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Experimental dapsone administration induces infertility in male Wistar rats: Mechanisms and clinical implications

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ABSTRACT

Dapsone (4, 4′-diaminodiphenylsulfone, DDS) is a potent anti-inflammatory and antibacterial compound which has been used in the treatment of leprosy, vasculitis and dermatitis herpetiformis, lupus erythematosus profundus and even as an antimalarial in combination with proguanil. This study investigated the effect of the administration of dapsone on the reproductive activities of male rats using in vivo and in vitro techniques. In the in vivo study, dapsone was administered orally to male Wistar rats for 5 days or 6 weeks after which their body weight, relative reproductive organ weights, sperm parameters and reproductive hormones were determined while testicular and epididymal histology were also assessed. Data were compared using analysis of variance and Students-Newman-Keuls multiple comparison test. For the in vitro study, Sertoli cells were cultured and treated with varying doses of dapsone at different durations, thereafter Sertoli cell viability and nuclei integrity were determined. Also, the genetic expressions of Glial cell line-derived neurotrophic factor (GDNF) and transferrin were assessed. The results obtained from the in vivo study showed a duration-dependent significant decrease in body and reproductive organ weights, sperm parameters and serum testosterone concentration. Testicular and epididymal histology also showed duration-dependent degenerative changes. However, all these changes were restored towards control values in the recovery experiment. The viability and deoxyribonucleic acid (DNA) integrity of the treated Sertoli cells showed dose and duration-dependent adverse effects while GDNF and transferrin showed normal genetic expressions. These results suggest that dapsone could induce male reproductive stress by affecting testicular and epididymal structure and function.

1. Introduction

Dapsone (4, 4′-diaminodiphenylsulfone, DDS) is a potent anti-inflammatory and antibacterial compound which has been used in the treatment of leprosy [1,2] and in treating malaria as chlorproguanil-dapsone and artesunate–dapsone–proguanil combinations [3,4]. It is also used in the treatment of vasculitis and dermatitis herpetiformis, lupus erythematosus profundus as well as infections with *Pneumocystis carinii, Pneumocystis jiroveci* [5–7] and *Toxoplasma gondii* in AIDS patients [8,9]. However, dapsone has been reported to cause some adverse side effects. Oral administration of dapsone has been shown to cause methemoglobinemia as well as hemolysis [10]. Other less common side effects of oral dapsone administration include cutaneous eruptions, leukopenia, peripheral neuropathy, minor neurological and gastrointestinal complaints [11]. Male reproductive toxicity can be linked to the adverse effects observed in his reproductive organs and related endocrine systems which may affect sexual maturation, gamete production and thereby affect fertility [12]. Some reproductive dysfunction associated with male infertility includes decreased reproductive organ weight and serum testosterone level with the occurrence of abnormal sperm parameters. It may also include destruction of both spermatogenic and Sertoli cells which may eventually lead to the arrest of spermatogenesis. Sertoli cells are 'nursing cells' which help to nurture developing spermatogenic cells during spermatogenesis [13]. Genes such as Glial cell-line derived neurotropic factor (GDNF) and transferrin are required for proper growth and development of Sertoli cells [14,15].

A variety of chemical and physical agents have been linked with male reproductive toxicity and infertility [16–18]. They include antibiotics, antimalarial drugs and even antiepileptic drugs. Tetra-

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cycline and doxycycline have been shown to exert toxic effects on testicular tissue and sperm parameters in treated mice by increasing apoptosis in sperm cells and lowering the number of acrosome-intact sperms [19]. Artemether, an antimalarial drug, has been reported to significantly reduce sperm parameters, viability and serum testosterone levels in the treated rats [20]. Sodium valproate and carbamazepine were also reported to have antifertility activities. Sodium valproate significantly decreased testicular weight and sperm parameters [21] while carbamazepine caused accentuated damage in the ventral prostate of the treated rats [22]. Sulphasalazine was reported to significantly reduce sperm motility and acrosome reaction. It also decreased the CD59 protein expression on the acrosomal membrane of the sperms [23]. Dapsone has been reported to have antifertility activities by reducing fecundity in rats by 38.3%, however this was not systematically investigated [24]. Although the effect of dapsone on rat fecundity has been investigated and reported, its detailed activities on male reproduction remain unknown. Considering the wide usage of this drug, this present study was designed to investigate the effects of dapsone on male reproductive activities in male rats using in vivo and in vitro techniques.

2. Materials and methods

2.1. Animals

Wistar strain albino rats (10–12 weeks; 180 g – 220 g) used for the in vivo study were obtained from the Central Animal House, College of Medicine, University of Ibadan, Ibadan while Sprague – Dawley albino male rats (16–18 days old; 18–22 g) obtained from the Laboratory Animal Division, Central Drug Research Institute, Lucknow, India were used for the in vitro study. They were housed in wire mesh cages under standard laboratory conditions of 12 h light/dark cycle. Rat feed (Standard rat chow, Ladokun Feeds) and water was provided *ad libitum* all through the study. The Ethical Research Committee reviewed and approved the study (Ref. No. 96/08/Endo/I.A.E.C.) and the animals were cared for in accordance with the "Guide for the Care and Use of Laboratory Animals" [25]. The animal model used for this study was the rat because the biological effects of gonadotropins and the process of testicular steroidogenesis can be easily analyzed and studied in them. [26].

2.2. In vivo research

Thirty male rats were divided into 5 groups containing 6 rats per group. They were matched weight for weight and administered dapsone as follows. Rats in group 1 received distilled water (vehicle) for 5 days and served as the control. Group 2 contained rats that were administered 1.4 mg/kg body weight of dapsone (Jacobus, U.S.A.) for 5 days to mimic its use as an antimalarial drug [4,27]. Group 3 rats received distilled water (vehicle) for 6 weeks. They served as the control animals for the long-term study. Group 4 rats were administered 1.4 mg/kg body weight of dapsone daily for 6 weeks. This was to mimic the use of dapsone in the long term treatment of leprosy, *Dermatitis herpetiformis* and also to expose the gametes to the chronic effects of the drug [28–30]. Group 5 rats received this same dose of dapsone daily for 6 weeks and were allowed a recovery period of 6 weeks (Table 1). The dose administered in this study was deduced from that used in humans. Dapsone was administered to rats orally using the oral cannula. The rats were closely observed during the period of dapsone administration for signs of toxicity.

Table 1
Summary of animal grouping and treatment.

Experimental group	Treatment
1	Vehicle (Distilled water for 5 days)
2	Dapsone (1.4 mg/kg B.W for 5 days)
3	Vehicle (Distilled water for 6 weeks)
4	Dapsone (1.4 mg/kg B.W. for 6 weeks)
5	Dapsone (1.4 mg/kg B.W. for 6 weeks and Recovery for 6 weeks)

2.3. Organ and blood sample collection

At the end of the scheduled experimental periods, the body weight of each rat was taken and they were killed by exsanguination under 25% urethane anesthesia (0.6 ml/100 g body weight). Urethane has been reported not to have any spermatogenic or antifertility effect in rats [31]. Blood was collected via cardiac puncture and serum was obtained from it. The testes, epididymis and seminal vesicles were harvested and weighed while the testes and epididymis were used for further investigations.

2.4. Measurement of hormone concentration

Follicle stimulating hormone, luteinizing hormone and testosterone concentrations were measured from the serum by the Enzyme-immunoassay (E.I.A.) technique using the E.I.A. kits (Immunometrics, London U.K) following the manufacturer's instructions [20]. The optical density was read using a spectrophotometer (Jenway, UK) at between 492 nm and 550 nm.

2.5. Analysis of sperm parameters

One of the testes was carefully removed together with its epididymis. The testis was immediately frozen for further investigation while semen from the caudal epididymis was used for the determination of the progressive sperm motility, sperm viability (live/dead ratio), sperm count and sperm morphology as earlier described [32–34]. Briefly, progressive sperm motility was carried out immediately by placing 2 drops of semen on a pre-warmed microscope slide. Thereafter, 2 drops of warm sodium citrate (2.9%) were added and covered with a cover slip. This was then examined under a light microscope (x40 objective). Eosin/Nigrosin stain was used to determine the sperm viability (live/dead ratio) while Walls and Ewas stain was used to determine the morphology of the sperms. Sperm count was obtained using the new improved Neuber's hemocytometer counting chamber.

2.6. Daily sperm production

This procedure assessed the number of homogenization – resistant spermatids present in the testis as earlier described [35,36]. Briefly, the frozen testis was thawed at room temperature and homogenized in 1 ml buffer solution containing 0.25 M sucrose and 0.02 M Tris (Sigma-Aldrich). The resulting homogenate was then made up to 5 ml with the same buffer solution. A few drops of the solution were then applied onto the Mackler chamber (Sefi Medical Instrument, Haifa, Israel) and the spermatids were then counted under light microscopy (x40).

