green et al., 2013; Nada et al., 2014). Several studies have reported their pharmacological activity as radical scavengers.

The aim of this study was to evaluate in animal models, the role of aqueous zest extract of *Citrus sinensis* (AZECS) on cisplatin-induced testicular degeneration using histomorphometric, histochemical, immunohistochemical, biochemical and physiological approaches.

MATERIALS AND METHODS

Preparation of aqueous zest extracts of Citrus sinensis (AZECS)

Five (500) hundred orange fruits were peeled with a zester or grater. The white portion of the peel under the zest (mesocarp) was generally avoided by limiting the peeling depth (Liogier, 1988). The zest was thoroughly rinsed in distilled water and dried at room temperature for about 2 weeks. It was then reduced to a powdered form by grinding. The aqueous extraction was done as described by Saalu et al. (2011). Briefly, the powdered sample was mixed with a calculated volume of distilled water and allowed to stand for 30 minutes before filtration. It was then centrifuged at about 3000 rmp for 5 min and the supernatant collected. The supernatants were cleaned of particles by suction filtration using Whatmann no 1 filter paper and cellulose filter paper. The extracts were subsequently concentrated to dryness *in vacuo* at 40oC using a rotary evaporator and stored in a desiccator. A fresh solution of the different extracts was prepared in normal saline as vehicle when required.

Experimental procedure

Twenty male Wistar rats (10-12 weeks old) weighing 230-306 g were divided into five groups containing four rats each. The rats in group A served as the negative control group and were treated orally (using gavage needle) with 2.5 ml/kg body weight of distilled water/daily, group B rats were treated with 40 mg/kg body weight of AZECS. To induce testicular degeneration, Group C rats were treated intraperitoneally with a single dose (10 mg/kg body weight) of CIS **(**Cherry et al., 2004). Group D and E rats after induction of testicular degeneration as done with group C were post-treated (orally) with 10 and 40 mg/kg body weight of AZECS respectively. The procedure lasted for 8 weeks (Duration of spermatogenesis in rat being 51.6-56 days (Jegou et al., 2002).

Animal sacrifice and sample collection

The rats were first weighed and then sacrificed by cervical dislocation. The testicular weights of each animal were evaluated with an electronic analytical and precision balance. The testes volumes were measured by water displacement method. The two testes of each rat were measured, and the average value obtained for each of the two parameters was regarded as one observation. One of the testes of each animal was fixed in Bouin's fluid for histological and morphometric analysis. Serum and the remaining testes of each animal were stored at -25°C for subsequent biochemical assays.

Tissue preparation for histology and histochemistry

The organs were processed for Haematoxyline and Eosin as described by Akunna et al. (2012) for histochemical study. Sections were stained with Periodic Acid-Schiff (PAS) reaction with hematoxylin counterstaining as described by Sheehan and Hrapchak (1987).

The slides were viewed under an integrated digital microscope (DN-117M, Zhejiang, China) with 1280x1024 resolution, connected to a computer monitor for qualitative and quantitative evaluation.

Determination of morphometric parameters

Histological slides were prepared from the formol -saline fixed testes. However, before embedding, it was ensured that the sections were placed perpendicular to their long axes, and chosen as "vertical sections".

For each testis, seven "vertical sections" from the polar and the equatorial regions were sampled (Qin and Lung, 2002). Seven "vertical sections" per testis were selected by a systematic sampling method that ensured fair distribution between the polar and equatorial regions of each testis. Briefly, a section was taken at the equator of each testis; one on each side of the equator, three quarters of the distance between the pole and the equator;

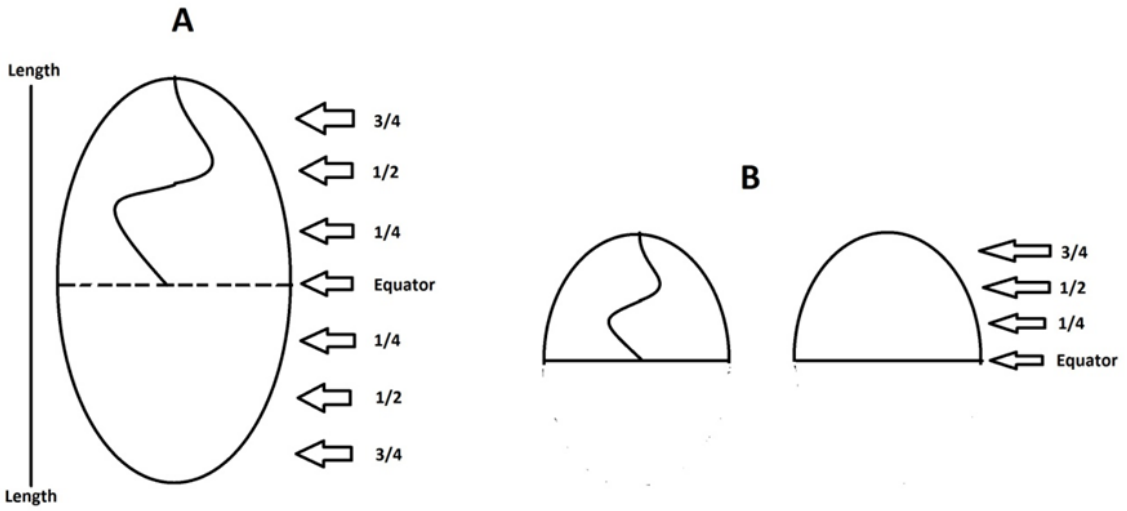

Fig 1. A) Schematic showing the longitudinal axis along which the length measurement was taken, where each testes was cut in two (------) and the position of sections to be taken (⇦) .**B**) Arrangement of the testes halves in the paraffin block and the position of the sections taken (⇦).

