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Research Article

Zinc Sulfate Ameliorates Testicular Degeneration Induced by Sodium Flouride in Male Wistar Rats

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Abstract

Environmental exposure to sodium fluoride (NaF) is a growing concern due to its adverse effects on reproductive health. In this study we investigated the reproductive effects of sodium fluoride and the therapeutic potential of zinc sulfate in male albino rats. Twenty male albino rats were divided into four groups of five animals each; Group A (healthy control) received distilled water, Group B received 50mg/kg Zinc Sulfate, group C received 50mg/kg Sodium fluoride and group D received concurrent administration of Sodium fluoride and Zinc Sulfate at 50mg/kg each. Treatments were administered orally for 28 consecutive days after which the rats were euthanized for sample collection and analysis. The collected data were subjected to analysis using descriptive statistics and ANOVA with GraphPad Prism version 5.0. The findings from this study concluded that the sodium fluoride (NaF) has significant effect on hematology, serum chemistry, semen quality, testosterone levels, and testicular architecture whilst the administration of zinc sulfate (ZnSO4) was effective in ameliorating the toxic effects induced by NaF on blood parameters, serum chemistry, and the histopathological damage in the testes.

Keywords: Sodium Fluoride; Zinc Sulfate; Male Wistar Rats; Testicular Degeneration

Abbreviations

ZnSO4: Zinc Sulfate; NaF: Sodium Fluoride; BPA: Bisphenol A; EDTA: Ethylene Diamine Tetra-Acetic Acid; ELISA: Enzyme-Linked Immunosorbent Assay; ANOVA: One-Way Analysis of Variance; PCV: Packed Cell Volume; HB: Hemoglobin; RBC: Red Blood Cell; Neut: Neutrophils; mono: Monocytes; lymph: Lymphocytes; WBC: White Blood Cells; MCH: Mean Corpuscular Hemoglobin; MCV: Mean Corpuscular Volume; MCHC: Mean Corpuscular Hemoglobin Concentration; AST: Aspartate Aminotransferase; ALT: Alanine Aminotransferase

Introduction

In recent years, there has been growing concern regarding the adverse effects of various environmental contaminants on male reproduction. Fluoride, a widely distributed natural pollutant, has been shown to decrease fertility in most animal species tested. It can cause testicular degeneration, structural defects in spermatozoa, decreased sperm count, and impact hormone levels [1-3]. Sodium fluoride has been found to decrease the activities of antioxidant enzymes and increase lipid peroxidation in the testes and spermatozoa indicating potential reproductive hazards associated with fluoride exposure [4]. This highlights the need for further

investigation into the mechanistic underpinning implications for human health. Symptoms of testicular degeneration from exposure to toxic chemicals can manifest as various reproductive health issues in males. These symptoms may include compromised sperm count, quality, and other changes in male reproductive health due to exposure to environmental toxicants [5]. Environmental contaminants can induce oxidative stress in the testes, affecting testicular histology and function leading to reduced fertility and impaired spermatogenesis. For example, exposure to bisphenol A (BPA) and phthalates has been associated with urogenital cryptorchidism, histological alterations in the fetal testis, and hormonal disruptions that can impact testicular function and sperm quality [6,7]. Sodium fluoride often induces oxidative stress which leads to decreased antioxidant enzyme activities and increased lipid peroxidation in testes and spermatozoa, a significant factor affecting male reproductive health [8,9].

On the other hand, zinc, an essential trace element plays a crucial role in male reproduction by influencing spermatogenesis, sperm motility and testosterone production [10,11]. Zinc deficiencies have been associated with gonadal dysfunction and impaired spermatogenesis. It has also been suggested to be a key regulator of spermatogenesis, influencing sperm motility, stabilizing sperm membranes, and modulating sperm functions [12]. Furthermore, it has been reported to show potentiated activities in positive feedback effects on male reproduction when administered with other ingredients, such as Vitamin E and other compounds. Several studies have demonstrated the enhancement of sperm quantity and quality by the administration of zinc, either alone or in combination with other substances [13]. There are limited reports on the potential use of zinc sulfate as an antioxidant in the management of testicular degeneration induced by exposure to NaF. Hence, in this study, we evaluated the possible ameliorative effect of zinc sulfate against sodium fluoride-induced testicular degeneration in male albino rats.