2.7. Histomorphometric analysis

The testis and epididymis were earlier prefixed in Bouin – Hollande's solution prior to histological studies. They were processed, embedded in paraffin and sectioned at a thickness of 5 microns.

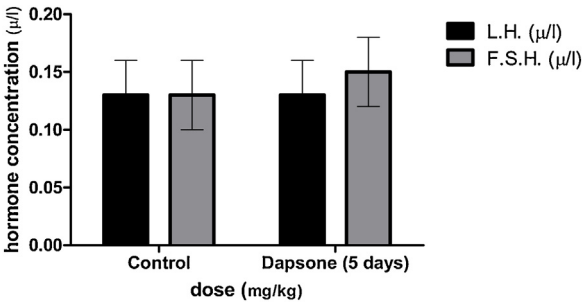


Fig. 1. Serum concentration of luteinizing hormone and follicle stimulating hormone in rats after 5 days of dapsone treatment. Values are plotted as mean ± SEM. Dapsone (5 days) indicates administration of dapsone to rats for 5 days. n = 6s.

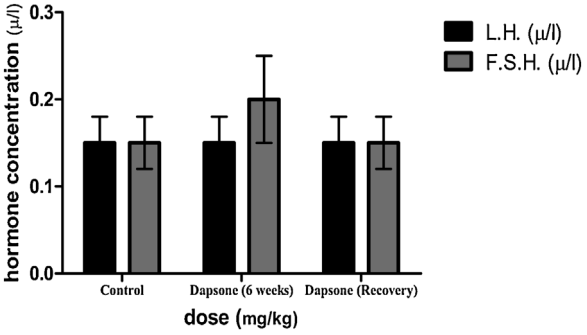


Fig. 2. Serum concentration of luteinizing hormone and follicle stimulating hormone in rats after 6 weeks of dapsone treatment and recovery. Values are plotted as mean ± SEM. Dapsone (6 weeks) indicates administration of dapsone to rats for 6 weeks while Dapsone (recovery) indicates a recovery period of 6 weeks after treatment. n = 6.

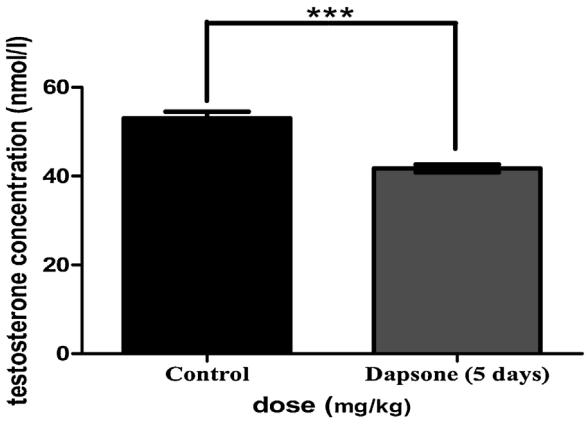


Fig. 3. Serum concentration of testosterone in rats after 5 days of dapsone treatment. Values are plotted as mean ± SEM. Dapsone (5 days) indicates administration of dapsone to rats for 5 days. n = 6. ***P < 0.001. (*) indicates significant difference between the treated group and control.

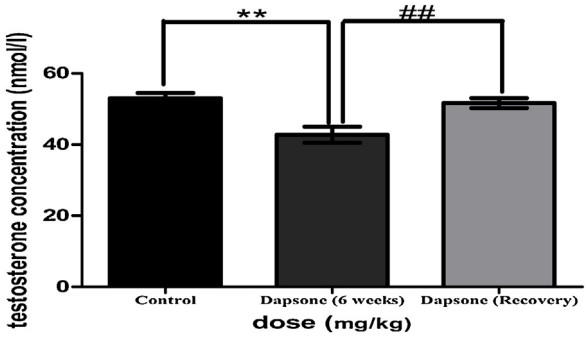


Fig. 4. Serum concentration of testosterone in rats after 6 weeks of dapsone treatment and recovery. Values are plotted as mean ± SEM. Dapsone (6 weeks) indicates administration of dapsone to rats for 6 weeks while Dapsone (recovery) indicates a recovery period of 6 weeks after treatment. n = 6. **P < 0.01, ##P < 0.01. (*) indicates significant difference between the treated group and control while (#) indicates significant difference between the treated group and recovery group.

The sections were further processed and stained with hematoxylin – eosin and examined under light microscopy [37,38].

2.8. Mating studies

Male rats were administered dapsone for 6 weeks and mated with untreated proestrus female albino rats (ratio of 1 male : 2 females). Successful mating was confirmed by the presence of a vaginal plug or sperms in the vaginal smear. The number of pups delivered was noted and recorded.

2.9. In vitro research

Sertoli cells were isolated from the testes of Sprague–Dawley rats (16–18 days old) as earlier described [39]. They were cultured in DMEM/F-12 medium (Sigma-Aldrich). It was supplemented with transferrin (5 mg/L), vitamins A and E (200 ng/ml), insulin (5 mg/L), gentamicin sulfate (25 mg/L) (Sigma-Aldrich) and sodium bicarbonate (1.2 g/L) in a humidified atmosphere of 5% CO_2/95% air at 34 °C at a density of 1×10^6 cells/25 mm^2 area. 48 h later, the cells were treated with 20 mM Tris buffer (pH 7.6) for 3 min to remove the contaminating germ cells. The Sertoli cells were then plated in 48-well tissue culture plates and fresh DMEM/F-12 medium were added and further supplemented with follicle stimulating hormone

Table 2
Body and relative organ weight of rats after 5 days of dapsone treatment.

	Body Weight (g)	Testes (%)	Epididymis (%)	Seminal Vesicles (%)
Control	208.00 ± 9.41	1.44 ± 0.14	0.21 ± 0.03	0.51 ± 0.04
Dapsone (5 days)	205.00 ± 5.30	1.39 ± 0.12	0.20 ± 0.01	0.44 ± 0.05

Values are presented as mean ± SEM. Dapsone (5 days) indicates administration of dapsone to rats for 5 days. n = 6.

Table 3
Body and relative organ weight of rats after 6 weeks of dapsone treatment and recovery.

	Body Weight (g)	Testes (%)	Epididymis (%)	Seminal Vesicles (%)
Control	286.00 ± 9.80	1.49 ± 0.03	0.28 ± 0.01	0.57 ± 0.03
Dapsone (6 weeks)	233.00 ± 6.78***	1.26 ± 0.04**	0.19 ± 0.01***	0.44 ± 0.03*
Dapsone (Recovery)	262.00 ± 5.83#	1.37 ± 0.04	0.21 ± 0.01	0.49 ± 0.03

Values are presented as mean ± SEM. Dapsone (6 weeks) indicates administration of dapsone to rats for 6 weeks while Dapsone (recovery) indicates a recovery period of 6 weeks after treatment. n=6. *P < 0.05, **P < 0.01, ***P < 0.001. #P < 0.05. (*) indicates significant difference between the treated group and control while (#) indicates significant difference between the treated group and recovery group.

Table 4
Sperm analysis after 5 days of dapsone treatment.

	Progressive Sperm Motility (%)	Sperm Viability (%)	Sperm Count (10^6/ml)	Sperm Morphology (% Abnormal Sperms)
Control	72.40 ± 1.30	75.00 ± 1.40	43.11 ± 1.82	3.90 ± 1.12
Dapsone (5 days)	57.20 ± 1.39	60.20 ± 1.53	39.17 ± 1.90	6.50 ± 1.92

Values are presented as mean ± SEM. Dapsone (5 days) indicates administration of dapsone to rats for 5 days. n = 6.

Table 5
Sperm analysis after 6 weeks of dapsone treatment and recovery.

	Progressive Sperm Motility(%)	Sperm Viability(%)	Sperm Count (10^6/ml)	Sperm Morphology (% Abnormal Sperms)
Control	81.40 ± 1.86	85.80 ± 1.80	48.04 ± 0.92	2.00 ± 0.63
Dapsone (6 weeks)	49.60 ± 1.44***	53.80 ± 1.16***	31.77 ± 0.83***	4.50 ± 1.36
Dapsone (Recovery)	66.60 ± 2.36###	71.00 ± 2.41###	41.31 ± 1.43###	3.90 ± 1.18

Values are presented as mean ± SEM. Dapsone (6 weeks) indicates administration of dapsone to rats for 6 weeks while Dapsone (recovery) indicates a recovery period of 6 weeks after treatment. n = 6. ***P < 0.001. ###P < 0.001. (*) indicates significant difference between the treated group and control while (#) indicates significant difference between the treated group and recovery group.