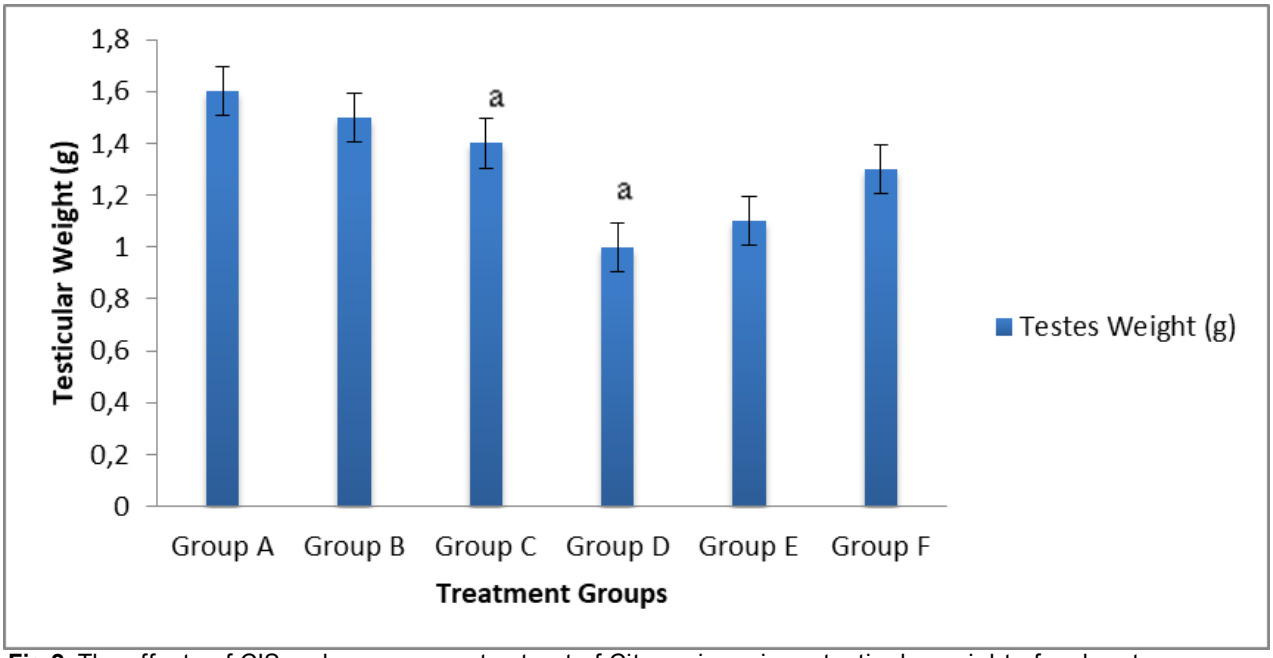

Fig 2. The effects of CIS and aqueous zest extract of Citrus sinensis on testicular weight of male rats.

a,b represent significant increases or decreases at $p < 0.05$ when compared to negative control (Group A) and positive control (Group B) respectively. Values are means ± SD. n = 4 in each group.Group A rat (5 ml/kg NS), Group B rats (10 mg/kg AZECS), Group C rats (40 mg/kg AZECS), group D rats (10 mg/kg CIS), group E rats (10mg/kg AZECS+ 10mg/kg CIS) and group F rats (40mg/kg AZECS+ 10mg/kg CIS) for 8 weeks.

another half-way between each pole and the equator; and one on each side of the equator, a quarter of the distance from each of the pole (Fig. 1).

Diameter, perimeter, length, width, roundness, lumen diameter and germinal epithelia height of seminiferous tubules of the testes were estimated with a digimizer software programme. Unbiased numerical estimation of the following morphometric parameters was determined using a systematic random scheme (Gundersen and Jenson, 1987): cross-sectional area of the seminiferous tubules (AC); number of profiles of seminiferous tubules per unit area of testis (NA); and numerical density of the seminiferous tubules (NV) were determined.

For each stereological parameter (*D*, *AC*, *NA* and *NV*), five randomly selected fields from all the seven sections of a single testis was viewed, and estimation on each carried out. The average from a total of seventy readings from five fields in seven sections of the two testes of one rat was obtained and this was recorded as one observation (Akunna et al., 2012).

