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ORIGINAL ARTICLE

Co-Administration of Vitamin E and Homtamin Ginseng Prevented and Reversed Sodium Fluoride-Induced Testicular Dysfunction Through Inhibition of Oxidative Influx and Hormonal Modulation Mechanism in Wistar Rats

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Abstract

Background: Despite the concern over reproductive damage from sodium fluoride exposure, there is limited scientific data on the synergistic effect of Homtamin ginseng and vitamin E. A potential remedy could include Homtamin ginseng and vitamin E supplementation, but further research is needed. This research was conducted to investigate the effects of Homtamin ginseng and vitamin E on the reproductive damage induced by sodium fluoride exposure. Different treatment protocols were employed, such as administering drugs alone, preventive measures, and reversal approaches.
Methods: In the drug alone or preventive-protocol, rats received oral treatment of Saline (2 mL/kg), Homtamin ginseng (40 mg/kg/day), vitamin E (150 mg/kg/day) or both alone repeatedly for 28 days, or in combination with sodium fluoride (100 ppm/kg/P.O./day) from days 15-28. In the reversal-protocol, the animals received NaF for 28 days prior to saline, Homtamin ginseng, vitamin E or Homtamin ginseng + vitamin E from days 15-28.
Results: The result showed an increase ($P < 0.05$) in testicular malondialdehyde and a decrease in the activities of testicular superoxide dismutase, catalase and glutathione peroxidase in the group exposed to sodium fluoride. These changes were significantly ($P < 0.05$) ameliorated by vitamin E and Homtamin ginseng. More so, sodium fluoride altered testicular and brain weight, spermatogenesis, hormonal profile, fertility test as well as testicular architecture negatively following treatment with sodium fluoride. However, Homtamin ginseng and vitamin E or combination of both prevented and reversed the effects of sodium fluoride on spermatogenesis, hormonal profile, fertility, and mating indices in rats.
Conclusion: In conclusion, Homtamin ginseng and vitamin E prevented and reversed sodium fluoride-induced testicular dysfunction through inhibition of oxidative influx and hormonal modulation mechanism in Wistar rats.

Keywords: NaF, HG, Vitamin E, Reproduction, Dysfunction, Spermatogenesis and Gonadotropin

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INTRODUCTION

Excessive exposure to sodium fluoride (NaF) can lead to numerous health risks, such as skeletal and dental fluorosis, and bone deformation [1,2,3,4,5,6]. The World Health Organization (WHO) recommends an optimal fluoride concentration of 0.5 to 1.0 mg/L in drinking water [6], however, in Nigeria, the tolerable limit is 1.5 to 2 mg/L. To counteract the impacts of sodium fluoride exposure, a tailored remedy plan should be implemented to reduce the level of sodium fluoride present. Exposure to a NaF concentration greater than 10 mg/L has the potential to cause fluoride poisoning, which can lead to cancer [7,8,9]. Moreover, NaF poisoning can harm a number of soft tissues, including the liver, kidney, muscle, brain, heart, thyroid, testis, and ovaries [10,11,12,13]. Studies have shown that long-term exposure to NaF poses a potential risk to reproductive health, particularly in male subjects. Studies

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have linked chronic exposure to NaF with infertility or impairment of reproductive organs, such as the testes and ovaries, as well as certain parts of the brain. Over the last decade, studies done on populations have determined that fertility levels have been decreasing globally [14,15], leading to increased attention to the potential risks posed by environmental toxins on male reproductive function [16,17]. To counteract the impacts of NaF exposure, a tailored remedy plan comprising of dietary changes, activity modifications and natural supplements is recommended. NaF exposure has a variety of adverse effects on human reproductive health, particularly in males. Men exposed to NaF may experience a decrease in reproductive hormones and organ weight, abnormal spermatogenesis, and sperm deformities [16, 18]. Females may be at an elevated risk of miscarriage [19]. NaF has the potential to enter the brain and accumulate in neurons and the neuroglial cells located in the hippocampus, leading to morphological changes, and ultimately, to neurodegeneration. This can cause learning disabilities and memory deficits, in addition to changes in the activity of enzymes in the brain [20]. Exposure to NaF can have serious impacts on human health. It has been found to cross the placental barrier, resulting in low birth weights in the fetus, as well as morphological and neurotransmitter abnormalities. Additionally, during the developing stage, NaF can be transferred from the mother to the weanling pup through breast milk, resulting in changes to the pup's neuronal structure and morphology. Various studies have been conducted to analyze the effects of NaF exposure on testicular functions, and the results have indicated a correlation between high levels of NaF and impaired reproductive health in males. However, there are currently few drugs with proven clinical efficacy for the treatment of testicular dysfunction as a result of NaF exposure [21]. Investigating drugs like vitamin E and homtamin ginseng might be a critical part of dealing with reproduction issues caused by NaF exposure.

Vitamin E (vit E) is an effective antioxidant which helps protect the body from oxidative stress. Its ability to target the unsaturated fatty acids found in the phospholipid layer of testicular cells is especially beneficial, providing an added layer of protection against NaF exposure. Vitamin E has been observed to reduce the presence of free radicals due to its donation of a hydrogen atom (H). This inactivates lipid peroxidation, which helps to protect cells from damage. In addition, research has demonstrated that the intake of vitamin E can significantly raise testosterone levels. Studies have demonstrated that Vitamin E exposure has the potential to enhance the quality of sperm cells and augment the size of seminiferous tubules [22, 23].

Homtamin ginseng, a type of white ginseng referred to as the "King of Herbs", has been found to offer promising benefits for overall health. In both animal and human case-control studies, there was a positive association between this plant and libido, as well as copulatory performance. Studies indicate that ginseng can help improve the sperm quality and count both for healthy individuals and those experiencing infertility caused by treatment.

Given the potential adverse effects of sodium fluoride on male fertility, it is necessary to investigate the role of co-administration of vitamin E and homtamin ginseng in order to prevent and reverse any negative impacts of NaF-induced testicular dysfunction. To do this, a study should be conducted to observe the reproductive anomaly and reproductive hormone modulation of rats after NaF exposure, in comparison to rats given only vitamin E and homtamin ginseng. This will provide a better understanding of the effects of NaF on male fertility, and the positive effects of vitamin E and homtamin ginseng in helping to counteract them.

METHODS

Animal's model

For the experiment, 114 adults male Wistar strain rats with a weight range of 150-200g were acquired. The rats were then placed in six per plastic cage at 25 ± 1°C and exposed to a 12-hour light/dark cycle. During the experiment, all rats had access to standard rat pellet and water ad libitum. The study adhered to the legal and ethical requirements established by the Institution's Animal Care and Use Research Ethics, while strictly following the National Institute of Health's Guidelines for the Use and Care of Laboratory Animals (Publication No. 85-23, revised).

Drugs, chemicals purchased and dose administration

Vitamin E (150 mg/kg) and Homtamin ginseng (40 mg/kg) from Kule Ara Pharmaceuticals (Ibadan, Oyo State, Nigeria) and Sodium Fluoride (NaF, 100ppm/kg) from Sigma-Aldrich (St. Louis, USA) were used in the study. Corn oil (obtained from Shoprite, Ibadan) was utilized as the vehicle for the Vitamin E and Homtamin ginseng, with each being dissolved in 20 ml of corn oil prior to use [24]. A total of 221 mg of sodium fluoride (NaF) was dissolved in 1000ml of tap water to reach the desired concentration of 100 ppm of fluoride ions. The fluoride water was provided to the animals freely, following the findings of previous studies and preliminary investigation. Additionally, 150mg/kg of Vitamin E was administered, in accordance with previous research. This tailored remedy plan was designed to counteract the impacts of sodium fluoride exposure. Homtamin ginseng from Neimeth International Pharmaceutical Plc was administered at two different dosages: 40mg/kg body weight per day, and 80mg/kg body weight per day. This dosage was in accordance with the manufacturer's instructions. A study protocol based on the research of Burses et al. [25] and Ilodigwe [26] was followed which entailed administering 10µg/100g of estradiol benzoate and 0.5mg/100g of progesterone by subcutaneous injection over a 48-hour period and 4 hours prior to pairing, respectively. Naive rats were divided into two groups, one serving as a normal control and the other receiving corn (2 mL/kg per oral [P.O.]) and distilled water (10 mL/kg P.O.) as a vehicle.