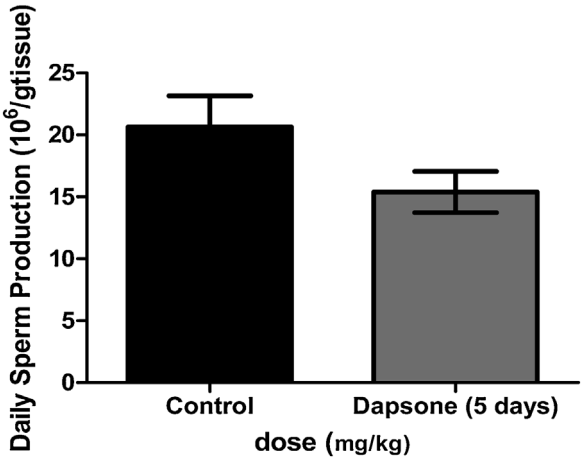


Fig. 5. Daily Sperm production after 5 days of dapsone treatment. Values are plotted as mean ± SEM. Dapsone (5 days) indicates administration of dapsone to rats for 5 days. n = 6.

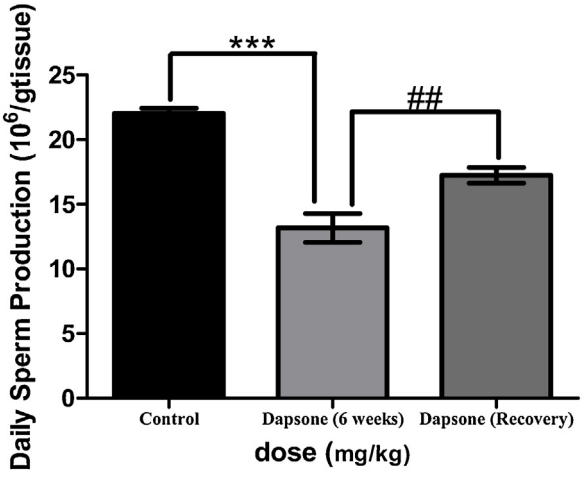


Fig. 6. Daily Sperm production after 6 weeks of dapsone treatment and recovery. Values are plotted as mean ± SEM. Dapsone (6 weeks) indicates administration of dapsone to rats for 6 weeks while Dapsone (recovery) indicates a recovery period of 6 weeks after treatment. n = 6. ***P < 0.001, ##P < 0.01. (*) indicates significant difference between the treated group and control while (#) indicates significant difference between the treated group and recovery group.

(0.05 Units/ml) and testosterone (10^{-6} M). The cultured Sertoli cells were then subjected to the following treatments and test procedures

2.9.1. Sertoli cell viability test using the M.T.T. Assay technique

The cultured Sertoli cells were treated with 0.3 μM, 0.6 μM, 1.25 μM, 2.5 μM, 5 μM, and 10 μM of the pure form of dapsone (97%; Sigma-Aldrich Inc, St. Louis, U.S.A.) which was first dissolved in dimethyl sulfoxide (D.M.S.O.) (final concentration was < 1%) for 24 h, 72 h and 120 h in vitro at 34 °C and 5% CO_2/95% air inside a CO_2 incubator. After the duration of each treatment, 20 μl of M.T.T. [3-(4, 5-dimetylthiazol-2-yl)-2, 5-diphenyl tetrazolium Bromide] (Sigma-Aldrich) was added to each well of the 48 – well tissue culture plates [40]. The live Sertoli cells took up the M.T.T. stain forming formazan crystals. This was further incubated for 4 h and the medium was removed from each well. 200 μl of Dimethyl Sulfoxide (D.M.S.O.) was immediately added to each well and kept at 37 °C for 30 min. The absorbance was measured using a microplate reader (Microquant Biotech Instruments Inc.) at 540 nm.

2.9.2. Determination of dsDNA integrity using fluorescence microscopy

This procedure assessed the integrity of the double stranded deoxyribonucleic acid (dsDNA) of the treated Sertoli cells. They were isolated as earlier outlined [39], plated on sterile cover slips in 12 – well tissue culture plates at a density of 2.5×10^6 cells/well. They were then treated with 2.5 μM, 5 μM, and 10 μM of the pure form of dapsone 24 h, 72 h and 120 h. The positive control group contained Sertoli cells treated with Nonoxynol – 9 (N-9), a known inducer of DNA fragmentation [41]. The medium was then carefully removed at the end of the treatment and washed with phosphate buffered saline (Sigma-Aldrich) twice. 500 μl of phosphate buffered saline together with 1 μl of 4′-6-Diamidino-2-phenylindole (DAPI) were added to each well and then incubated for one hour on a plate shaker. The medium was removed and the cells were washed again with phosphate buffered saline. The cover slips were then carefully extracted from each well and mounted on slides. They were observed under the fluorescence microscope (Nikon Eclipse 80i) at a wavelength of 350 nm. The nuclei that had undergone apo-

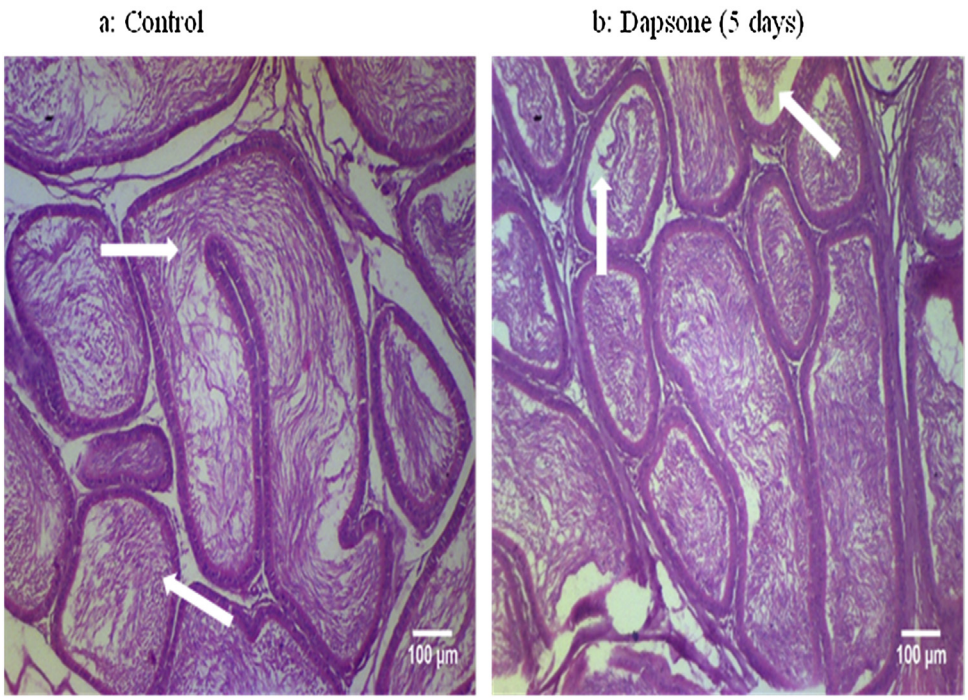


Fig. 7. Histology of the epididymis of rats treated with dapsone for 5 days. Fig.7*a shows the epididymis of control rats while* Fig7*b shows the epididymis of rats treated with dapsone for 5 days. Mag. x100 (ARROWS ARE SHOWING STORED SPERM CELLS IN 7a WHILE SHOWING VACUOLIZATION 7b.*