Estimation of volume density of testicular components and number of seminiferous tubules was

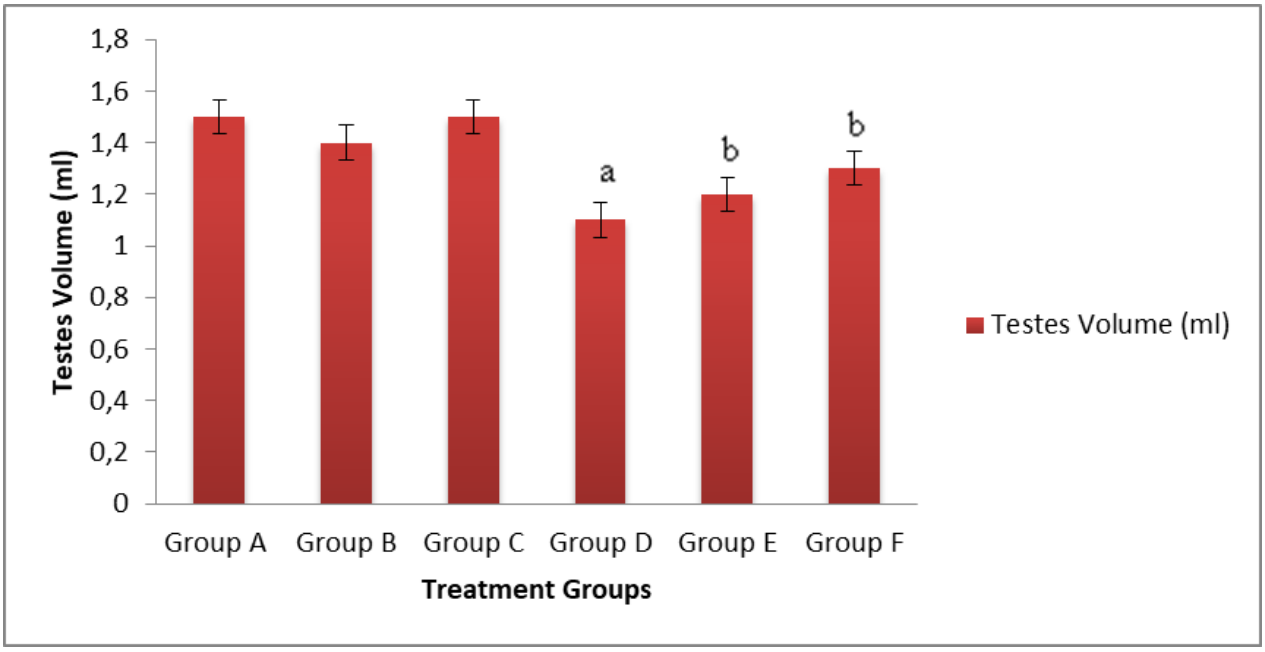

Fig 3. The Effects of CIS and aqueous zest extract of Citrus sinensis on volume of male rats.

a,b represent significant increases or decreases at $p < 0.05$ when compared to negative control (Group A) and positive control (Group B) respectively. Values are means ± SD. n = 4 in each group. Group A rat (5 ml/kg NS), Group B rats (10 mg/kg AZECS), Group C rats (40 mg/kg AZECS), group D rats (10 mg/kg CIS), group E rats (10mg/kg AZECS+ 10mg/kg CIS) and group F rats (40mg/kg AZECS+ 10mg/kg CIS) for 8 weeks. Values are means ± SD. n = 4 in each group.

done on a computer monitor onto which a graph sheet was superimposed and on which slides were projected from an integrated digital microscope (DN-117M, Zhejiang, China) with 1280x1024 resolutions.

Determination of epididymal sperm morphology

Briefly, the testes from each rat were exposed, removed and were trimmed free of the epididymides and adjoining tissues. From each epididymis, the caudal part was cut and placed in a beaker containing physiological saline solution. Each section was quickly macerated with a pair of sharp scissors and left for a few minutes to liberate its spermatozoa into the saline solution. Sperm morphology was determined as earlier described by Saalu et al. (2011) and Akunna et al. (2012). Caudal sperm was diluted 1:20 with 10% neutral buffered formalin (Sigma-Aldrich, Oakville, ON, Canada), placed on slides covered with a cover slip and examined under the microscope using x 400 objective. Five hundred spermatozoa from the sample were scored for morphological abnormalities. Briefly, in wet preparations using phase-contrast optics, spermatozoa were categorized. In this study a spermatozoon was considered abnormal morphologically if it had one or more of the following features: rudimentary tail, round head and detached head and was expressed as a percentage of morphologically normal sperm.

Statistical analysis

The data were statistically analyzed and expressed as Mean ± SD. Analysis was carried out using analysis of variance (ANOVA) with Scheffe's post hoc test. The level of significance was considered at $p < 0.05$.

RESULTS

Gross anatomical parameters

The animals treated with CIS-alone had a significant ($p<0.05$) decrease in testis weight, testis weight/body weight ratio and testes volume when compared to the negative control group (Group A) (Fig. 3).

There was a significant decrease in the testes weight of rats treated alone with AZECS when compared to that of the negative control (Fig. 2). However, the testes volume of rats treated alone with AZECS showed values comparable to that of the negative control (Fig. 2).

There was a significant ($p<0.05$) increase in testis weight and testis volume of rat treated with AZECS after CIS exposure when compared to the positive control (Group D) (Fig. 3).

Testes histological profiles

The cross-sections of the seminiferous tubules of the control group and those treated alone with AZECS were moderately circular or oval in outline, with normal seminiferous epithelium and numerous spermatozoa within their lumen (Fig. 4).

Rats that were treated with CIS alone showed marked degeneration and atrophied seminiferous tubules, interstitial edema, degenerated and vacuolated germinal epithelium, absence of late stage germ cells, degenerated spermatogenic cells and absence of sperm bundles in most tubules (Fig. 4).