Experimental Procedures

The study was carried out in three different experiments Unit. Experiment Unit 1 consisted of treatment with drugs alone (n = 6). Accordingly, rats in Group 1 were given saline (2 mL/kg). Group 2 received corn oil (2 mL/kg), group 3 had Vit.E (150 mg/kg), group 4 was treated with HG (40 mg/kg), group 5 was treated with HG (80 mg/kg), while group 6 received the combination of Vit. E (150 mg/kg) and HG (40

mg/kg) orally (P.O.) once daily for 28 days. However group 7 received the combination of Vit. E (150 mg/kg) and HG (80 mg/kg) orally (P.O.) once daily for 28 days. In Unit 2, which is the prophylactic protocols, rats were randomly distributed into six groups (n = 6). Animals in group 1 received 2 mL/kg of saline and served as normal control. Group 2 received corn oil (2 mL/kg, P.O.) and served as vehicle control. Rats in group 3 received saline (2 mL/kg) but served as negative control. Group 4 was pre-treated with Vit E (150 mg/kg, P.O.), group 5 with HG (40 mg/kg, P.O.), while group 6 was pre-treated with the combination of Vit.E (150 mg/kg, P.O.) and HG (40 mg/kg, P.O.) once daily for 28 days. However, from the 14th to 28th day onward, rats in groups 3–6 additionally received NaF (100 ppm/kg) once daily. But, in experiment Unit 3 (n=6) which is the curative protocol, a direct opposite of the prophylactic protocol in experiment 2, animals from group 1 were given saline (2 mL/kg, P.O.) as normal control, and group 2 received corn oil (2 mL/kg, P.O.) as vehicle control. Groups 3–6 were pre-treated with NaF (100 ppm/kg) once daily for 28 days. Then from days 14 to 28 onward, rats in group 3 additionally received saline (2 mL/kg, P.O.). Group 4 received Vit. E (150 mg, P.O.). Group 5 was treated with HG (40 mg/kg, P.O.), while group 6 received the combination of Vit. E (150 mg/kg, P.O.) and HG (40 mg/kg, P.O.) once daily, respectively.

During the experiment, all of the animals' body weights were measured weekly, and at the end of the experimental protocol the animals were euthanized under a light thiopentone sodium anesthesia and their blood was collected via cardiac puncture after laparotomy for hormonal analysis. The testes and brain tissues were dissected for histological studies.

Fertility study

Male rats (n=6) were paired with pre-oestrous female rats in a 1:2 ratio and allowed to cohabitate overnight on days 24, 26, and 28 of the experiment. This mating process was conducted to comply with the method of Frietal et al., 2002 and included a 4 day acclimatization period to the mating environment. Fertility testing was then performed on all male rats. Following the confirmation of successful mating, the mated females were separated and kept in individual cages in order to track the number of pregnancies and the total number of live implantations that occurred 28 days after coitus. These data were used to calculate the fertility index.

Sample Collection

At the conclusion of the experiment, the animals were made to go without food for an entire night. Subsequently, they were put under light thiopentone sodium anesthesia and samples of blood were drawn from their hearts. This blood was then centrifuged and the serum was stored in a refrigerator at 20°C to be used for analysis of male reproductive hormones. After this, the testes of the animals were removed and rinsed in saline that was chilled with ice. After the testes were weighed, they were placed into Bouin's Solution for histological examination.

Reproductive hormones analysis

Serum samples were tested for LH, FSH, and testosterone using ELISA kits from Monobind Inc. in Lakeforest, CA, per the manufacturer's instructions.

Sperm analysis

The left epididymis was detached from the testis after being harvested. Then, the caudal epididymis was cautiously separated and transferred onto a slide pre-heated to 27°C, using a dissecting blade to release a few sperm onto the slide. The sample was immediately analyzed after being collected [27].

Histology

The harvested testes were subjected to Bouin's fixative, followed by a dehydration process with 50-100% ethanol. Xylene was utilized to rinse the tissues to rid them of the dehydrant and then the tissue was embedded in paraffin for both increased structural integrity and to facilitate dissection. The tissue samples were prepared for microphotographic viewing by first rinsing them in xylene to remove the paraffin, followed by washing in decreasing concentrations of ethanol (100–50 percent). Following this, the samples were rehydrated using water. Sectioning of the tissues into 6 μm thick slices was then performed, and the sections were stained with the hematoxylin-eosin (H-E) stain. Once stained, the sections were observed under a light microscope at 400x magnification, as described by Naiho et al [28].

Statistical analysis

The data analysis for the study was conducted using Graph Pad Prism version 5 software, which is produced by Graph Pad Software, Inc. located in La Jolla, California. The results were presented as the Mean ± Standard Error of the Mean (SEM). The comparison between the groups was conducted by using one-way analysis of variance (ANOVA), with Bonferroni's post hoc test for pair-wise comparison being employed afterward. A p value of less than 0.05 was regarded as statistically significant.

RESULTS

Effect of Vitamin E and Homtamin Ginseng in naïve and sodium fluoride-induced changes on body, testicular and whole brain weights in male Wistar rats.

The study found that treatment with sodium fluoride alone resulted in a significant decrease in body, testes, and brain weights in rats compared to the control group. However, when vitamin E and HG were given repeatedly, the final weight, testes, and whole brain weight increased significantly. The combination of Vitamin E and HG, administered individually or as a combination, prevented the decrease in final body, testicular, and brain weights caused by NaF. Pre-treatment with both Vitamin E and HG was found to be more effective than either Vitamin E or HG alone. The combination of Vitamin E and HG alone resulted in a notable increase in body weight and testes weight.

Vitamin and Homtamin ginseng and their combination mitigated sodium fluoride induced-alteration on sperm indices in the preventive and reversal treatments in rats

The preventive protocol showed that NaF significantly decreased sperm count, motility, viability, and abnormal morphology in rats compared to the control group (Fig. 1). Vit. E and HG had a positive effect on preventing sperm indices, and a combination of both showed a significant increase in sperm indices compared to their individual effects.

The study found that sodium fluoride (100 ppm/kg) significantly decreased sperm count, motility, and viability, and increased the number of abnormal morphology sperm cells compared to normal controls (Fig. 2). Vitamin E (150

mg/kg orally) and HG (40 mg/kg orally) were effective in reversing the effects of sodium fluoride exposure, as indicated by the alteration in sperm count, motility, viability, and morphology. A combination of vitamin E and HG had a significantly greater recovery effect on these altered sperm counts.

Table 1a. Prophylactic effect of Vitamin E and Homtamin ginseng and their combination in naïve and sodium fluoride invoked changes on body, testicular and brain weights in male Wistar rats

Group	Initial bodyweight (g)	Final body weight (g)	Testes weight (g)	Brain weight (g)
Control	195.60 ± 14.64	243.10 ± 26.01	2.54 ± 0.45	2.02 ± 0.07
Corn oil (2 mL/kg)	196.20 ± 17.05	241.20 ± 25.98	2.52 ± 0.41	2.07 ± 0.03
NaF (100 ppm/kg)	194.90 ± 13.87	126.30 ± 7.06****	0.98 ± 0.03****	0.83 ± 0.05****
Vit E (150 mg/kg)+NaF (100 ppm/kg)	196.30 ± 17.01	198.19 ± 3.43####	1.84 ± 0.16####	1.82 ± 0.05####
HG (40 mg/kg) +NaF (100 ppm/kg)	195.80 ± 16.93	210.40 ± 20.30####	2.04 ± 0.14####a	1.81 ± 0.05####
Vit, E (150 mg/kg)+HG (40 mg/kg) + NaF (100 ppm/kg)	197.60 ± 18.03	240.60± 24.31####ab	2.49 ± 0.35####ab	2.13 ± 0.02####ab

Values are expressed as Mean ± SEM (n=6) (One-way ANOVA followed by bonferroni's post hoc test), ****statistically significant at p<0.0001 when compared with the control group;* ####*Statistically significant at p<0.0001 when compared with the NaF group;* a*Statistically significant at p<0.05 when compared with the Vit. E + NaF group;* b*Statistically significant at p<0.05 when compared with the HG + NaF group*