Materials and Methods

Chemicals

Sodium fluoride and zinc sulphate were obtained from Sigma-Aldrich company (St. Louis, MO, USA).

Experimental animals and housing conditions

Adult male albino rats (Rattus rattus) weighing 200 ± 10g were obtained from a local breeder in Ibadan, Oyo State, Nigeria at 12 weeks of age. The animals were stabilized for two weeks at the animal house section, Veterinary Biochemistry Department, Faculty of Veterinary Medicine, University of Ibadan, Nigeria under normal photoperiod of (12 hours light and 12- hours dark), controlled conditions of temperature (25 ± 2.0°C) and relative humidity (50 ± 15%). All procedures were performed under the guidelines of the University of Ibadan Animal Care and Use Committee (ACUREC) with number UI-ACUREC/102-1021/18.

Study design and animal selection

Twenty male albino rats were randomly divided into four groups (A-D) of five animals each. Animals in Group A served as control groups which were given distilled water, animals in Group B received 50 mg/kg of Zinc sulfate following the protocol by [13], animals in Group C received 50 mg/kg of sodium fluoride as described by [14]. Group D received a combination of 50 mg/kg of zinc sulfate and 50 mg/kg of sodium fluoride. All treatments were given daily by oral gavage for 28 consecutive days.

Blood sample collection and analysis

Terminal blood samples were aseptically collected from the rats' retro-orbital plexus using sterile heparinized capillary tubes. Blood was collected into sample bottles containing EDTA for hematology analysis and plain bottles for serum biochemistry. Hematological profiling was conducted using conventional methods as described by [15]. For serum biochemistry, blood samples were centrifuged at 2500g for 12 minutes to obtain serum. The harvested serum was used for biochemical analysis including measurements of total protein, albumin, globulin, creatinine, triglycerides, and liver enzymes using analytical grade reagent kits according to the manufacturer's instructions. This comprehensive analysis provided detailed insights into the physiological and biochemical status of the animals, essential for assessing the impact of the treatments.

Testosterone assessment

Blood collected in EDTA sample bottle was centrifuged at 2500g for 12 minutes to obtain plasma. Plasma testosterone levels was quantified using a commercially available enzyme-linked immunosorbent assay (ELISA) kit following the manufacturer's protocol. The ELISA method, known for its high sensitivity and specificity, allows for the precise measurement of hormone concentrations in biological samples. Plasma samples were

collected from the rats and prepared according to the ELISA kit instructions, ensuring that the assay conditions were optimal for accurate hormone detection.

Semen analysis and gonadometric assessment

After euthanizing the animals, we assessed semen motility, sperm count, sperm viability, and morphological abnormalities as described by [16,17]. We then excised the epididymis, right testis, and left testis to measure and record their respective weights, lengths, and diameters.

Histopathology and testicular histomorphometry measurements

The testicular tissues were fixed in Bouin's solution, followed by processing and staining of slides using a standardized procedure as described [18]. Histomorphometry measurements, such as seminiferous tubular diameter, germinal height, and luminal diameter, were then assessed at 100X magnification using Moticam software.

Johnsen's score

The Johnsen score was developed by Johnsen in 1970. It is a ten-point scoring system used for assessing testicular function by quantifying spermatogenesis [19]. It categorizes spermatogenesis activity based on the types of cells observed along the seminiferous tubules, where a score of 10 indicates maximum spermatogenesis, while a score of 1 indicates the complete absence of germ cells [20,21].

Statistical analysis

Data obtained were presented as mean ± standard error of mean. The statistical analysis was performed in GraphPad prism version 5.0 using one-way analysis of variance (ANOVA) followed by Turkey's test at a 95% confidence limit. 5.0.