The rats that had AZECS after CIS showed a

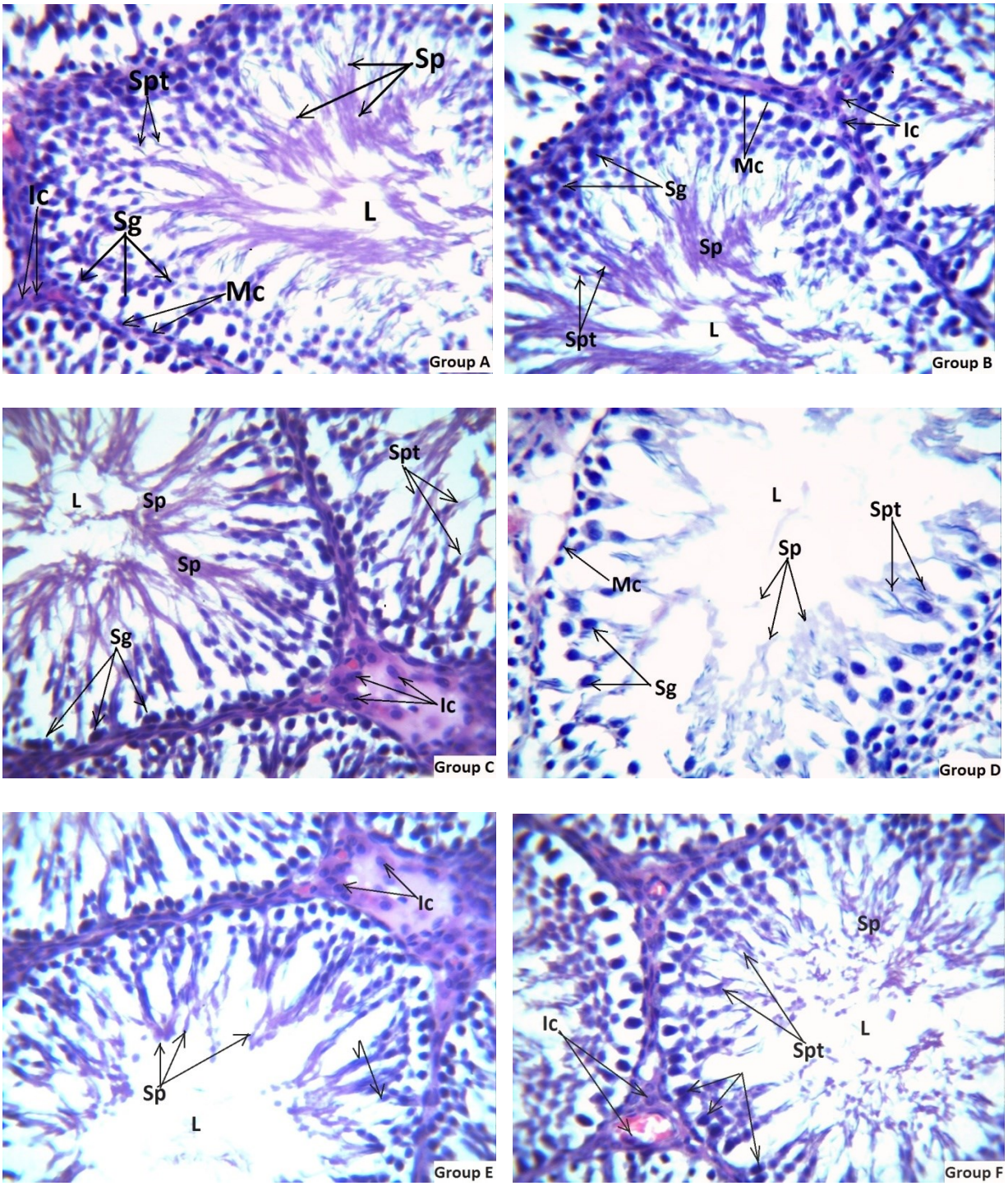

Fig 4. Histological micrograph of a cross-section of testis of Group A rat (5 ml/kg NS), Group B rats (10 mg/kg AZECS), Group C rats (40 mg/kg AZECS), group D rats (10 mg/kg CIS), group E rats (10mg/kg AZECS+ 10mg/kg CIS) and group F rats (40mg/kg AZECS+ 10mg/kg CIS) for 8 weeks. Stain: H&E. Slide showing the L: Lumen, Sp: Spermatozoa, Sg: Spermatogonia, Spt: Spermatid, Ic: Interstitial cells, Mc: Myoid cell. Magnification: x 400.

remarkable preservation of their seminiferous epithelium (Fig. 4).