Table 1b. Curative effect of Vitamin and Homtamin ginseng and their combination in naïve and sodium fluoride invoked changes on body weight, testicular weight and whole brain weight in male Wistar rats

Group	Initial bodyweight (g)	Final body weight (g)	Testes weight (g)	Brain weight (g)
Control	199.30 ± 13.14	263.30 ± 24.01	2.63 ± 0.04	2.01 ± 0.03
Corn oil (2 mL/kg)	198.90 ± 12.81	262.40 ± 23.14	2.63 ± 0.04	1.99 ± 0.06
NaF (100 ppm/kg)	198.60 ± 11.36	114.30 ± 8.14****	0.43 ± 0.01****	0.33 ± 0.02****
NaF (100 ppm/kg) + Vit. E (150 mg/kg)	199.10 ± 14.01	189.60 ± 6.31####	1.92 ± 0.05####	1.67 ± 0.07####
NaF (100 ppm/kg) + HG (40 mg/kg)	197.70 ± 13.60	216.50 ± 16.03####a	2.02 ± 0.04####a	1.72 ± 0.15####
NaF (100 ppm/kg) +Vit. E (150 mg/kg) +HG (40 mg/kg)	197.60 ± 18.03	253.40 ± 20.03####ab	2.57 ± 0.14####ab	2.12 ± 0.18####ab

Values are expressed as Mean ± SEM (n=6) (One-way ANOVA followed by bonferroni's post hoc test), ****Statistically significant at p<0.0001 when compared with the control group;* ####*Statistically significant at p<0.0001 when compared with the NaF group;* a*Statistically significant at p<0.05 when compared with the NaF + Vitamin E group;* b*Statistically significant at p<0.05 when compared with the NaF + HG group*

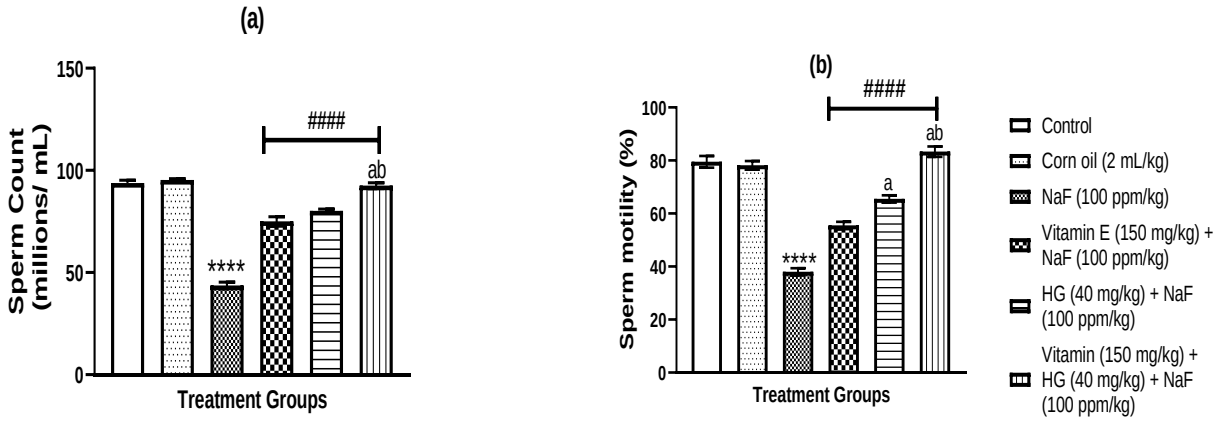
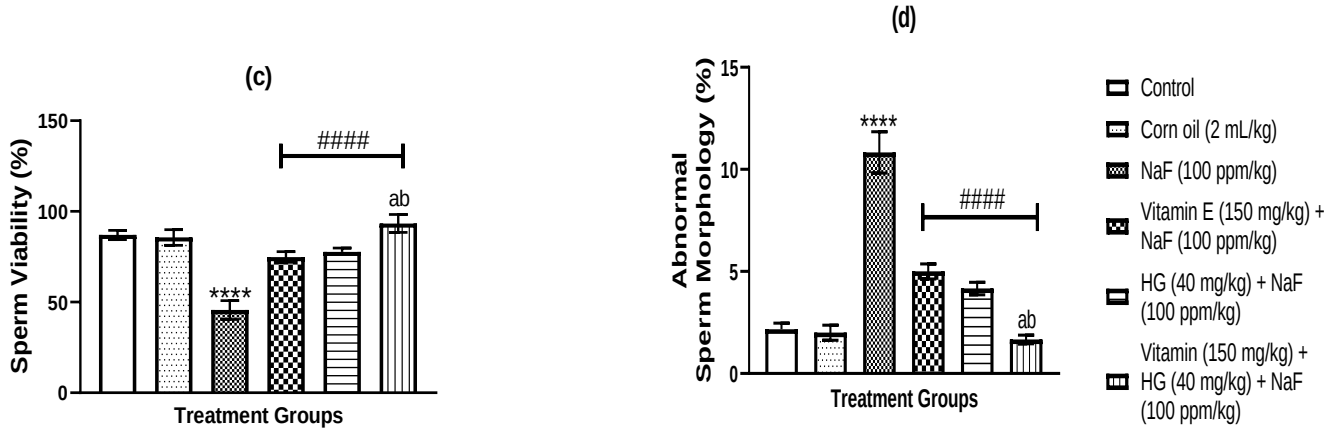


Fig 1. Prophylactic effect of Vitamin E, Homtamin ginseng and in combination on naïve and sodium Flouride-induced alterations in sperm count (a), motility (b), viability (c), and morphology (d) in male rats.
Data are expressed as mean ± S.E.M. (n = 6) (One-way ANOVA followed by Bonferroni's post hoc test). ****P<0.0001 when compared with controls;* ####*P<0.0001 when compared with NaF;* a*p<0.05 when compared with the group given Vitamin E + NaF;* b*p<0.05 when compared with the group given HG + NaF. HG; Homtamin Ginseng; NaF: Sodium Flouride.*



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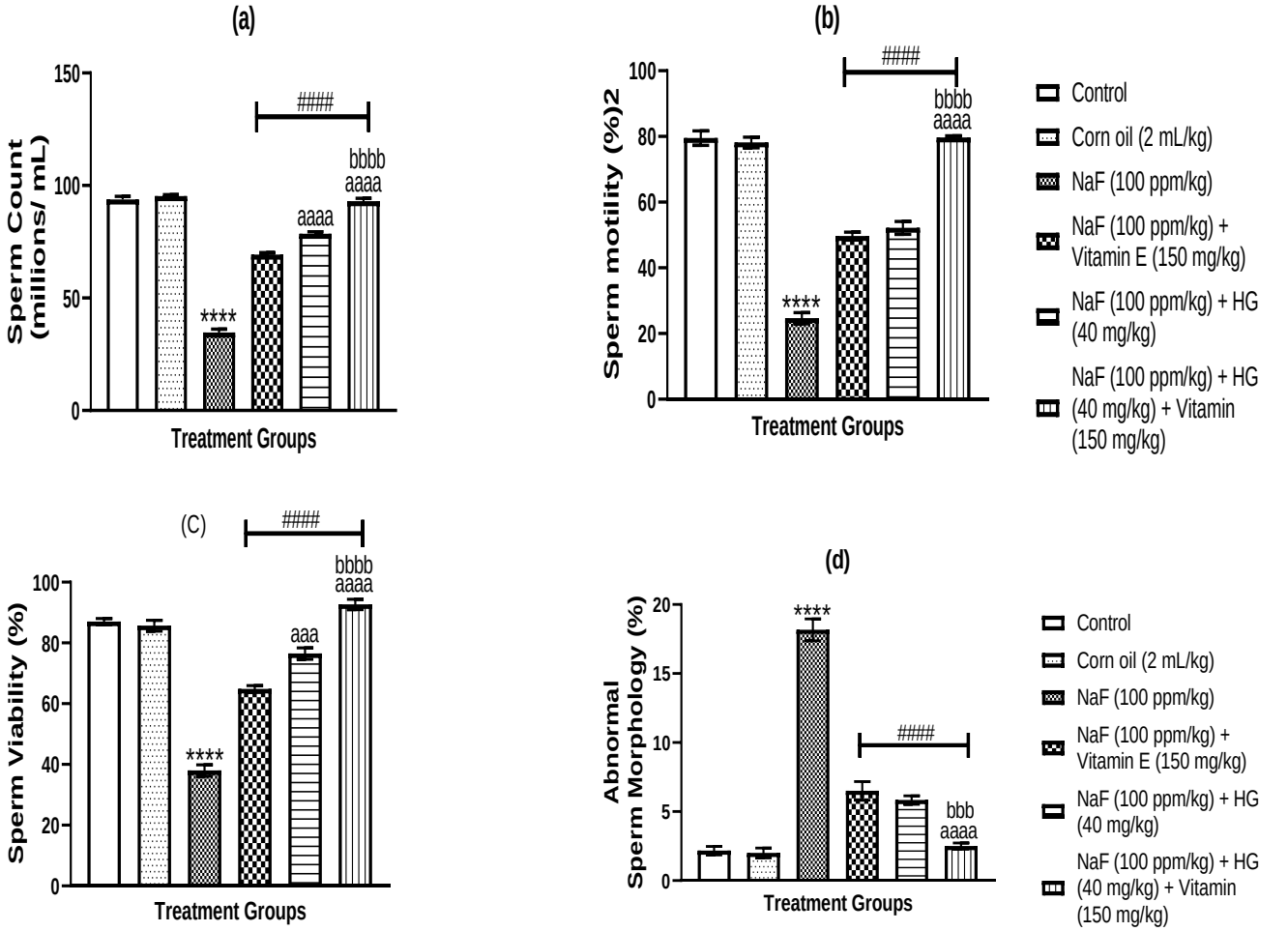