Results

Hematological parameters of the male albino rats treated with Zinc sulfate and sodium fluoride.

The packed cell volume (PCV), hemoglobin (HB), red blood cell (RBC), white blood cells (WBC), Lymphocyte, Neutrophils, mean corpuscular hemoglobin (MCH) and mean corpuscular volume (MCV) results showed significant difference ($p < 0.05$) across the treatment groups (A, B, C and D). There was also a significant difference between Group B (Zn only), Group C (NaF only) and Group D (NaF+ZnSO4) as compared to the control group (Group A). Furthermore, Platelet, monocytes and mean corpuscular hemoglobin concentration (MCHC) parameters showed no significance difference across the groups ($p > 0.05$) as represented in table 1.

GROUPS	GROUP A (control)	GROUP B (50mg/kg $ZnSO_4$)	GROUP C (50mg/kg NaF)	GROUP D (50mg/kg $ZnSO_4$+ (50mg/kg NaF)	F- stats	P-value	REMARK
PCV (%)	42.00 ± 2.03^{a}	41.80 ± 2.01^{a}	33.40 ± 2.36^{b}	34.60 ± 0.60^{ab}	5.989	0.0062	****
HB (%)	13.9 ± 0.70^{a}	13.82 ± 0.67^{b}	11.04 ± 0.79^{c}	11.46 ± 0.20^{d}	5.762	0.0072	****
RBC ($X10^{6}\mu l$)	12.40 ± 0.45^{a}	9.98 ± 0.30^{b}	10.036 ± 0.15^{c}	9.46 ± 0.26^{b}	17.92	<0.0001	****
WBC ($10^{3}\mu l$)	9.32 ± 0.27^{a}	10.28 ± 0.40^{a}	10.08 ± 0.61^{a}	12.08 ± 0.38^{b}	7.253	0.0027	****
Platelet	11.2 ± 0.49^{a}	11.6 ± 0.40^{a}	10.8 ± 0.49^{a}	10.0 ± 0.00^{a}	2.917	0.0662	NS
LYMPH (%)	41.8 ± 1.77^{a}	44.6 ± 1.44^{a}	52.6 ± 1.50^{b}	41.2 ± 1.46^{a}	11.47	0.0003	***
NEUT (%)	56.8 ± 1.88^{a}	53.4 ± 1.08^{a}	46.2 ± 1.39^{b}	58.2 ± 1.77^{a}	11.75	0.0003	***
MONO (%)	1.4 ± 0.25^{a}	2.00 ± 0.45^{a}	1.2 ± 0.37^{a}	0.6 ± 0.4^{a}	2.381	0.1078	NS
MCH (pg)	10.6 ± 0.24^{a}	13.6 ± 0.75^{b}	10.6 ± 0.93^{a}	12.0 ± 0.45^{c}	4.857	0.0137	***
MCV (fl)	33.6 ± 0.6^{a}	41.4 ± 2.07^{bc}	32.8 ± 2.42^{c}	36.2 ± 1.24^{a}	5.022	0.0122	***
MCHC (%)	32.6 ± 0.24^{a}	32.6 ± 0.24^{a}	$33.0 \pm {}^{0}a$	33.0 ± 0^{a}	1.778	0.1919	NS

Table 1: Hematological profile of the male albino rats in different treatment groups. Group A-D with n=5 for each group. Values are reported as mean ± SEM. NS means no significant mean difference at 5% level. ***Means at least two treatment groups are significantly different at 0.05 level. abc: Means the same row with different superscript differ significantly ($P < 0.05$).

Serum chemistry of the male albino rats treated with zinc sulfate and sodium fluoride.

Following treatment with zinc sulfate and sodium fluoride, total protein, AST, ALT, creatinine, and triglycerides across all the study groups are statistically significant ($p < 0.05$) as compared to the control group. The serum chemistry values of albino rats treated with NaF only (Group C) were significantly lower than the control (Group A) group. However, the group treated with NaF+ZnSO4 was also significantly lower than the control group. The results show that the serum parameters increased in the ZnSO4 treated group compared to the NaF group (Table 2). Thus, ZnSO4 treatment could improve serum chemistry in NaF induced testicular degeneration in rats.