Testes histochemical profiles

Polysaccharides

Strong PAS-positive materials appeared in the *tunica albuginea,* as well as in the intertubular connective tissue of the testes of those rats treated alone with AZECS, as well as rats in the negative control group (Fig. 5). It showed normal cytoplasmic carbohydrate supplement in nearly all of the cells which were participated in spermatogenesis process. Accordingly, the Sertoli cells were manifested with well PAS stained cytoplasm showing normal carbohydrate supplement. Also the cells in

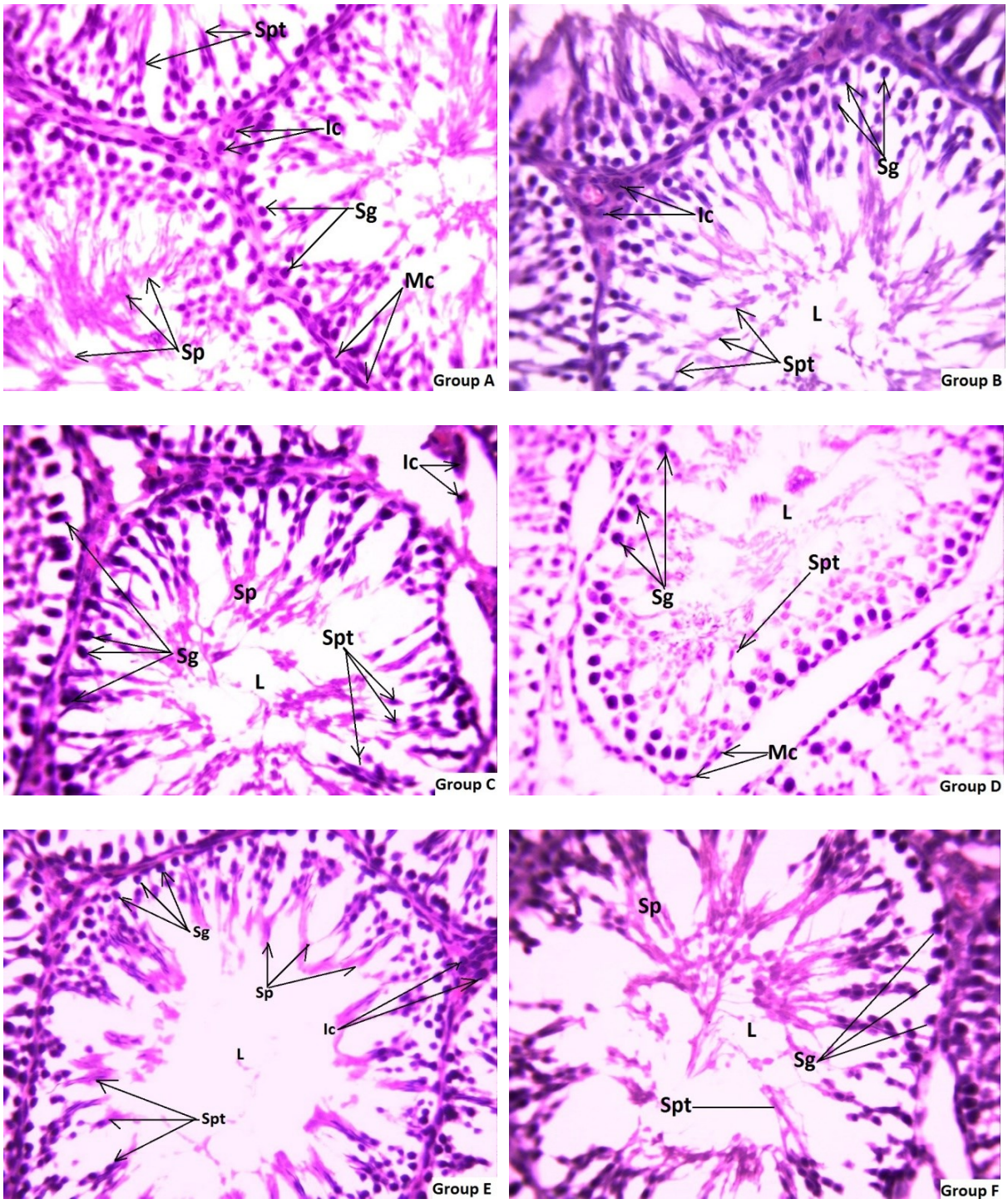

Fig 5. Histochemical micrograph of a cross-section of testis of Group A rat (5 ml/kg NS), Group B rats (10 mg/kg AZECS), Group C rats (40 mg/kg AZECS), group D rats (10 mg/kg CIS), group E rats (10mg/kg AZECS+ 10mg/kg CIS) and group F rats (40mg/kg AZECS+ 10mg/kg CIS) for 8 weeks. Stain: H&E. Slide showing the L: Lumen, Sp: Spermatozoa, Sg: Spermatogonia, Spt: Spermatid, Ic: Interstitial cells, Mc: Myoid cell. Magnification: x 400.

spermiogenesis process were observed with normal lipid foci accumulation with low reaction to PAS.

The testes of rats treated with CIS alone revealed a decrease of PAS-positive materials. In these specimens, *tunica albuginea*, the boundaries of the seminiferous tubules as well as the intertubular connective tissue had weak PAS-positive materials (Fig. 5).

More or less normal polysaccharides content was illustrated after post-treatment with AZECS (Fig. 5). However, the spermatogenic cells exhibited weak reaction while the sperms showed strong reaction.

Testes histo-morphometry

The mean diameter, perimeter and length of the seminiferous tubules in the negative control rats

Table 1. Effects of cisplatin and aqueous zest extract of Citrus sinensis on Diameter (D), perimeter of seminiferous tubules (PST) cross-sectional area (Ac), number of profiles per unit area (NA) and length of seminiferous tubules (LST) of Wistar rats.