Fig 2. Curative effect of Vitamin E, Homtamin ginseng and in combination on naïve and sodium Flouride-induced alterations in sperm count (a), motility (b), viability (c), and morphology (d) in male rats.

Data are expressed as mean ± S.E.M. (n = 6) (One-way ANOVA followed by Bonferroni's post hoc test). $^{****}P<0.0001$ when compared with controls; $^{####}P<0.0001$ when compared with NaF; $^{aaaa}p<0.0001$, $^{aaa}p<0.001$ when compared with the group given Vitamin E + NaF; $^{bbbb}p<0.0001$, $^{bbb}p<0.0001$ when compared with the group given HG + NaF. HG; Homtamin Ginseng; NaF: Sodium Flouride.

Vitamin E and Homtamin ginseng abate NaF-induced alterations in serum hormonal indices in the preventive and reversal treatments in rats

The study found that exposure to sodium fluoride significantly affected FSH, LH, and testosterone activities compared to normal control groups (Fig 3 & 4). The combination of Vitamin E and HG, with doses of 150 mg/kg and 40 mg/kg, showed a more significant effect on preventing and reversing these effects compared to Vitamin E or HG alone.

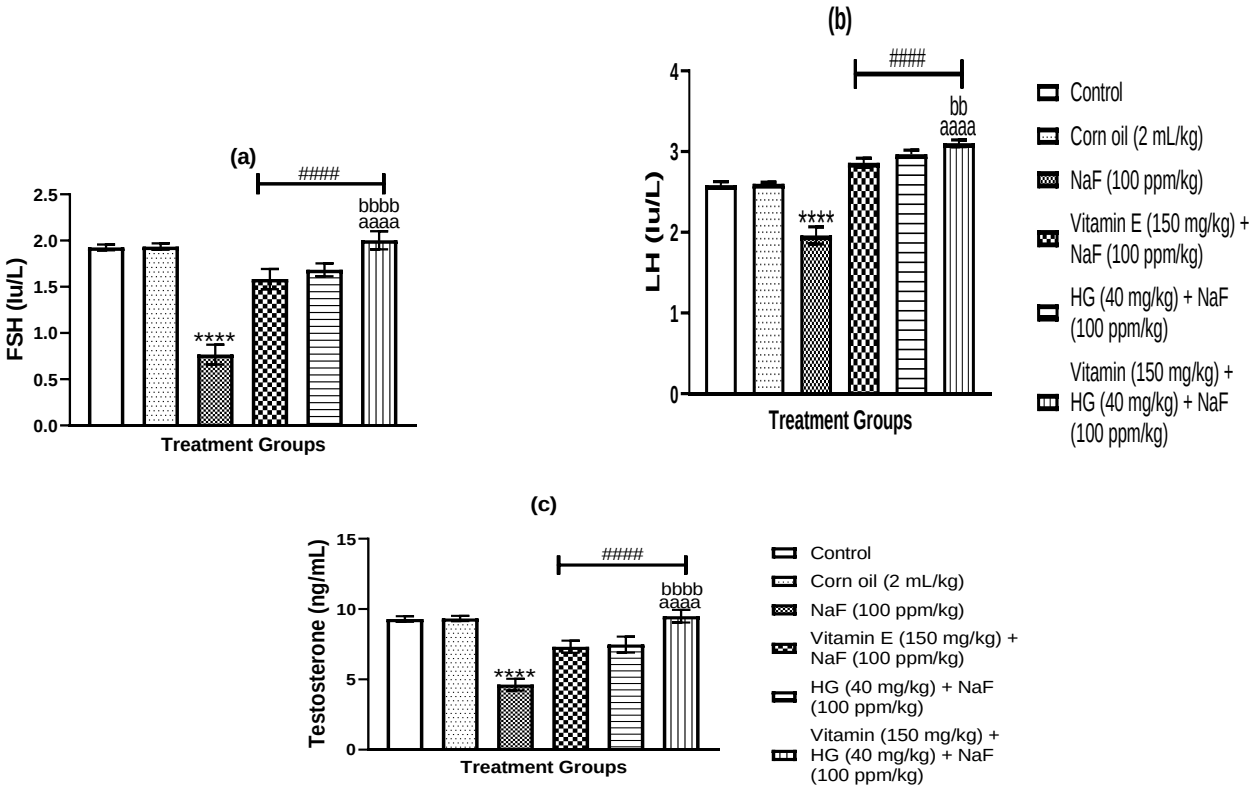

Fig 3. Prophylactic effect of Vitamin E, Homtamin ginseng and in combination on naïve and sodium Flouride-induced alterations in hormonal indices, FSH (a), LH (b), and Testosterone (c) in male rats.
Data are expressed as mean ± S.E.M. (n = 6) (One-way ANOVA followed by Bonferroni's post hoc test). $^{****}P<0.0001$ when compared with controls; $^{####}P<0.0001$ when compared with NaF; $^{aaaa}p<0.0001$ when compared with the group given Vitamin E + NaF; $^{bbbb}p<0.0001$, $^{bb}p<0.01$ when compared with the group given HG + NaF. HG; Homtamin Ginseng; NaF: Sodium Flouride.