GROUPS	GROUP A (control)	GROUP B (50mg/kg $ZnSO_4$)	GROUP C (50mg/kg NaF)	GROUP D (50mg/kg $ZnSO_4$+ (50mg/kg NaF)	F- stats	P-value	RE-MARK
Total protein (g/dl)	5.24 ± 0.16^a	5.2 ± 0.20^a	4.12 ± 0.08^b	3.54 ± 0.07^c	35.52	< 0.0001	****
Albumin (g/dl)	2.06 ± 0.04^a	2.0 ± 0.20^a	1.06 ± 0.04^b	1.16 ± 0.02^c	25.30	< 0.0001	****
Globulin (g/dl)	3.0 ± 0.08^a	3.2 ± 0.03162^a	3.06 ± 0.04^a	2.43 ± 0.07^b	32.05	< 0.0001	***
AST (µl)	62.6 ± 1.07^a	54.6 ± 0.50^b	42.8 ± 1.02^c	36.6 ± 0.6^d	193.1	< 0.0001	****
ALT (µl)	53.4 ± 0.92^a	44.6 ± 0.50^b	34.6 ± 0.81^c	26.8 ± 0.58^d	254.1	< 0.0001	****
Creatinine (mg/dl)	2.61 ± 0.05^a	2.37 ± 0.013^b	2.10 ± 0.05^c	1.18 ± 0.03^d	270.1	< 0.0001	****
Triglycerides	61.2 ± 0.8^a	63.2 ± 0.49^a	53.4 ± 0.6^b	42.4 ± 0.75^c	197.8	< 0.0001	****

Table 2: Serum chemistry profiling of the male albino rats in different treatment groups. Group A-D with n = 5 for each group. Values are reported as mean ± SEM. NS means no significant mean difference at 5% level. ***Means at least two treatment groups are significantly different at 0.05 level. abc: Means the same row with different superscript differ significantly ($P < 0.05$).

Zinc sulfate treatment improves testicular degeneration induced by sodium fluoride and improve testosterone hormonal level.

The semen characteristics (sperm motility, sperm livability and sperm count) of the study groups after treatment with zinc sulfate and sodium fluoride showed a significant difference ($p < 0.05$). The sperm motility, sperm livability and sperm count significantly reduced following NaF treatment as compared to the control group. However, there was also an improvement in the group treated with zinc sulfate only and combination of sodium fluoride (NaF) and zinc sulfate (ZnSO4) as presented in Figure1. The testosterone level after treatment with zinc sulfate and sodium fluoride shows significant difference across the study groups ($p < 0.05$) also presented in Figure1. The testosterone level of the NaF group are significantly lower than the control group and the zinc sulfate group. The group treated with NaF and ZnSO4 group D) also significantly differ from NAF group showing ZnSO4 ameliorates the toxic effect induced by NaF.

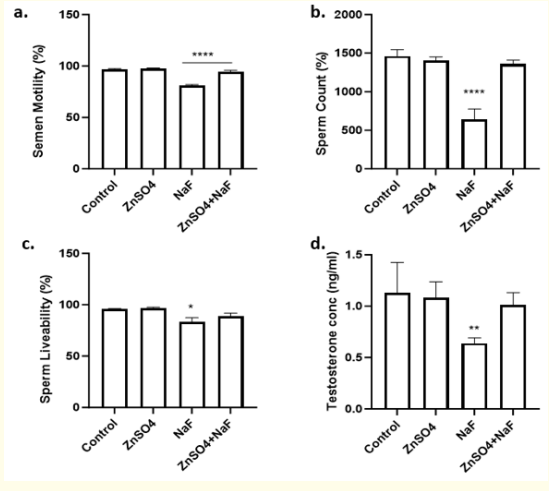

Figure 1: Semen characteristics and testosterone level of male albino rats in different treatment groups. a) percentage of sperm motility b) percentage of sperm count c) percentage of sperm livability and d) concentration of testosterone level across all the groups. Group A-D with n=5 for each group. Values are reported as mean ± SEM. NS means no significant mean difference at 5% level. ***Means at least two treatment groups are significantly different at 0.05 level. abc: Means the same row with different superscript differ significantly ($P < 0.05$).