Treatment Groups	D (µm)	PST (µm)	Ac (x 10^3µm^3)	N_A (x10^{-8} µm^{-2})	LST (µm)
GROUP A	278.5 ± 91.6	900.4 ± 121.0	23.62 ± 1.12	27.11 ± 8.13	342.7 ± 72.3
GROUP B	256.2 ± 31.6[a]	910.1 ± 113.0	24.12 ± 2.21	28.31 ± 5.11	351.1 ± 71.1
GROUP C	251.1 ± 11.1[a]	913.2 ± 141.1	24.23 ± 2.21	27.31 ± 6.11	347.1 ± 41.1
GROUP D	206.7 ± 32.0[a]	611.9 ± 149.4[a]	15.63 ± 6.1[a]	12.16 ± 5.1[b]	328.7 ± 57.2[a]
GROUP E	263.6 ± 9.72[b]	871.7 ± 25.3[b]	32.13 ± 0.33[b]	31.46 ± 5.03[b]	393.0 ± 6.22[b]
GROUP F	251.2 ± 9.6[b]	817.5 ± 83.3[b]	38.8 ± 6.33[b]	36.26 ± 6.9[b]	355.7 ± 9.3[b]

a,b represent significant increases or decreases at p < 0.05 when compared to negative control (Group A) and positive control (Group B) respectively. Values are means ± SD. n = 4 in each group.

Table 2. Effects of cisplatin and aqueous zest extract of Citrus sinensis on width (WST), roundness (RST) germinal height (GEH), numerical density (NV) and lumen diameter (LD) of seminiferous tubules of Wistar rats.

Treatment Groups	WST (µm)	RST (µm)	GEH (µm)	N_V (x10^{-10}µm^{-2})	LD (µm)
GROUP A	232.7 ± 24.6	0.84 ± 0.1	33.4 ± 3.23	12.06 ± 3.2	87.9± 10.9
GROUP B	233.1 ± 14.1	0.82 ± 0.1	34.1 ± 2.21	12.14 ± 2.1	86.4± 11.2
GROUP C	231.3 ± 21.1	0.83 ± 0.1	32.1 ± 3.11	11.12 ± 3.3	88.6± 11.3
GROUP D	200.9 ± 31.6[a]	0.88±0.1	3.8 ± 1.8[a]	10.07 ± 3.3[a]	190.4± 59.6[a]
GROUP E	276.5 ± 11.73[b]	0.94 ± 0.0	33.4 ± 3.03[b]	10.37 ± 0.5	151.3± 45.43[b]
GROUP F	230.0 ± 33.0[b]	0.91 ± 0.1	25.3 ± 2.8[b]	17.16 ± 4.9	87.2± 32.33[b]

a,b represent significant increases or decreases at p < 0.05 when compared to negative control (Group A) and positive control (Group B) respectively. Values are means ± SD. n = 4 in each group.

were 278.5 ± 91.6 µm, 900.4 ± 121.0 µm and 342.7 ± 72.3 µm, respectively (Table 1). The group that had CIS-alone had a significant decrease in tubular diameter (p<0.05), perimeter (p< 0.05 and) and length of the seminiferous tubules (p<0.05) when compared to the negative control. However, the animals that were treated with AZECS after exposure to CIS showed significant changes in their tubular diameter when compared to the positive control (Table 1).

The width, roundness and lumen diameter of the seminiferous tubules in the control rats were 232.7 ± 24.6 µm, 0.84 ± 0.1 µm and 87.9± 10.9 µm respectively (Table 2).

There was a significant reduction (p<0.05) in width and increase (p<0.05) in lumen diameter of the tubules in rats treated with CIS (200.9 ± 31.6and 190.4± 59.6 µm respectively) (Table 2).

The animals that had AZECS after CIS treatment had significant increase in width, and lumen diameter of the tubules when compared to the positive control group.

The mean numbers of germinal epithelia height, cross-sectional area, number of profiles per unit area and numerical density of seminiferous tubules in negative control groups were 25.3 **±** 2.8 µm, 38.8 **±** 6.33 Ac (x103 µm3), 36.26 **±** 6.9 NA (x10-8 µm-2) and 17.16 **±** 4.9 (x10-10 µm-2) respectively (Table 3).

However, there was a significant (p<0.05) increase in germinal height, cross-sectional area, number of profiles per unit area and numerical density in rats treated with AZECS after CIS when compared to the positive control (Table 2).

Sperm parameters

Sperm Morphology in Wistar rats

Results show a significant (p<0.05) decrease in normal sperm morphology and increase in abnormal sperm morphology in rats treated with CIS alone when compared to the negative control. Although there was a significant (p<0.05) decrease in normal sperm morphology and decrease in abnormal sperm morphology in rats treated AZECS after CIS treatment, these values were comparable to those of the negative control group (Fig. 6).

DISCUSSION

Cancer treatment usually comes with diverse side effects (Jordan and Carmo-Fonseca, 2000; Cohen and Lippard, 2001; Cepeda et al., 2007; Maghous et al., 2017). Spermatogenic cells are targeted partly because of their high mitotic activities (Atessahin et al., 2006).