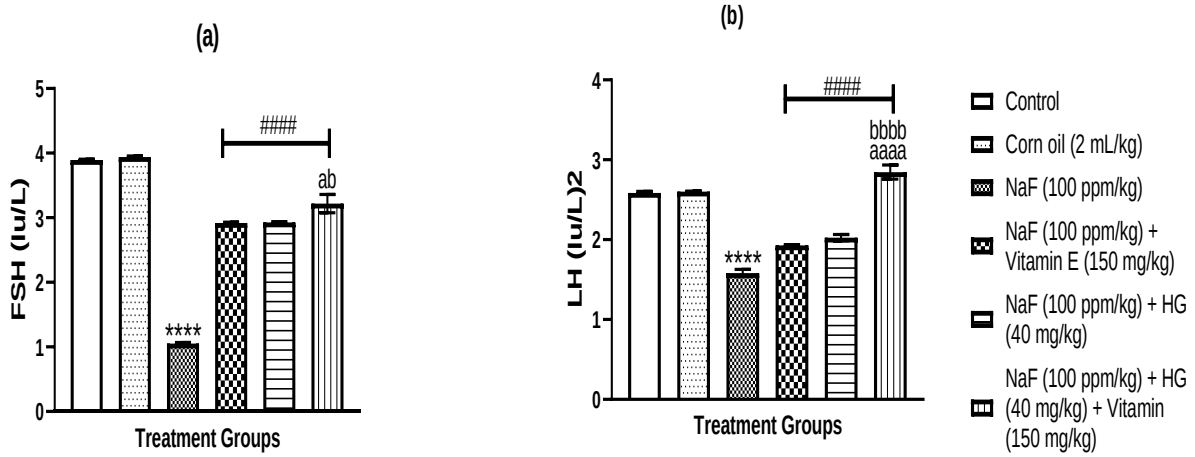

Fig 4. Curative effect of Vitamin E, Homtamin ginseng and in combination on naïve and sodium Flouride-induced alterations in hormonal indices, FSH (a), LH (b), and Testosterone (c) in male rats.
Data are expressed as mean ± S.E.M. (n = 6) (One-way ANOVA followed by Bonferroni's post hoc test). $^{****}P<0.0001$ when compared with controls; $^{####}P<0.0001$ when compared with NaF; $^{aaaa}p<0.0001$ when compared with the group given Vitamin E + NaF; $^{bbbb}p<0.0001$ when compared with the group given HG + NaF. HG; Homtamin Ginseng; NaF: Sodium Flouride.

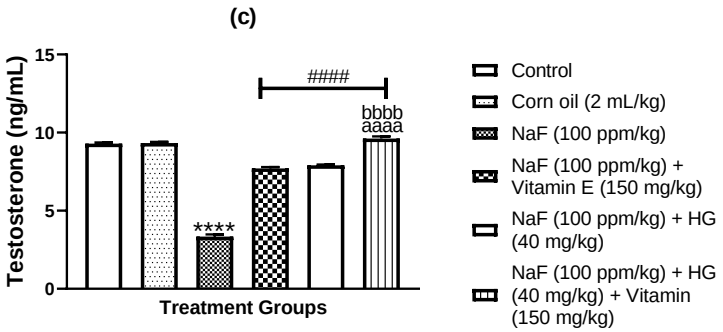

Fig 4. Continued.

Vitamin E and Homtamin gingeng abate NaF-induced reduction in fertility in the preventive and reversal treatments in rats

A reproductive study conducted over 14–28 days was undertaken to assess the impacts of sodium fluoride exposure. For this study, both naive (rats receiving vitamin E and Homtamin ginseng) and control rats were examined. Results from the study indicated that out of the 6 rats that were mated, 5 of them became pregnant, equating to a 100% mating index, and a 83.3% fertility index. The fertility of rats treated with sodium fluoride (NaF) at a dose of 100ppm/kg/day for 14 days was significantly decreased, as illustrated by a 16.7% decrease in fertility of male Wistar rats. The male rats' capacity to pair was also severely compromised, with just one of the six NaF-treated males mating with cohabitated females (resulting in a mating index of 1% and a fertility index of 1%). Table 2a demonstrated that fertility in rats treated with NaF could be prevented by providing vitamin E and HG. Importantly, the combination of vitamin E and HG was associated with a higher rate of normal pregnancy outcomes than that seen in rats solely treated with vitamin and NaF or HG and NaF. In Table 2b, it was observed that treating rats exposed to Sodium Fluoride (NaF) with Vitamin E and HG was able to prevent the decline in fertility that was otherwise seen. Specifically, among the NaF treated rats, 100ppm/kg/day for 28 days, 0/6 mated and 0/6 became infertile, resulting in a mating index of 0% and fertility index of 0%. In contrast, treating rats with Vitamin E and HG revealed no decrease in fertility, with a mating index of 100%, and fertility index of 100%. When compared to the control group, the combination of vitamin E and HG had a greater effect on normal pregnancy outcomes than when just vitamin or HG were used in conjunction with sodium fluoride (NaF).

Vitamin E and Homtamin gingeng mitigates NaF-induced oxidative stress in the preventive and reversal treatments in rats' testes

The results from Figures 5 and 6 indicate that exposure to sodium fluoride (NaF) caused oxidative stress. This was illustrated by an increase in malondialdehyde (MDA) levels when compared to the control group ($p < 0.05$). Additionally, the levels of superoxide dismutase (SOD), glutathione peroxidase (GPx) and reduced glutathione (GSH) were lower than in the control group. Analysis revealed that administering a combination of Vitamin E (150 mg/kg, orally) and HG (40 mg/kg) to rats' testes substantially reduced MDA levels and increased activities of SOD, CAT, GPx, and GSH. These effects were found to be significant (p 0.05) for reversing and preventing NaF-induced oxidative stress. Compared to individual groups of vitamin E (150 mg/kg, orally) and HG (40 mg/kg, orally), a combination of the same amounts of both substances produced a more

Table 2a. Prophylactic effect of Vitamin and Homtamin ginseng and their combination in naïve and sodium fluoride induced reduction in fertility of male Wistar rats treated for 14 days.

Group	Number of males put for mating	Number of females put for mating	Mating Index	Fertility Index
Control	3	6	5/6 (83.3%)	5/6 (83.3%)
Corn oil (2 mL/kg)	3	6	4/6 (66.7%)	4/6 (66.7%)
NaF (100 ppm/kg)	3	6	1/6 (16.6%)	1/6 (16.7%)
Vit. E (150 mg/kg) + NaF (100 ppm/kg)	3	6	4/6 (66.7%)	4/6 (66.7%)
HG (40 mg/kg) + NaF (100 ppm/kg)	3	6	5/6 (83.3%)	4/6 (66.7%)
Vit. E (150 mg/kg) + HG (40 mg/kg) + NaF (100 ppm/kg)	3	6	6/6 (100%)	5/6 (83.3%)

Mating Index = No of females mated/no of females put for mating; Fertility Index = No of pregnant females/no of cohabitated females; % = Percentage; HG = Homtamin ginseng.

considerable effect in preventing and reversing NaF-induced changes in SOD, CAT, GPX, and GSH values (p less than

Table 2b. Prophylactic effect of Vitamin and Homtamin ginseng and their combination in naïve and sodium fluoride induced reduction in fertility of male Wistar rats treated for 14 days

Group	Number of males put for mating	Number of females put for mating	Mating Index	Fertility Index
Control	3	6	6/6 (100%)	5/6 (83.3%)
Corn oil (2 mL/kg)	3	6	5/6 (83.3%)	5/6 (83.3%)
NaF (100 ppm/kg)	3	6	0/6 (0%)	0/6 (0%)
NaF (100 ppm/kg) + Vitamin E (150 mg/kg)	3	6	3/6 (50%)	3/6 (50%)
NaF (100 ppm/kg) + HG (40 mg/kg)	3	6	4/6 (66.7%)	3/6 (50%)
NaF (100 ppm/kg) + Vit. E (150 mg/kg) + HG (40 mg/kg)	3	6	5/6 (83.3%)	4/6 (66.7%)

Mating Index = No of females mated/no of females put for mating; Fertility Index = No of pregnant females/no of cohabitated females; % = Percentage; HG = Homtamin ginseng.