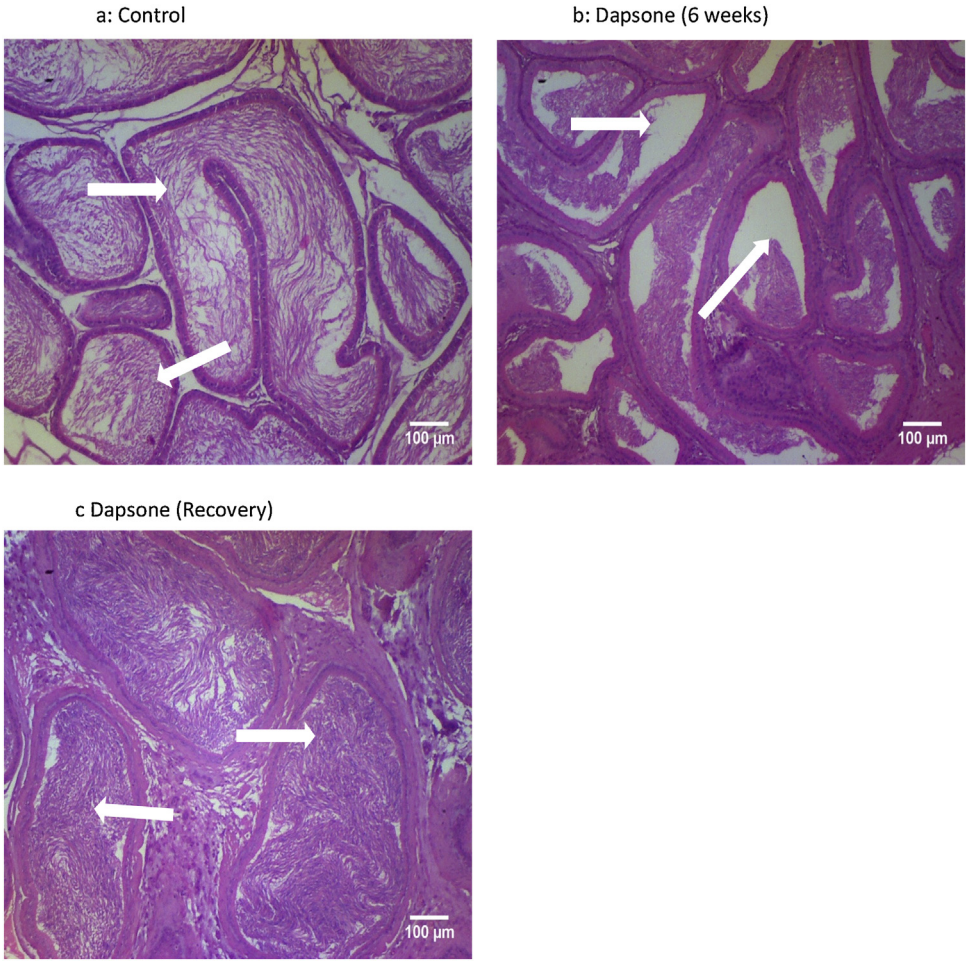


Fig. 8. Histology of the epididymis of rats treated with dapsone for 6 weeks and recovery. Fig. 8a shows the epididymis of control rats. Fig. 8b shows the epididymis of rats treated with dapsone for 6 weeks while Fig. 8*c shows the epididymis of rats in the recovery group. Mag. x100 (ARROWS ARE SHOWING STORED SPERM CELLS IN 8a WHILE SHOWING VACUOLIZATION IN 8b. IT IS SHOWING REGENERATION IN 8c).*

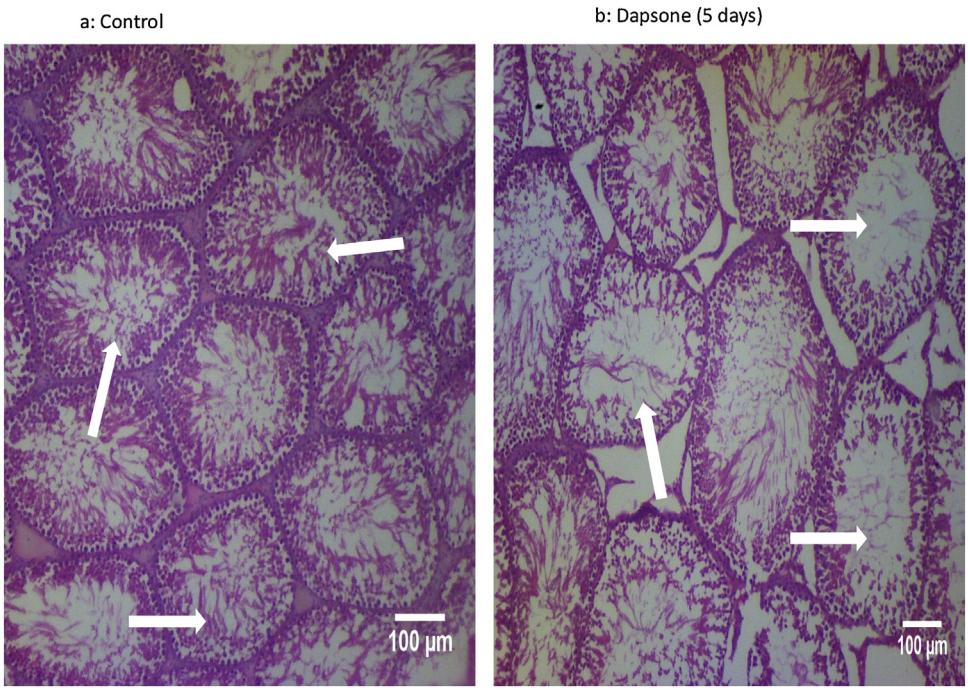


Fig. 9. Histology of the testis of rats treated with dapsone for 5 days. Fig. 9a shows the seminiferous tubules of control rats showing normal architectural arrangement of the cells while Fig. 9*b shows the seminiferous tubules of rats treated with dapsone for 5 days with mild degenerative changes. Mag. x100 (ARROWS ARE SHOWING SEMINIFEROUS TUBULES).*

ptosis were identified by the presence of condensed chromatin at the periphery of the nuclear membrane or a total fragmented morphology of nuclear bodies.

2.9.3. Quantitative reverse transcriptase - polymerase chain reaction (RT-PCR)

Sertoli cells were isolated as earlier described [39] and plated into 6 – well tissue culture plates (5×10^6 cells /well). They were treated with 1.25 µM, 2.5 µM and 5 µM of pure dapsone for five days. After the treatment, RNA was extracted from the cells and complementary DNA was formed from 2.5 µg of each RNA sample using standard procedures [42]. β-actin integrity was checked and the expression of transferrin and GDNF (Glial cell line-derived neurotrophic factor) genes were determined using the Reverse Transcriptase Polymerase Chain Reaction (RT-PCR) technique. It was further resolved using the electrophoresis technique on a horizontal agarose resolving gel assembly. Ethidium bromide stain was used to visualize the product obtained in a gel duct under ultraviolet light. The rat β-actin primer (Sigma Genoys) used had the following forward and reverse sequences;

F = 5′ AGGCATCCTGACCCTGAAGTA 3′
R = 3′ TCTTCATGAGGTAGTCTGTCA 5′

The forward and reverse sequences for the rat transferrin and GDNF gene primers (Sigma Genoys) used are;

Transferrin gene
F = 5′ GCTGTGGCCAGTTTCTTCTC 3′
R = 3′ CCACATCTCCACCTCCATCT 5′
GDNF (Glial cell line-derived neurotrophic factor) gene
F = 5′ CCAATATGCCCGAAGATTATC 3′
R = 3′ TTCGTAGCCCAAACCCAAG 5′

2.10. Statistical analysis

The data obtained were presented as mean ± standard error of mean (X ± S.E.M) and analyzed using the analysis of variance (ANOVA) with Students-Newman-Keuls multiple comparison test [43] on the Graphpad Instat Statistical software package. A probability value of $p < 0.05$ was considered significant.

3. Results

3.1. Body and relative organ weights

Administration of dapsone to rats for 5 days did not cause any significant decrease in their body and relative organ weights when compared with the control (Table 2) whereas there were significant reductions observed in these parameters in rats administered dapsone for six weeks when compared with their control ($P < 0.01$). Rats in the recovery group did not show any significant change in their body and relative organ weights when compared with the treated group (Table 3).

3.2. Serum hormone concentration

There was no significant change in the serum concentrations of luteinizing hormone and follicle stimulating hormone in rats treated with dapsone for 5 days when compared with the control. This was also observed in rats treated for 6 weeks when compared with their control (Figs. 1 and 2). Administration of dapsone to rats for 5 days and 6 weeks respectively significantly decreased ($P < 0.01$) their serum testosterone level when compared with their control. However, there was a significant increase ($P < 0.01$) in serum testosterone concentration of rats in the recovery group when compared with the rats treated for 6 weeks (Figs. 3 and 4).

3.3. Sperm parameters

Administration of dapsone to rats for 5 days did not cause any significant change in their progressive sperm motility, sperm viability (live/dead ratio), sperm count and sperm morphology when compared with the control (Table 4). A significant decrease ($P < 0.001$) was observed in the progressive sperm motility, sperm