In this study, we observed a significant (p<0.05) decrease in testicular weight and testicular weight/ body weight ratio, but not testicular volumes of rat treated with CIS-alone. Our result is in accordance with that of Rekha et al. (2011), who state that derangement in some testicular structures such as seminiferous tubules and leydig cells could lead to about loss of 70% to 80% of testicular mass (Setchell and Brooks, 1998). Also, Arai et al. (1998) have suggested a possible correlation be-

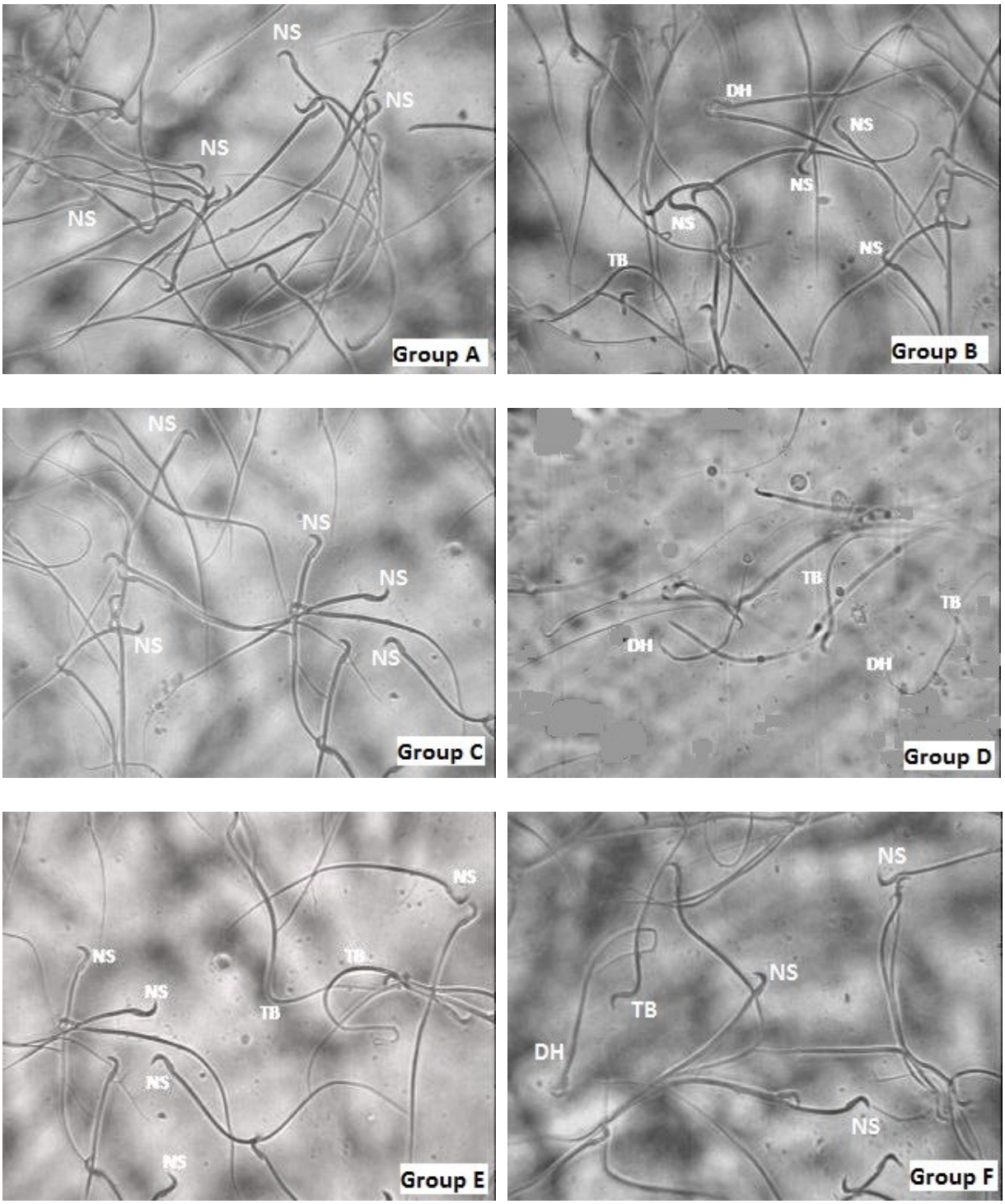

Fig 6. Sperm Morphology of Group A rat (5 ml/kg NS), Group B rats (10 mg/kg AZECS), Group C rats (40 mg/kg AZECS), group D rats (10 mg/kg CIS), group E rats (10mg/kg AZECS+ 10mg/kg CIS) and group F rats (40mg/kg AZECS+ 10mg/kg CIS) for 8 weeks showing DH: Detached, NS: Normal sperm, TB: Twisted body at X400. No Stain was used.

tween testicular volume and testicular function which was also observed in this present study.

In a study to determine the role of Mesenchymal stem cells in cisplatin-induced testicular toxicity in rats and the protective effect of Arjunolic acid in cisplatin-Induced testicular toxicity in rats, Iman et al. (2014, 2016) reported a significant decrease in testicular weight.

Reductions of testicular weights in CIS-control animals shown in this study have also been reported by Malarvizhi and Mathur (1995) and Majid (2015). This could be due to marked parenchymal atrophy and severe degeneration, necrosis, and reductions in seminiferous tubule and germinal cell thickness seen in the testes of rats treated with CIS-alone. This is line with studies done by Iman

Table 3. Effects of cisplatin and aqueous zest extract of Citrus sinensis on sperm morphology of Wistar rats.