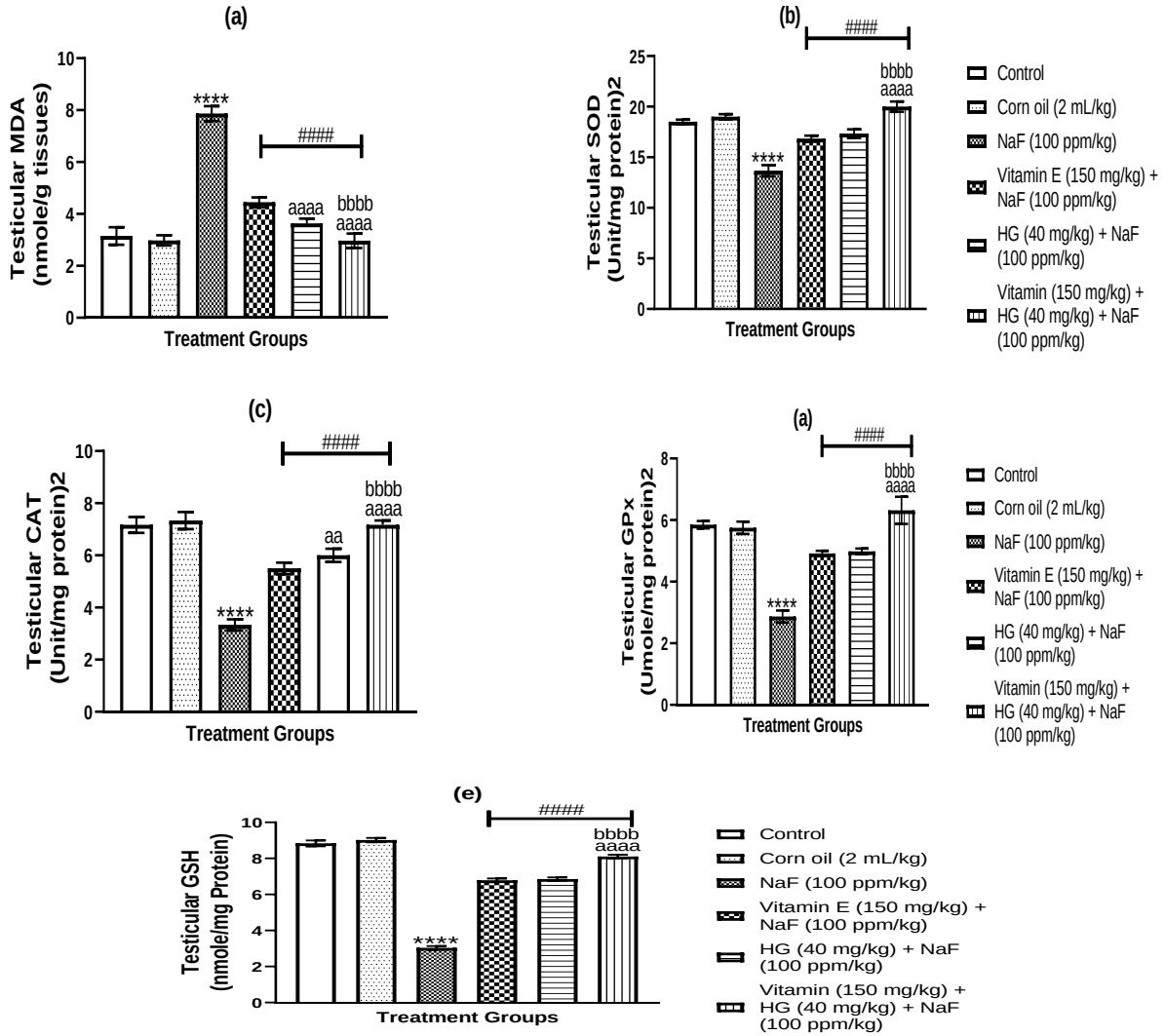

Fig. 5 a-e. Prophylactic effect of Vitamin E and Homtamin ginseng on NaF-induced oxidative stress in rats' testes: malondialdehyde, MDA (a), superoxide dismutase, SOD (b), catalase, CAT (c), glutathione, GPx (d) and glutathione peroxidase, GSH (e) Bars represent the mean ± S.E.M (n = 6).
*One way ANOVA followed by Bonferroni's post-hoc test revealed that there are significant differences between various treatment groups. ****$p < 0.0001$ as compared with control group; ####$p < 0.0001$ as compared with NaF group; aaaa$p<0.0001$ when compared with the group given Vitamin E + NaF; bbbb$p<0.0001$ when compared with the group given HG + NaF. HG; Homtamin Ginseng; NaF: Sodium Flouride (Prophylactic protocol of test drug administration for 28 days followed by NaF treatments from days 15-18).*

0.05).

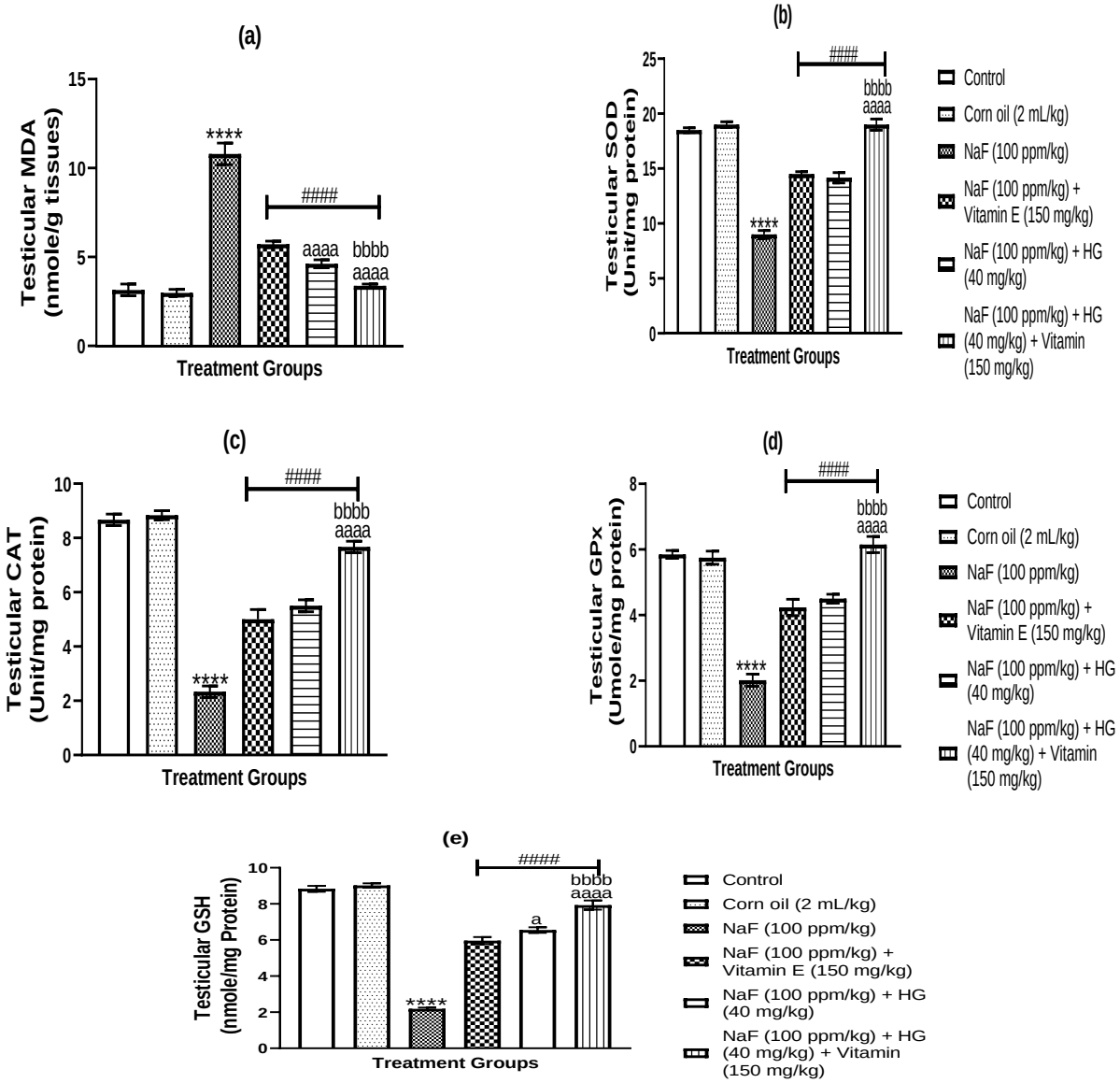

Fig. 6 a-e. Curative effect of Vitamin E and Homtamin ginseng on NaF-induced oxidative stress in rats brain: malondialdehyde, MDA (a), superoxide dismutase, SOD (b), catalase, CAT (c), glutathione, GPx (d) and glutathione peroxidase, GSH (e) Bars represent the mean ± S.E.M (n = 6). One way ANOVA followed by Bonferroni's *post-hoc* test revealed that there are significant differences between various treatment groups. ****$p < 0.0001$ as compared with control group; $^{\#\#\#\#}p < 0.0001$ as compared with NaF group; $^{aaaa}p<0.0001$, $^{a}p<0.05$ when compared with the group given Vitamin E + NaF; $^{bbbb}p<0.0001$ when compared with the group given HG + NaF. HG; Homtamin Ginseng; NaF: Sodium Flouride (Prophylactic protocol of NaF treatments for 28 days followed by test drug administration from days 15-18).