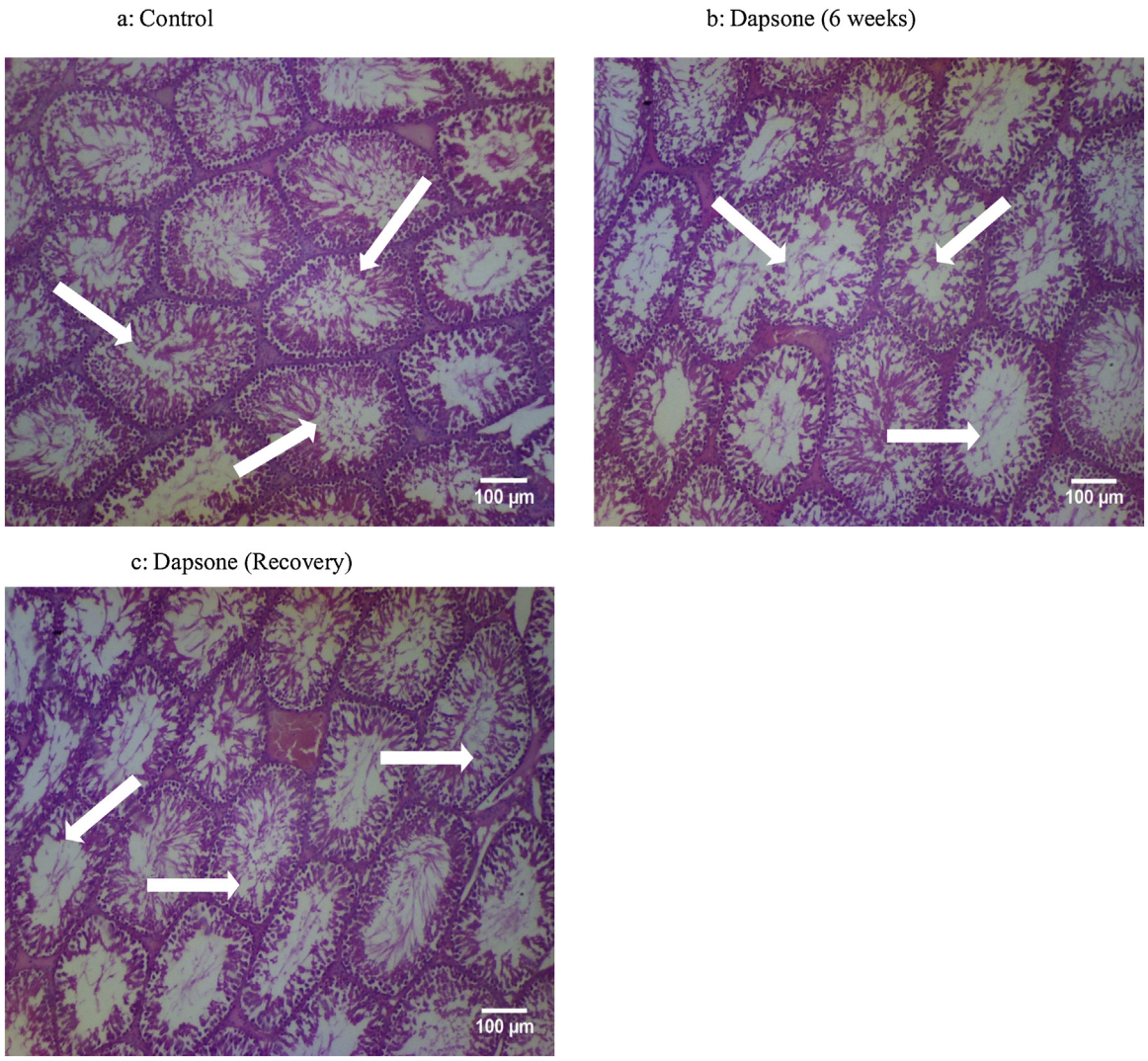


Fig. 10. Histology of the testis of rats treated with dapsone for 6 weeks and recovery. Fig. 10a shows the seminiferous tubules of control rats with normal architectural arrangement of the different cells. Fig. 10b shows the seminiferous tubules of rats treated with dapsone for 6 weeks with great tubular degeneration while Fig. 10c *shows regeneration in the seminiferous tubules of rats in the recovery group. Mag. x100 (ARROWS ARE SHOWING SEMINIFEROUS TUBULES).*

viability (live/dead ratio) and sperm count of rats treated for 6 weeks when compared with their control. However, there was a significant increase ($P < 0.001$) in these parameters in rats in the recovery group when compared with those treated for 6 weeks. There was no significant change in the number of abnormal sperms (morphology) in these treated rats when compared with the control (Table 5).

3.4. Daily sperm production

There was no significant change in the daily sperm production of rats administered dapsone for 5 days when compared with their control (Fig. 5) whereas a significant decrease ($P < 0.001$) was observed in the daily sperm production of rats treated with dapsone for 6 weeks when compared with their control. However, rats in the recovery group showed a significant increase ($P < 0.01$) in their daily sperm production when compared with the treated group (Fig. 6).

3.5. Histomorphometric analysis

Administration of dapsone to rats caused duration-dependent degenerative changes in their epididymis and testis. Rats treated with dapsone for 5 days showed none to mild degenerative changes

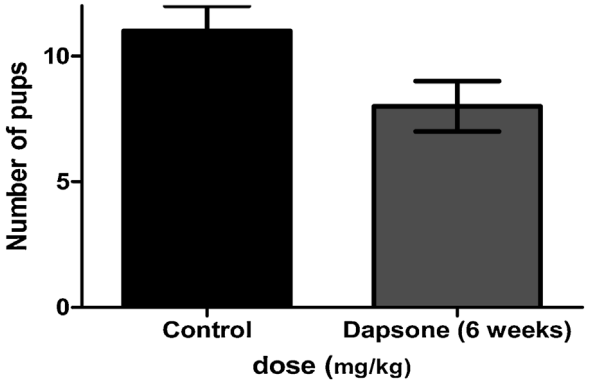


Fig. 11. Effect of dapsone on litter size. Values are plotted as mean ± SEM. Control indicates the average number of pups delivered by female rats cohabited with control male rats while dapsone (6 weeks) indicates the average number of pups delivered by female rats cohabited with male rats administered dapsone for 6 weeks.

when compared with the control while the epididymis and testis of rats treated for 6 weeks showed severe degenerative changes. There was visible vacuolization within the epididymal structure as evidenced by reductions in the sperm content. The testicular his-

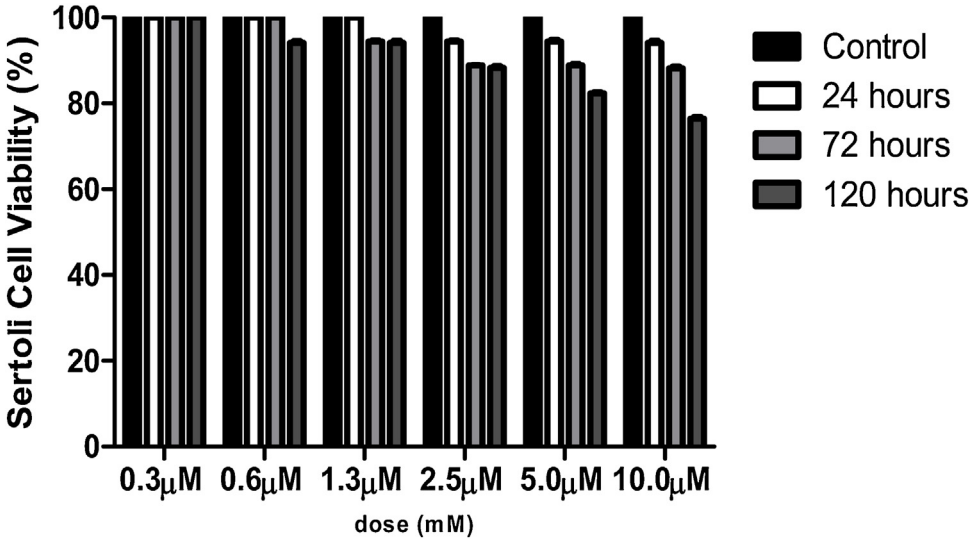


Fig. 12. Sertoli cell viability assay. Values are plotted as mean ± SEM.