Sperm morphological abnormalities of the male albino rats treated with zinc sulfate and sodium fluoride.

Table 3 shows that there is a significant difference in the mean percentage difference of curved midpiece, abnormal head, looped tail and total abnormal cells across all groups ($p < 0.05$). On the other hand, percentage headless tail, tailless head, and bent tail are not significantly different when compared between the groups.

GROUPS (%)	GROUP A (control)	GROUP B (50mg/kg $ZnSO^4$)	GROUP C (50mg/kg NaF)	GROUP D (50mg/kg $ZnSO^4$+ (50mg/kg NaF)	F- stats	P-value	REMARK
Curved midpiece	17.43 ± 3.79^a	5.96 ± 0.37^b	6.68 ± 2.38^c	1.36 ± 0.55^d	8.453	0.0016	*
Abnormal Head	0.98 ± 0.31^a	0.34 ± 0.31^a	0.00 ± 0.00^b	0.00 ± 0.00^c	4.821	0.0152	*
Headless Tail	1.02 ± 0.69^a	0.065 ± 0.06^a	1.34 ± 0.55^a	0.4 ± 0.24^a	1.365^a	0.2913	NS
Tailess head	2.23 ± 1.54^a	1.60 ± 0.50^a	2.93 ± 0.67^a	6.36 ± 2.19^a	2.071	0.1471	NS
Bent tail	3.64 ± 2.09^a	2.23 ± 0.67^a	20.01 ± 4.35^b	8.35 ± 1.45a	9.091	0.0011	NS
Looped tail	0.81 ± 0.21^a	0.13 ± 0.07^b	0.00 ± 0.00^c	0.00 ± 0.00^d	11.97	0.0003	****
Total Abnormal Cell	24.25 ± 5.88a	10.32 ± 0.61^a	29.19 ± 3.43^{ab}	11.80 ± 1.28^a	6.297	0.0056	****
Percentage Abnormalities	6.12 ± 1.40^a	2.50 ± 0.18^a	6.62 ± 0.49^a	2.74 ± 0.28^c	7.269	0.0031	**
Total Cell Count	400.4 ± 17.22^a	436.2 ± 30.59^a	437.8 ± 29.49^a	430 ± 9.35^a	0.5569	0.6510	NS

Table 3: Sperm Morphological Abnormalities of the male albino rats in different treatment groups. Group A-D with n=5 for each group. Values are reported as mean ± SEM. NS means no significant mean difference at 5% level. ***Means at least two treatment groups are significantly different at 0.05 level. abc: Means the same row with different superscript differ significantly ($P<0.05$).

Testicular biometry and histomorphometry assessment of testis of the male albino rats treated with zinc sulfate and sodium fluoride.

The testicular and epididymal biometry observed after treatment with zinc sulfate and sodium fluoride showed no significant difference across the groups ($p > 0.05$) as represented in table 4. However, the seminiferous tubular diameter and germinal thickness of the testes shows significant difference across the study groups ($p < 0.05$) as shown in figure 2. Microscopical examination of testis of the control rats (group A) revealed normal histological structures with Johnsen score 10 showing complete spermatogenesis with many spermatozoa. Zinc sulfate (group B) revealed Johnsen score 8-9; many spermatozoa are present but with slight sloughing off of the lumen, NaF only treated rats (group C) revealed Johnsen Score 3 showing only a few spermatozoa present, also marked sloughing and obliteration of the lumen and the co-administration of ZnSO4 and NaF (Group D) showed Johnsen Score 7 many spermatids are present. This indicates that despite the testicular impairment initiated by NaF, ZnSO4 was able to ameliorate this toxic effect.