Vitamin E and Homtamin ginseng prevented and reversed NaF-induced histopathological changes of the testes of rats

Compared to control rats, rats given NaF (at a concentration of 100 ppm/kg/day) had a reduced ability to produce sperm, with fewer spermatocytes present in the seminiferous tubules. This was demonstrated by incidences of degeneration, necrosis, and severe blockages in the process of germinal maturation, leading to congestion in the interstitial spaces. Vitamin E (150 mg/kg) and HG (40 mg/kg) appear to be an effective remedy for reducing the detrimental effects of sodium fluoride exposure upon testicular architecture, seminiferous tubules, and spermatozoa (spermatogenesis). This was demonstrated through both prophylactic (Plate 1) and curative (Plate 2) protocols with more improvement in the combined treatment than in the NaF groups.

Slides A and B revealed normal testicular architecture with seminiferous tubules, normal maturation stages with presence of spermatozoa within their lumen (white arrow) and normal interstitial spaces of leydig cells (slender arrow). Slide C revealed very poor testicular architecture with several atrophic seminiferous tubules, cessation of germinal maturation with lack of spermatozoa within their lumen (Black arrow) and interstitial space showing area with congestion and degenerated/necrotic germ cells of the

seminiferous tubules (spanned arrow) and some normal interstitial spaces (slender arrow). Slide D is characterized by a poor seminiferous tubules (black arrow) and more seminiferous tubules with normal maturation stages with presence of spermatozoa within their lumen (white arrow). The interstitial spaces show normal leydig cells with few area

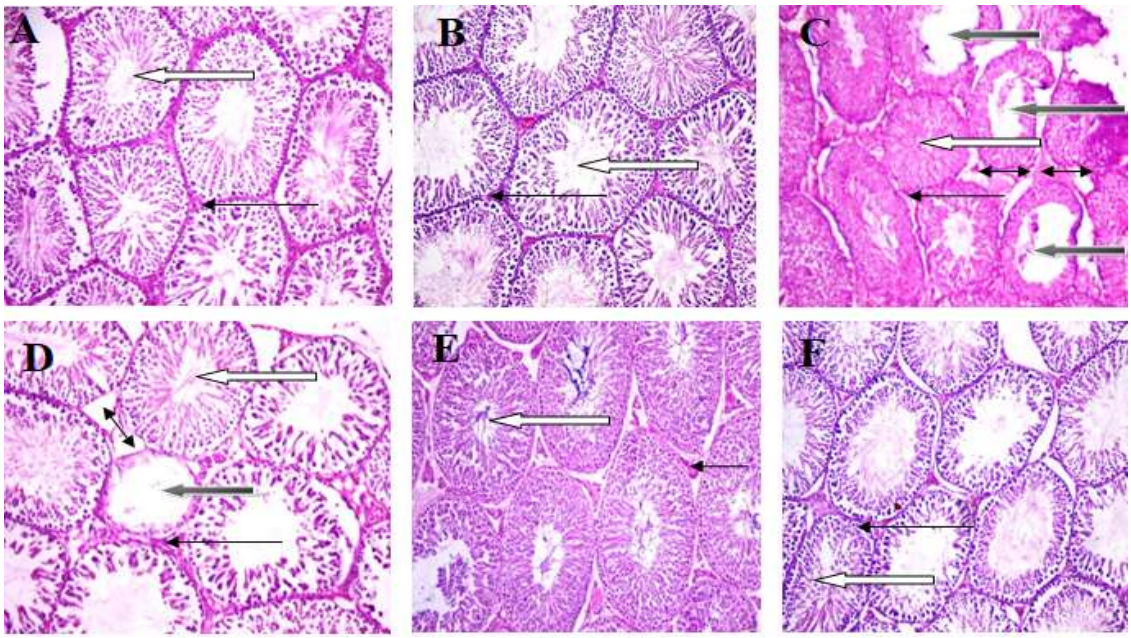

Plate 1. Photomicrographs showing the prophylactic effect of vit. E, Homtamin ginseng (HG) and in combination on NaF-induced histomorphological changes in the testes of rats. Haematoxylin-eosin stain: Original magnification x400, Calibration bar = 0.01 mm (10 µm) for all plates. A: Control (Normal saline); B: 2 mL/kg of corn oil; C: NaF (100 ppm/kg); D: vitamin E (150 mg/kg) + NaF (100 ppm/kg); E: HG (40 mg/kg) + NaF (100 ppm/kg); F: vitamin E (150 mg/kg) + HG (40 mg/kg) + NaF(10 mg/kg).

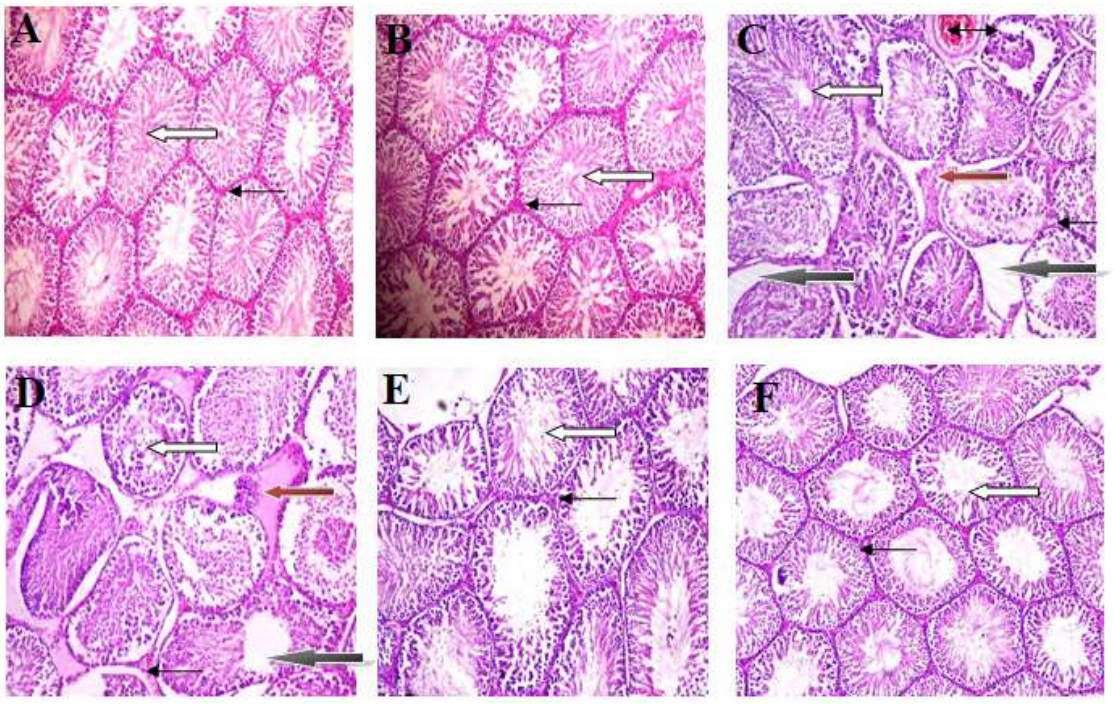

Fig. 5 a-e. Prophylactic effect of Vitamin E and Homtamin ginseng on NaF-induced oxidative stress in rats testes: malondialdehyde, MDA (a), superoxide dismutase, SOD (b), catalase, CAT (c), glutathione, GPx (d) and glutathione peroxidase, GSH (e) Bars represent the mean ± S.E.M (n = 6).
*One way ANOVA followed by Bonferroni's post-hoc test revealed that there are significant differences between various treatment groups. ****p < 0.0001 as compared with control group; $^{\#\#\#\#}$p < 0.0001 as compared with NaF group; ^{aaaa}p<0.0001 when compared with the group given Vitamin E + NaF; ^{bbbb}p<0.0001 when compared with the group given HG + NaF. HG; Homtamin Ginseng; NaF: Sodium Flouride (Prophylactic protocol of test drug administration for 28 days followed by NaF treatments from days 15-18).*

showing necrotic germ cells (spanned arrow). Slide E is associated with normal testicular architecture with normal germ cell layer of the seminiferous tubules and normal maturation stages with presence of spermatozoa within their lumen (white arrow) and normal leydig cells (slender arrow). Slide F revealed seminiferous tubules with normal germ cell layer and normal maturation stages with presence of spermatozoa within their lumen (white arrow) with interstitial spaces showing normal leydig cells (slender