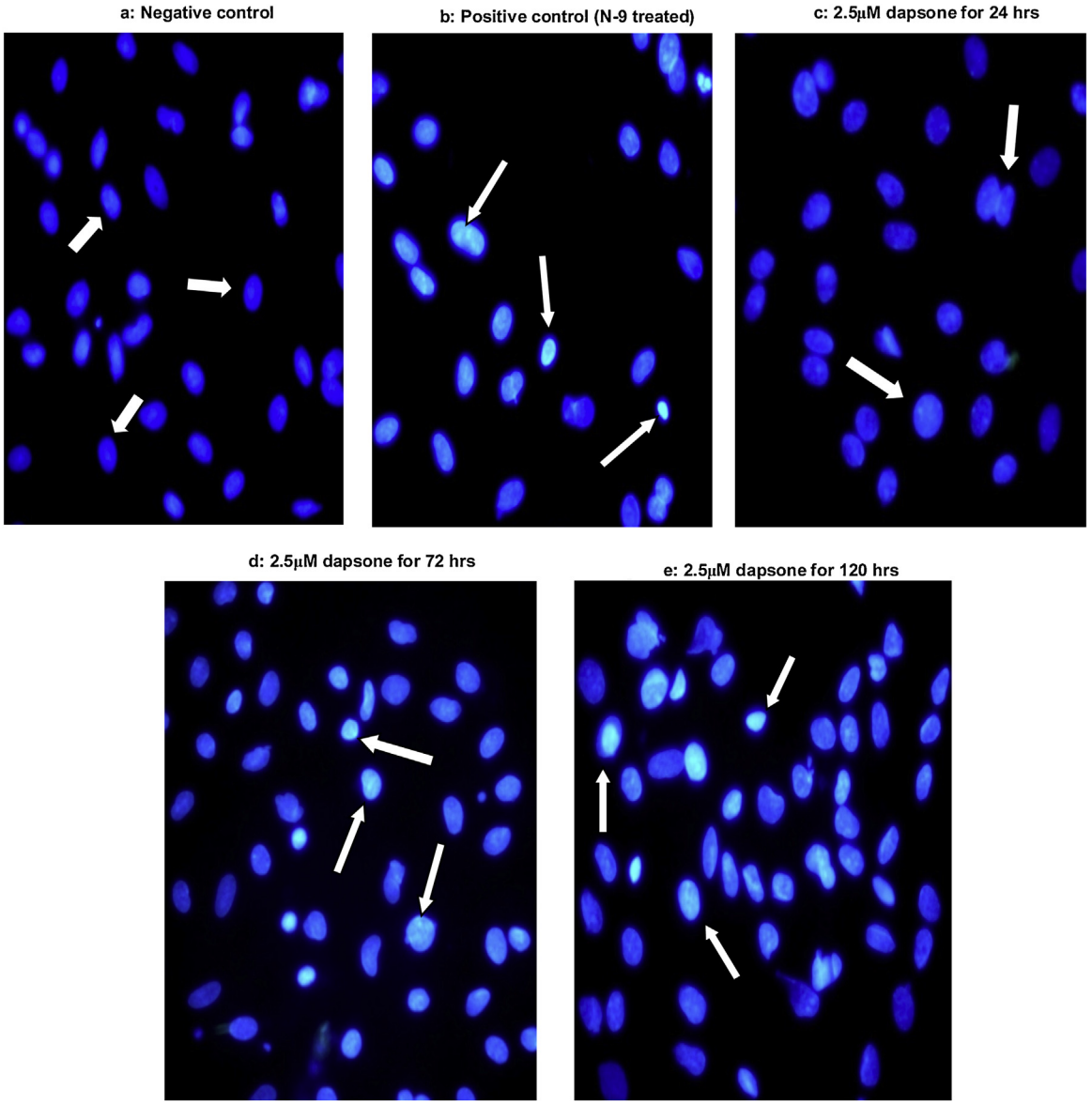


Fig. 13. Effect of 2.5 μM dapsone administration on ds-DNA integrity of Sertoli cells· (a) Negative control (untreated) (b) Positive control (N-9 treated) (c) 2.5μM of dapsone for 24 h (d) 2.5μM of dapsone for 72 h (e) 2.5 μM of dapsone for 120 h (ARROWS ARE SHOWING SERTOLI CELL NUCLEUS) (Mag. x40).

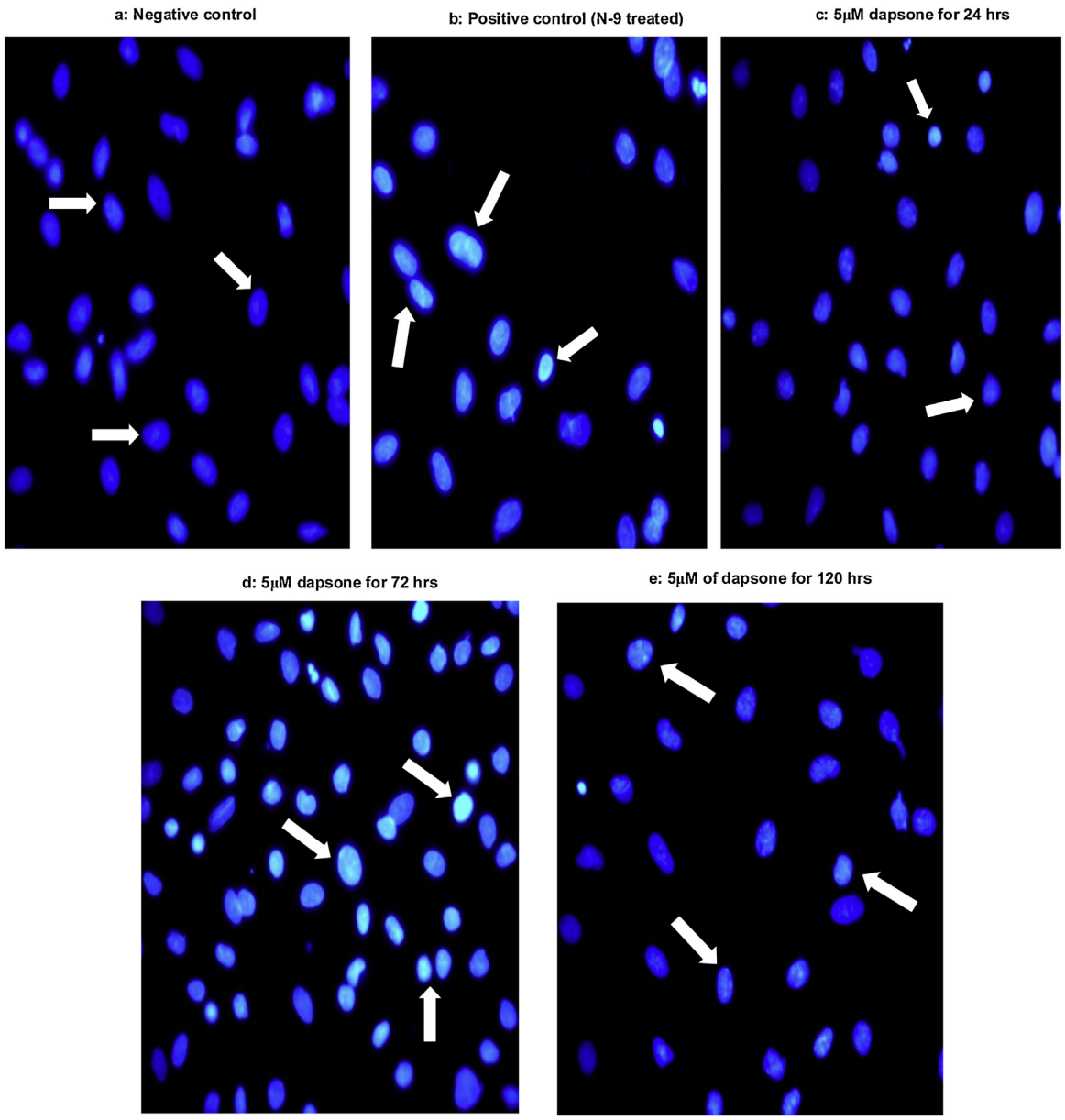


Fig. 14. Effect of 5 μM dapsone administration on ds-DNA integrity of Sertoli cells· (a) Negative control (untreated) (b) Positive control (N-9 treated) (c) 5μM of dapsone for 24 h (d) 5μM of dapsone for 72 h (e) 5 μM of dapsone for 120 h (ARROWS ARE SHOWING SERTOLI CELL NUCLEUS) (Mag. x40).

tology showed distortion and disorganization of the seminiferous tubular architecture, degeneration of the plasmalemma with possible arrest of spermatogenesis. These degenerative changes where however observed to be reversed in the epididymal and testicular histology of rats in the recovery group (Fig. 7–10).

3.6. Mating studies

The number of pups delivered by untreated females cohabited with male rats administered dapsone for 6 weeks was decreased when compared with the number of pups delivered by untreated female rats cohabited with control male rats although this decrease was not statistically significant (Fig. 11).

3.7. Sertoli cell viability

The results obtained from the M.T.T. assay in the determination of Sertoli cell viability showed a dose and duration-dependent inhibition of Sertoli cell growth. The least growth inhibition was observed at a dose of 0.3 μM for 24 h while the highest growth inhibition was observed at a dose of 10 μM for 120 h (Fig. 12).

3.8. Sertoli cell nuclei integrity

The effects of the administration of 2.5 μM, 5 μM and 10 μM of dapsone to cultured Sertoli cells for 5 days on ds-DNA integrity are presented in Figs. 13–15 respectively. The results showed a dose and duration-dependent fragmentation of their ds-DNA with 2.5 μM showing the least effect at 24 h while the greatest occurrence of fragmentation was observed at 10 μM for 120 h.