GROUPS	GROUP A (control)	GROUP B (50mg/kg $ZnSO^4$)	GROUP C (50mg/kg NaF)	GROUP D (50mg/kg $ZnSO^4$+ (50mg/ kg NaF)	F- stats	P-value	RE-MARK
Bodyweight (g)	227.80 ± 19.11[a]	233.83 ± 20.05[a]	225.2 ± 17.55[a]	220.5 ± 16.65[a]	0.1097	0.9535	NS
Left Testis Weight (g)	1.28 ± 0.04[a]	1.24 ± 0.05[a]	1.18 ± 0.05[a]	1.25 ± 0.08[a]	0.5279	0.6694	NS
Right Testis Weight (g)	1.28 ± 0.03[a]	1.26 ± 0.05[a]	1.21 ± 0.07[a]	1.20 ± 0.11[a]	0.2589	0.8539	NS
Left Testis Length (Mm)	18.64 ± 0.29[a]	18.37 ± 0.27[a]	18.08 ± 0.36[a]	17.98 ± 0.71[a]	0.4490	0.7214	NS
Right Testis Length (Mm)	18.11 ± 0.34[a]	18.1 ± 0.32[a]	17.64 ± 0.27[a]	17.62 ± 0.60[a]	0.4270	0.7364	NS
Left Testis Diameter (Mm)	11.11 ± 0.21[a]	11.13 ± 0.28[a]	11.04 ± 0.12[a]	11.18 ± 0.30[a]	0.06429	0.9780	NS
Right Testis Diameter (Mm)	10.96 ± 0.25[a]	11.40 ± 0.21[a]	10.94 ± 0.25[a]	10.87 ± 0.36[a]	0.7903	0.5169	NS
Epididymal Weight (g)	0.52 ± 0.04[a]	0.53 ± 0.03[a]	0.49 ± 0.03[a]	0.54 ± 0.03[a]	0.3937	0.7592	NS

Table 4: Testicular and Epididymal Biometry of the male albino rats in different treatment groups. Group A-D with n = 5 for each group. Values are reported as mean ± SEM. NS means no significant mean difference at 5% level. ***Means at least two treatment groups are significantly different at 0.05 level. abc: Means the same row with different superscript differ significantly (P < 0.05).

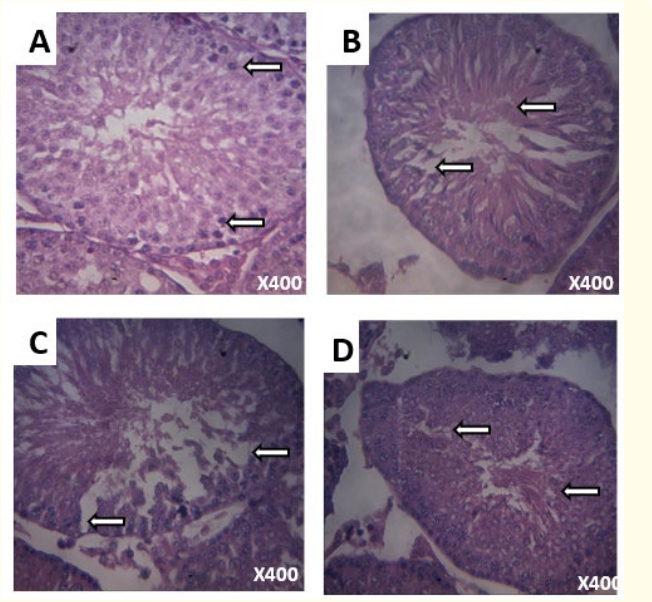

Figure 2: Histological Examination of the testis of the male albino rats treated with zinc sulfate and sodium fluoride using Johnsen's score. A-Johnsen Score 10, B- Johnsen Score 8-9, C-Johnsen Score 3 and D-Johnsen Score 7.