Treatment	Abnormal sperm	Normal sperm
Group A	12.5 ± 1.3	87.5 ± 2.1
Group B	8.5 ± 1.1[a]	91.5 ± 1.1[a]
Group C	10.5 ± 0.4[a]	89.5 ± 1.2[a]
Group D	71.5 ± 0.1[a]	28.5 ± 0.3[a]
Group E	36.2 ± 0.3[b]	63.8 ± 0.1[b]
Group F	21.6 ± 3.1[b]	78.4 ± 1.5[b]

a,b represent significant increases or decreases at $p < 0.05$ when compared to negative control (Group A) and positive control (Group B) respectively. Values are means ± SD. n = 4 in each group.

et al. (2016) and Fallahzadeh et al. (2017).

The effects of CIS on the testis may be due to their specific toxic effects on the target organ and not the result of their general toxicity. However, the groups of rat dosed with AZECS after CIS showed a remarkable improvement in testis weights, testis weight/body weight ratio.

Marked degeneration and atrophied seminiferous tubules, interstitial edema, degenerated and vacuolated germinal epithelium were observed in group of rats that received CIS alone. This agrees with the findings by many investigators (Xu et al., 1993; Chia et al., 1994; Zhang et al., 2001; Cherry et al., 2004). Atessahin et al. (2006) provide well-documented evidence of testicular morphologic and morphometric impairment following CIS challenge in animal models. As also observed in this study, there was a significant decrease in tubular diameter, perimeter, width, length, germinal height, cross-sectional area, number of profiles per unit area, numerical density and increase ($p<0.05$) in lumen diameter of the tubules in animals treated with CIS alone when compared to the negative control. Reduced seminiferous tubular diameters, depleted germ cells and irregular small seminiferous tubules with Sertoli cells post-cisplatin treatment were also reported by Iman et al. (2014).

CIS has been reported to cause apoptosis to testicular germ cells and Sertoli cells (Cherry et al., 2004). The resultant damage was also associated with upregulation of p53 expression. Elevation of p53 protein expression in response to DNA damage triggers either a transient cell cycle arrest or apoptosis (Gomez-Lazaro et al., 2004; Szoke et al., 2005). Sperm responds to exposure to a DNA-damaging agent by stirring p53 protein levels (Wang et al., 2006). It is therefore suggested here that p53 is an essential component in the CIS-mediated apoptotic pathway of testicular epithelia.

As was the case with the weights and for probably similar reasons, post-treatment with AZECS showed a remarkable improvement in the histomorphometric parameters.

Spermatogenic cells constitute one of the body tissues that are susceptible to CIS-induced oxidative stress. CIS toxicity by intracellular generation of free radicals and reactive oxygen species, along with intercalation with DNA and subsequent inhibition of topoisomerase (Hrdina et al., 2000). This increase oxidative stress damages the sperm membranes, proteins and DNA (Kirsi and Timo, 2001; Kalender and Yel, 2005).

This could explain the significant ($p<0.05$) normal sperm morphology, along with a significantly ($p<0.05$) increased abnormal sperm morphology rates in CIS alone group rats when compared to the control groups. This result is in line with previous reports that demonstrated testicular impairment (Garside and Harvey, 1992; Lafuente and Esquifino, 2000; Atessahin et al., 2006; Silici et al., 2009). Foote (1999), Iman et al. (2014) and Fallahzadeh et al. (2017) also reported decrease in progressive motility, sperm normality and testicular toxicity.

This effect may be attributed to testicular germ cell destruction and the toxic effects of these agents on the flagellum. It also has been reported that adenosine triphosphate (ATP) is an energy source for sperm motility, and its availability may be a limiting factor responsible for loss of sperm motility in CIS-treated rats (Vernet et al., 2004; Leon et al., 2005). Worthy of note is also the remarkable normalization of these parameters in the groups of rats that were post-treated with AZECS.

Studies have shown that CIS results in direct oxidative injury to DNA. The biochemical mechanism by CIS causes cytotoxicity is currently unclear. However, several mechanisms have been postulated to account for the effects of CIS, both in terms of anticancer potential and adverse effects (Hrdina et al., 2000; Kirsi and Timo, 2001; Kalender and Yel, 2005; Ronald et al., 2010).

CIS is known to generate free radicals either by the enzymatic pathway of redox cycling between a semiquinone form and a quinone form or by the non-enzymatic pathway of forming a DOX-Fe3+ complex (Davies and Doroshow, 1986; Cepeda et al., 2007).

Post treatment with AZECS helped to overcome the oxidative stress as demonstrated by the moderation of these biochemical markers. AZECS potent antioxidants could have attenuated the CC and CIS testicular derangement through a reduction of free radicals dependent lipid peroxidation.

Biochemical analysis of AZECS revealed the presence in high concentration of vitamin C, vitamin E, lycopene and polyphenolic flavonone glycosides, hesperiden, neohesperiden, nariratin, limonoid and naringin. These compounds are powerful antioxidants and free radical scavengers individually and collectively (Das et al., 2002; Yeh and Yen, 2003; Ateşşahin et al., 2006; Yusuf et al., 2009).

The normalization of the biochemical parameters in the group post-treated with AZECS could be due to the free radical scavenging activities of the various potent antioxidant components of these extracts, as reported by Ahmad et al. (2013), Ia et al. (2014) and Kais et al. (2017).

This study has provided evidence of the efficacy of aqueous zest extract of *Citrus sinensis* to protect and attenuate the testicular toxicity associated with cisplatin chemotherapy and testicular neoplasm.

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