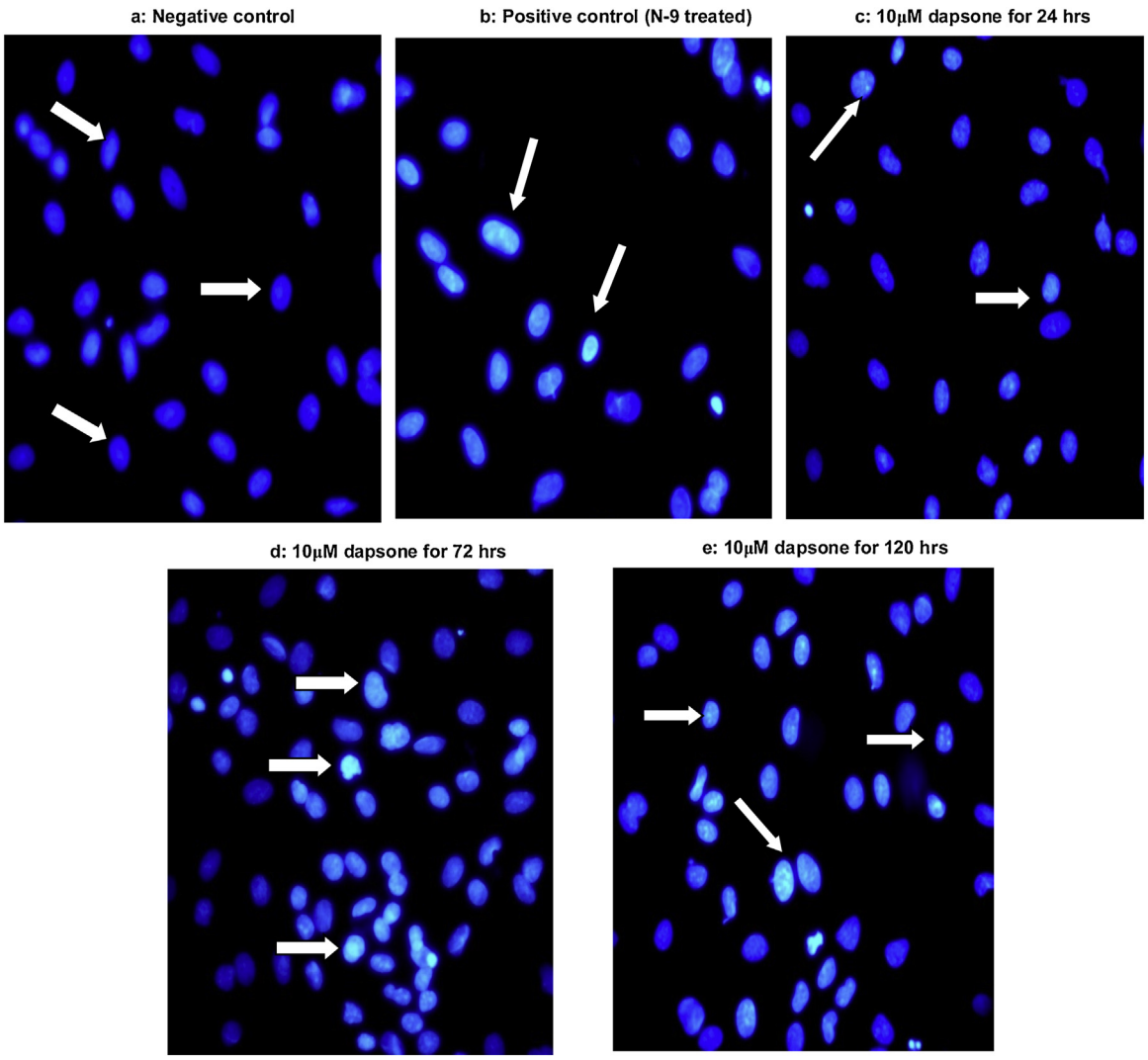


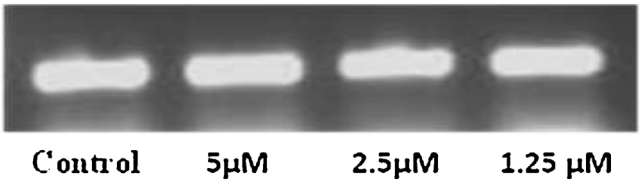
Fig. 15. Effect of 10 μM dapsone administration on ds-DNA integrity of Sertoli cells· (a) Negative control (untreated) (b) Positive control (N-9 treated) (c) 10μM of dapsone for 24 h (d) 10μM of dapsone for 72 h (e) 10μM of dapsone for 120 h (ARROWS ARE SHOWING SERTOLI CELL NUCLEUS) (Mag. x40).

3.9. *Sertoli cell genes expression*

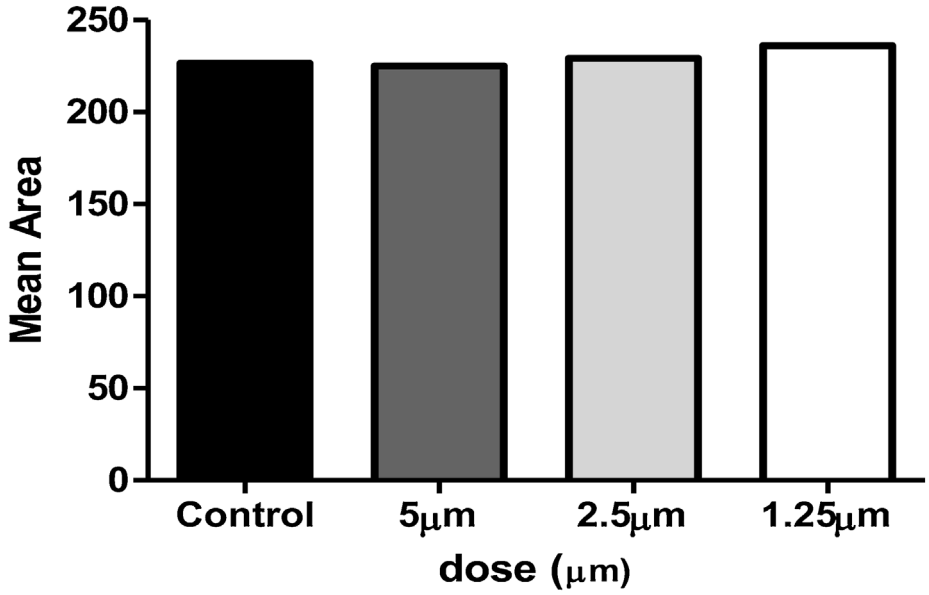
The expression of the housekeeping gene, β-actin in the Sertoli cells showed normal expression (Fig. 16). Glial cell line-derived neurotrophic factor (GDNF) and transferrin genes showed normal expression in Sertoli cells treated with 5 μM, 2.5 μM and 1.25 μM of dapsone when compared with the gene expression in control Sertoli cells (Figs. 17 and 18).

4. Discussion

This present study investigated the effects of dapsone (4, 4′-diaminodiphenylsulfone, DDS), a very important drug used in the treatment of various ailments and skin related diseases [3,4] on male reproduction. The results obtained from this study showed that short term administration of dapsone to rats did not have any significant effect on their body and relative reproductive organ weights however these parameters were significantly decreased with long term administration of dapsone. A reduction in the body weight, absolute or relative organ weight of treated animals is an indication of the toxic effect of the substance administered [44,45]. The toxic effect of dapsone was manifested in the reproductive organs of the treated rats such as testes, epididymis and seminal vesicles by significantly decreasing their relative weights. Experimental studies in reproduction uses the weight of male reproductive organs as an index of fertility risk assessment [20]. A similar finding by Sharma et al [46] showed that administration of cypermethrin for 4 weeks significantly decreased the weight of the testis and epididymis of the treated rats. They also reported a significant decrease in sperm motility, counts and viability with a significant increase in sperm abnormalities. Although, the short term administration of dapsone caused a non-significant decrease in the sperm parameters and daily sperm production of the treated rats however the long term administration significantly decreased these parameters. This may be attributed to the toxic effect of dapsone on spermatogenesis as evidenced by a reduction in testicular weight since spermatogenesis occurs within the tubules and germinal elements and they account for about 98% of the testicular weight [47]. The reduction in epididymal sperm count and daily sperm production could also be linked with the epididymal and testicular damage reported in this study. Chitra et al [48] reported a similar finding when they investigated the testicular and epi-



a. Beta-actin gene expression in Sertoli cells

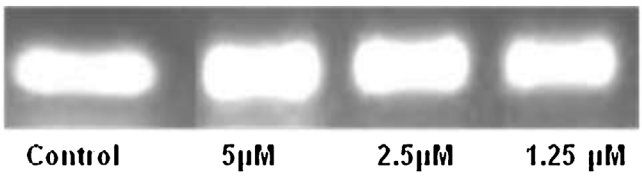


b. Graph of Beta-actin gene expression in Sertoli cells (using ImageJ software).