Discussion

Fluoride has been identified to induce destruction of peripheral circulating white and blood cells with subsequent replacement response by the myeloblastic elements of the bone marrow and outpouring of immature cells [22]. The present study showed that there is a significant difference between the hematocrit (PCV) of the animal groups administered with NaF with a reduced PCV value below the standard values. This finding is in accordance with that of [23] who reported marked decrease in the hematological profile of male rat following NaF administration. Also, the significance between the group administered with NaF and that of the ZnSO4 as indicated by the low RBC counts, hemoglobin concentration, decline in RBC are traceable to the toxic effects of NaF that could have resulted in inhibition of globin synthesis [24]. The effects of NaF on the leucocyte population (reductions in neutrophils and lymphocytes in this study aligns with the decline observed in the red blood cells) and PCV. [25] reported that reduction in RBC, white blood cell (WBC) counts, hemoglobin content and morphological abnormalities in RBCs (poikilocytosis) in fluoride exposed mice. This finding is further indicative of the toxic effects

of NaF on the ability of the male animal species to combat entrance and invasion of pathogens, an effect beyond the reproductive system as presented in this study. Zinc has been suggested to potentiate erythropoiesis in rats and helps mitigate toxic effects of heavy metals [26]. In alignment with this report, we observed in this study that ZnSO4 increases the level of the packed cell volume, leucocytes and other important hematological parameters which are indicative of enhanced erythropoietic activities by ZnSO4 and a counter effect against the observed toxicities of NaF. This indicates that ZnSO4 has ameliorative effect against NAF intoxication hence restoration of erythropoiesis.

Sodium fluoride has been reported to negatively affect protein synthesis and metabolism in animals [1]. Serum chemistry is generally used to assess liver homeostasis. The liver serves as the chief organ for metabolism and detoxification and is indirectly involved in hematopoiesis. In this study, we observed that NaF significantly reduced serum total protein, aspartate aminotransferase (AST), alanine transaminase (ALT), creatinine, and triglyceride levels. This report corroborates the reports by [27], who reported that fluoride inhibits protein synthesis following oral administration of sodium fluoride and demonstrates that fluoride at ribosomal levels tends to inhibit protein synthesis. Kanbur., *et al*. [28] suggested that the occurrence of significantly decreased alanine transaminase (ALT) and alkaline phosphatase (ALP) activities and ALT activities could have resulted from alterations in enzymatic activities. The overall effects of sodium fluoride can therefore be assumed to have both pathomorphological and metabolic changes in liver that is presented in decreased serum proteins as observed in this study and corroborated by several studies. In this study, ZnSO4 administered alone and alongside NaF was found to ameliorate the toxic effects of NaF, as revealed by the serum total protein, ALT, AST, creatinine, and triglyceride levels compared to those in the animal groups administered NaF and the healthy control. These results indicate that ZnSO4 ameliorates the harmful effects of agents, such as NaF. To our knowledge, this is the first study to investigate the ameliorative effects of ZnSO4 on the adverse effects of NaF. This result is indicative of the significant role of ZnSO4 in combating the harmful effects of NaF in domestic animals, including protection of the liver and maintenance of liver homeostasis.