DISCUSSION

Chronic exposure to fluoride has been shown to lead to its accumulation in various parts of the body, mainly in the teeth, bones, muscles, ligaments, and brain [29]. This study explored the effects of vitamin E and homtamin ginseng (HG) on sodium fluoride (NaF) toxicity in male rats [30]. While the therapeutic potential of vitamin E has been previously tested, the use of HG for this purpose has not been adequately addressed in scientific literature. The findings of this study revealed that no toxicity was found in rats when exposed to 40 and 80 mg/kg NaF for four weeks. Therefore, the chosen dose of 40 mg/kg for the prophylactic and curative protocols is likely to be safe for human consumption. Evidence has been provided that NaF does induce testicular dysfunction and reprotoxicity [16]. Previous research has demonstrated the ability for Vitamin E and Homtamin Ginseng to lessen the negative impacts of sodium fluoride exposure on fertility and reproductive function [31,32,33,34]. This study appears to be the first looking into the effects of Vitamin E and Homtamin Ginseng on the damage that NaF can cause on testicular dysfunction and reproductive impairment.

This research study showed that the weight of the testis and body weight, which are important assessments of testicular dysfunction and reproductive damage, decreased drastically in creatures that were given NaF. These findings are similar to the studies by Miltonprabu and Thangapandiyan [35] and Hong et al. [56]. In this research, it was determined that NaF caused harmful reproductive effects on male rats, such as decreased levels of gonadotropin and steroidogenesis. Additionally, there was a decrease in semen quantity and quality, along with lowered mating and fertility indices. This decrease in testicular weight reflects a damaging effect on the seminiferous tubules [37]. Research has indicated that decreased body weight can be attributed to the creation of reactive oxygen species (ROS) and increased breakdown of cellular proteins that result from exposure to sodium fluoride (NaF) [38,39]. However, incorporating Vitamin E and HG into the treatment plan can reverse these adverse effects, restoring body weight and organ weight back to normal values in comparison to the control group. It appears that a combination of Vitamin E and HG has the potential to lessen the effects of NaF-induced reproductive toxicity due to their powerful antioxidant and antiradical properties. Furthermore, the decreased testicular weight could be attributed to the reduction in testosterone levels and disruption of the sperm production process. Abnormal sperm morphology has been linked to fertility issues, as observed in laboratory bioassays [41, 42]. The effects of NaF administration could potentially lead to a wide range of reproductive impairments, including degraded sperm morphology. Therefore, it is important to understand the implications of NaF exposure and recommend tailored remedy plans to counteract its effects. A number of changes have been observed at the cellular level as a result of sodium fluoride exposure, such as abnormal sperm morphology, shifted basal and stimulated intracellular calcium concentration, elevated creatine kinase levels, and increased reactive oxygen species (ROS) production [42]. These changes can in turn, cause DNA fragmentation and a loss of sperm function [40].

Previous research on rats treated with NaF suggests that there is a considerable reduction in sperm count in the cauda epididymis. This is in agreement with Elghareeb et al's [43] and Pal [44]'s findings. Consequently, male fertility is significantly impacted due to the decreased sperm count and motility, which are essential in testicular spermatogenesis and epididymis sperm maturation. Long et al. [43] investigated the effects of sodium fluoride (NaF) exposure on rats, finding a significant decrease in motile and progressively motile sperm in the cauda epididymis. Additionally, the researchers noted a significant decrease in testes weights, as well as disturbances in the spermatogenesis process, including complete loss of all stages of germ cell maturation, atrophy, and degenerative changes in the seminiferous tubules. In rats that were pre-treated with both Vitamin E and HG, normal histological structures were present in the seminiferous tubules and intestinal tissues, in addition to a full spermatogenesis cycle seen in the lumen.

It is important to consider that environmental exposure to sodium fluoride (NaF) has been associated with reduced fertility in male animals. Additionally, spermatozoa with abnormal morphology have been observed in some cases of male sterility. During a trial period of 14-28 days, significant drops in fertility were found in male rats when exposed to NaF - a reduction of 83.4%. In the reversal protocol, it was found that when NaF-treated rats were subjected to a 28 day treatment schedule, the fertility of the rats decreased significantly. This was demonstrated by a sharp decrease in both the mating index and fertility index of female rats when paired with the NaF-treated male Wistar rats, with the mating index dropping to 0% and the fertility index also decreasing to 0%. Studies such as Long et al. [43] have demonstrated that sodium fluoride (NaF) can have a direct effect on the testes and epididymis, resulting in lower sperm counts, motility, and viability in rats. This mechanism has been attributed to NaF interfering with the tight junctions of the epididymis, which would alter the blood-epididymis barrier and modify the epididymis luminal fluids. Research from other sources [43,44] have provided further evidence to support this hypothesis. The current study suggested that the combination of Vitamin E and HG could help to reverse and protect against the effects of sodium fluoride exposure.

The results of the present study suggested that NaF-exposure causes oxidative stress in the testes [45,46], resulting in a reduction in serum testosterone levels and damaging the testicular architecture. Administration of

Vitamin E and HG have been proven effective in reversing this damage, by restoring the architecture of the testis.

The research findings suggested that the administration of sodium fluoride (NaF) to male rats may have caused oxidative damage to their testicular function. This was demonstrated by the presence of increased malondialdehyde (MDA) levels and decreased activity of antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) in the testicular tissue. Additionally, lower serum gonadotropin levels were observed, providing evidence of oxidative-induced testicular dysfunction.

Reports of decreased levels of gonadotropins and testicular steroids have been observed in male rats following administration of sodium fluoride (NaF). This reduction in these variables is indicative of suppression of the hypothalamic–pituitary–gonadal axis, and is consistent with previous studies that have documented decreases in these variables following NaF administration in male rats [46]. This study found that the co-administration of Vitamin E and medicinal honey (HG) restored serum gonadotropins and testosterone concentration, sperm quality and resulted in a high mating and fertility index, which may be attributed to the antioxidative properties of Vit. E and HG in the testicles. Additionally, the delivery of sodium fluoride (NaF) was associated with a decrease in antioxidant enzyme activities and an increase in testicular lipid peroxidation. It is likely that sodium fluoride (NaF)'s negative impacts on the testes could be associated with both its direct toxic effects on the testes cells as well as its indirect effects on testosterone production. Additionally, in trials examining preventative and curative measures, treatment with both vitamin E and HG have resulted in a significant increase in antioxidant enzyme activities (superoxide dismutase, glutathione peroxidase, and

CONCLUSION

Our current research has conclusively illustrated that exposure to sodium fluoride (NaF) has a number of detrimental effects, including inducing oxidative stress (through the suppression of antioxidant enzyme activity), decreasing body and testicular weight, hampering spermatogenesis, and reducing levels of gonadotrophins, steroid hormones, and fertility. The study concluded that the combination of HG and Vit. E provided significant protection against NaF-induced deterioration of testicular function. Vitamin E and homtamin ginseng has shown potential synergistic effects. This Study have shown that vitamin E can enhance the bioavailability and absorption of ginseng, leading to increased effectiveness. Additionally, both of these supplements have antioxidant properties which can work together to provide a stronger defense against free radicals and support overall health. Interestingly, their histo-cytoprotectant, antioxidant, and gonadotropin modulating properties could prove to be a potential remedy against NaF exposure. Thus, a tailored remedy plan of HG and Vit. E may be recommended to counter the impacts of sodium fluoride

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Data Availability: The authors confirm that the data supporting the study's conclusions are included in the article.

Ethical Approval: This study followed the National Institutes of Health's guidelines for the care and use of laboratory animals (NIH Publications No. 8023, revised 1996)

Consent to Participate: Not applicable.

Consent for Publication: All authors agreed to publication.

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