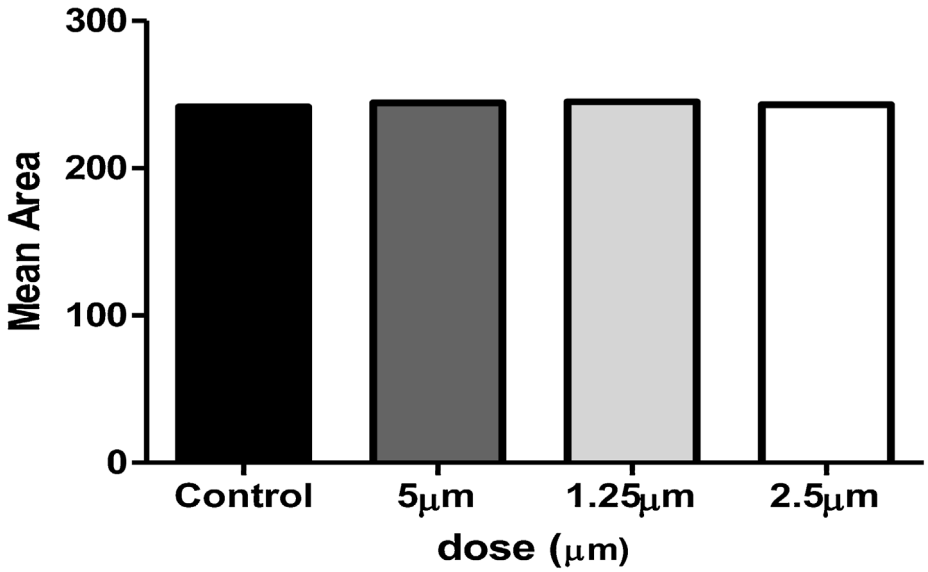
Fig. 16. a Beta-actin gene expression in Sertoli cells. b. Graph of Beta-actin gene expression in Sertoli cells (using ImageJ software).

didymal toxicity of carbaryl in Sprague-Dawley rats. They observed a significant decrease in testicular and epididymal weights with a concomitant significant decrease in epididymal sperm counts, viability and motility in the treated rats. Adequate testosterone levels are very important in the maintenance of spermatogenesis and hence fertility [49,50]. This hormone is in turn controlled by other hormones secreted by the hypothalamus and anterior pituitary gland which form the hypothalamic-pituitary-gonadal axis [51]. Although there were no significant changes observed in the serum concentrations of Luteinizing hormone (LH) and Follicle stimulating hormone (FSH) of the rats both at short and long term treatments, however the serum testosterone concentration was significantly decreased at both treatment durations. It implies that dapsone acted at the testicular level by affecting the production and release of testosterone from the Leydig cells thereby affecting spermatogenesis. This significant decrease observed in serum testosterone concentration of the treated rats might also be responsible for the significant decrease recorded in their epididymal sperm count and daily sperm production. Iamsaard et al [52] also reported a significant decrease in epididymal sperm count as well as a significant decrease in testosterone level when they administered monosodium glutamate to rats for 30 days. The significant decrease in epididymal sperm count and progressive sperm motility reported in our study was responsible for the decrease observed in the number of pups produced by female rats cohabited with long term treated male rats.

Histological studies revealed moderate to severe epididymal and testicular lesions at both durations of dapsone administration. The visible reduction in the sperm content observed in the epididymal histology was corroborated by the decrease recorded in the sperm count of the treated rats. Testicular histology revealed degeneration within the architecture of the seminiferous tubule implying that dapsone was able to penetrate the blood-testis barrier formed by the Sertoli cells [53,54] and thus affecting spermatogenesis. These results were similar to those published by Tumkiratiwong and Lerkchundhakriat [55] when they administered a combination of pyrimethamine-sulfanilamide to male Wistar rats. They reported desquamation in the seminiferous tubules and degeneration within the epididymis with infertility occurring in the treated rats. Pholpramool et al [56] also reported a similar finding when they investigated the effect of sulphonamide administration to male rats. In our study, it was noted that upon withdrawal of dapsone administration in the recovery experiments, all these parameters were observed to have similar values



a. GDNF gene expression in Sertoli cells



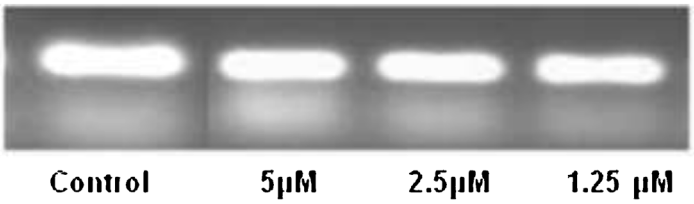
b. Graph of GDNF gene expression in Sertoli cells (using ImageJ software).

Fig. 17. a GDNF gene expression in Sertoli cells. b. Graph of GDNF gene expression in Sertoli cells (using ImageJ software).

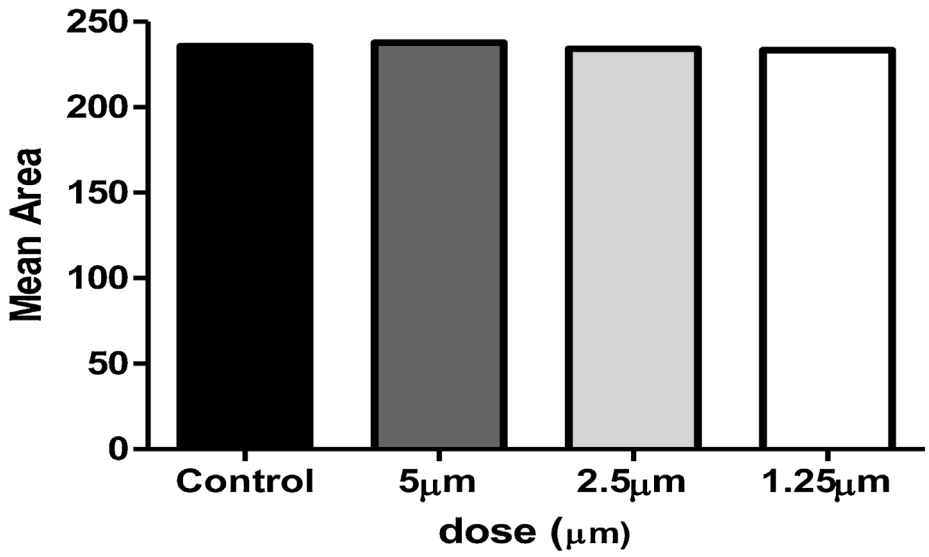
like those of the untreated, control rats which implies that the toxic effect of dapsone were not permanent but reversible upon cessation of drug administration.

Sertoli cells make up the blood-testis barrier and are also responsible for regulating spermatogenesis [57,58]. The results obtained from Sertoli cell viability study showed a dose and duration dependent decrease in the dapsone treated Sertoli cells. This reduction in treated Sertoli cells will result in disruption of the blood-testis barrier and a direct exposure of the germ cells, spermatocytes, spermatids and spermatozoa to the toxic effects of the drug. This will affect spermatogenesis and thus the sperm count as evidenced by the significant decrease in epididymal sperm count observed in this study. FSH has been reported to play an important role in Sertoli cell differentiation [59,60]. The non-significant increase observed in the serum FSH concentration of the long term treated rats will imply that a reduction in Sertoli cells due to dapsone toxicity will trigger Sertoli cell proliferation hence more FSH will be secreted since FSH facilitates Sertoli cell proliferation [61]. The toxic activity of dapsone in the treated Sertoli cells was further evidenced by the dose and duration dependent degeneration of their double stranded deoxyribonucleic acid (ds-DNA). The greatest occurrence of nuclear fragmentation and chromatin condensation was observed in Sertoli cells treated with 10μM of dapsone for 120 h. A similar finding was reported when Sertoli cells were treated with Nonylphenol. It was shown that the damage caused to the Sertoli cells was induced by endoplasmic reticular stress leading to apoptosis [62,63]. The expressions of Transferrin and Glial cell-line derived Neurotrophic factor (GDNF) were also investigated in this study. Transferrin gene has been reported to code the formation of transferrin, which is important in the delivery of iron to the germinal cells within the seminiferous tubules and maintenance of Sertoli cell differentiation [14,64] while GDNF plays an important role in Sertoli cell proliferation [15,65]. Normal gene expressions were observed in our study which implies that dapsone did not affect the expressions of Transferrin and GDNF at the tested doses despite causing nuclear fragmentation and chromatin condensation at the same doses.

The results obtained from both the in vivo and in vitro studies revealed that dapsone caused reversible toxicity on reproductive functions in a dose and duration dependent manner. The in vivo study of biological phenomena can be often complicated by the interaction of various systems operating within the living organism. In vitro culture models on the other hand, though highly artificial, provide controllable environmental conditions and the effect of various factors on specific cells can be directly studies. However, some factors such as changes in pH, temperature, culture medium and atmospheric conditions may affect the morphological and functional characteristics of the cultured cells but extreme care was taken to maintain these factors at physiological conditions suitable for the cultured cells in this study. Thus the findings from the in vivo experiment were largely supported by the findings of the in vitro experiment in this study.



a. Transferrin gene expression in Sertoli cells



b. Graph of Transferrin gene expression in Sertoli cells (using ImageJ software).

Fig. 18. a Transferrin gene expression in Sertoli cells. b. Graph of Transferrin gene expression in Sertoli cells (using ImageJ software).

5. Conclusion

In conclusion, dapsone administration has been shown to induce male reproductive toxicity at the testicular and epididymal levels. However, these deleterious changes appeared to be reversed upon cessation of dapsone administration. This might imply that continuous use of dapsone should be done with great caution as the greatest deleterious effects observed in this study were in the long term study. Further studies could be aimed at co-administering dapsone with other drugs which might minimize this adverse effect while still maintaining its potency and therapeutic activities.

Declaration of Competing Interest

The authors declare no conflicts of interest.

Acknowledgements

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