Sodium fluoride has been reported to be associated with a decrease in sperm count, sperm motility, and sperm viability. Several studies have demonstrated the toxic effects of sodium fluoride on the reproductive system of male animals. For instance, a study on male rats exposed to 2, 4, and 6 ppm sodium fluoride in their drinking water for 6 months showed a significant decrease in sperm motility and density in the cauda epididymis, as well as a decrease in the weight of the testis, epididymis, and ventral prostate [29]. Another study on male rabbits demonstrated a significant decrease in epididymal sperm count after fluoride exposure [30]. Additionally, excessive fluoride ingestion has been shown to interfere with spermatogenesis, lower sperm quality and reduced nuclear DNA integrity in humans and animals [31,32]. [33,34]. NaF have been implicated as a potent toxicant at concentrations ranging from 20mg/kg to as high as 300mg/kg causing different spermatozoa abnormalities. In contrast, Chioca., *et al*., [35] reported that fluoride does not alter spermatogenesis at 50 or 100 ppm, also Collin., *et al*. [36] reported that sodium fluoride in drinking water at 175 ppm (175mgkg) produced no developmental toxicity in fetuses and neonates of rats but at 250ppm (250mg/kg) may have some effects. However, this present study negates this claim as we have reported toxicity effects of NaF on spermatogenesis at 50 mg/kg (50ppm) in male albino rats. On the other hand, zinc is an essential element currently known as a potent protective agent against acute toxic effects of heavy metals such as cadmium and sodium fluoride. This study further affirms that Zinc sulfate is indicative for progressive increase in sperm motility, sperm concentration and livability as previously reported by [37]. The concurrent administration of NaF and ZnSO4 increases sperm motility, sperm concentration and livability indicative of the counter effects of ZnSO4 against NaF and portends ZnSO4 to ameliorates the toxic effects of NaF on male reproductive system. Whilst the toxic effects of NaF are indicative in this study, and ZnSO4 showed ameliorative effects against the NaF toxicity, the total abnormal cells recorded in this study further affirms that NaF induces increased sperm abnormalities while ZnSO4 showed reduced number of abnormal sperm cells. However, this present study shows that NaF has no significant effect on the testicular biometry as compared to the group treated with ZnSO4, NaF and ZnSO4 and the healthy control group.

The toxic effects of NaF on spermatogenesis have been associated with impairment in angiotensin-converting enzyme activity and subsequent reduced testosterone secretion [33].

Furthermore, Wan., *et al.,* [38] indicated that fluoride significantly reduces the secretion of Leydig cells and subsequently affects epididymal growth and receptor activity in male species. This study showed a reduction in testosterone concentration following NaF administration, corroborating the reports of Giri., *et al*., [14] and Ghosh., *et al.,* [33] who demonstrated the negative effects of NaF on Leydig cells and angiotensin-converting enzyme activities. Therefore, our findings affirm that NaF might cause damage to important organs necessary for testosterone secretion. The overall disruption of the integrity and homeostasis of the epididymis by NaF subsequently indicates its toxic effects, such as reduced sperm motility, viability, and count, as demonstrated in this study. Interestingly, zinc supplementation has been associated with the activation and secretion of testosterone, and subsequently increased spermatogenic activity and efficiency of germinal cells [39]. The effects of the concurrent administration of ZnSO4 and NaF in this study led to increased testosterone secretion, with evidence of the ameliorative effects of ZnSO4 whether administered alone or concurrently with NaF. This further suggests that the administration of NaF could be the main cause of the reduced testosterone secretion, alongside the sperm abnormalities previously mentioned in this study. In the present study, the accumulated doses of NaF reduced sperm activity, increases sloughing, necrosis, and damage to testicular tissues. Histopathological examination of rats treated with NaF showed apparent alterations in the testes, where it induced focal disorganization of seminiferous tubules, which was associated with a reduction in spermatogenic cells. The histopathological effects of NaF on testicular tissues indicated the presence of only spermatogonia based on the Johnsen scoring system [40] which is indicative of sloughing, disorganization, and incomplete spermatolytic arrest and atrophy. Meanwhile, treatment with ZnSO4 resulted in noticeable improvement in the histopathological changes induced by the toxic effects of NaF. This finding affirms the opinion of Sayed., *et al*., [41] which supports the fact that ZnSO4 minimizes the hazardous effects of NaF, and consequently, the germane role of ZnSO4 in maintaining and restoring the integrity of the reproductive system.

Conclusion

In conclusion, this study demonstrates that sodium fluoride (NaF) has detrimental effects on the male reproductive systems with consequential impediment to fertility potentials in male rats while the application of antioxidant agents like zinc sulfate (ZnSO4) can significantly mitigate NaF's toxicities.

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Conflict of Interest

The authors declares no conflict of interest.

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