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Original article

Effects of Ginger Extract on Reducing Abnormalities in Sperm Morphology Induced by Cyclophosphamide in Male Rats

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Abstract
The drug cyclophosphamide CP has harmful genetic effects when used at a dose of 5 mg/kg body mass. For this reason, this study was conducted to determine these damaging effects and to reduce them using ginger metabolic extract. The methodology, linked to four groups of seven animals each, was used in the experiment. The first group, the control group, consisted of negative control rats fed a regular meal and purified water. In contrast, the second group was given oral cyclophosphamide at a daily dose of 5 mg/kg body mass for four weeks. In the third group (the test group), rats received 200 mg of ginger extract per kilogram of body weight daily for four weeks. The fourth group, the protective group, received two phases of treatment: first, 200 mg/kg body mass of ginger extract was given, and then, two hours later, CP (5 mg/kg body mass daily) was administered orally for four weeks. In the CP group, notable changes in sex cells were observed. These results describe the chromosomal effects of CP free radicals. Chromosome aberrations are reduced compared to the test group. When ginger extract is used. CP negatively impacts sperm cells. Sperm cells indicate that CP at 5 mg / Kg causes an increase in these aberrations in sperm cells when rats are treated with CP. The current study concludes that the ginger extract had an antimutagenic effect when given at 200 mg / Kg before the CP. It can be used as a protective and therapeutic treatment agent since it has a preventive effect against the mutagenic effects of CP.
Keyword. Ginger Extract, Reducing Abnormalities, Sperm Morphology, Cyclophosphamide, Male Rats.

Introduction

Cyclophosphamide can cause oxidative stress in the reproductive system, which hurts sperm quality and quantity. It is frequently used in immunosuppressive diseases and cancer treatment in both young and elderly individuals (Al-Niwehee, 2019; (Floyd et al., 2005). Increased testicular oxidative stress is an important part of the genesis of infertility in men since it might result in modifications to endocrine signalling and testicular microvascular blood flow patterns (Turner et al., 2008). Also, oxidative stress on seminiferous tubules and Sertoli cells hinders androgenesis and spermatogenesis, producing germ cell apoptosis (Rezvanfar et al., 2008); (Turk et al., 2010). With a decrease in the size of the testicles and epididymis, a drop in the count of spermatogonia within the seminiferous tubules, a drop in testosterone levels, and ultimately infertility (Rezvanfar et al., 2008). Also decreases their ability to reproduce (Trasler et al.1986); (Ichikawa et al., 2010). The amount of germ cells produced determines the testicular weight, so weight reduction could be a sign of a decline in these (Katoh et al., 2008). By altering sperm morphology, decreasing concentration and motility, and raising sperm DNA fragmentation, oxidative stress can impact testicular functioning (Ali et al., 2020). The medication suppresses the immune system in smaller dosages (Avendano & Menendez,2008).

By altering DNA purine base cross-linking, CP prevents the production of DNA, RNA, and proteins and the demise of cells that divide quickly (Wetzels, 2004). Numerous diseases are known to be influenced by free radicals and the lipid peroxides they produce (Ayhanci et al., 2019). The accumulation of free radicals from an unbalanced antioxidant system may originate from these negative effects. Free radicals can lead to chromosomal abnormalities and DNA fragments (Mott et al., 2021); nonetheless, it is linked to several adverse effects on the reproductive system (Pavin et al., 2018). In many cultivated cells, cyclophosphamide causes sister chromatid exchanges in metabolic activity (Suchitra et al., 2011; Paithankar et al., 2014). Based on the study carried out by (Tripathi & Jena 2008) (ceribaşiet al. 2010), cyclophosphamide administration causes anomalies in the sperm head's morphology, revealing that mature male rats given cyclophosphamide developed aberrant tail sperm, while adult male mice treated with cyclophosphamide developed defective sperm head morphology (Nayak et al., 2015; Al-Niwehee, 2019). Zingiber officinale, known as ginger, is a member of the Zingiberaceae family and is an herb that has a strong, fragrant flavour. The roots of ginger serve as a flavouring in food and drinks and in traditional medicine to cure rheumatism, bronchitis, waist discomfort, and fever.

According to Stoilova et al. 2003), Chinese medicine uses ginger rhizomes to treat stomachaches and as a stimulant or tonic when macerated in alcohol. According to reports, plants in this family contain antihyperglycemic, anti-inflammatory, anti-ulcerating, antioxidant, and antimicrobial properties. The ginger rhizome (Zingiber officinale R., Zingiberaceae family) is a widely used spice. Ginger has both androgenic and antioxidant properties in animal studies (Phan et al., 2005). Vitamins such as B3 and B6, volatile oils, acids, resins, choline, folic acid, inositol, gingerol, pantothenic acid, and sesquiterpenes are among the many substances it contains, as well as elements similar to K, Mg, Ca, and P, K (Morakinyo et al., 2010).

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Ginger oil has demonstrated a dominant protective effect in preventing harm to DNA caused by H2O2, and it may be utilised as an oxygen radical scavenger and antioxidant (Phan et al., 2005; Zahedi et al., 2010).
Additionally, Zingiber contains various antioxidants, including flavonoids, flavone glycosides, rutin, alkaloids, terpenoids, and beta-carotene (Ghasemzadeh et al., 2010). It was shown that phenolic compounds may stop lipid peroxidation by reactive oxygen species and scavenge reactive oxygen species, such as hydroxyl radicals or superoxide anions. Strong antioxidants like ginger can prevent or reduce the production of reactive oxygen species. It is considered a safe, natural medication with limited unfavourable effects that positively impacts rats' male reproductive systems in doses (Al-Yahya et al., 2022). After consuming ginger, the author noted a rise in testosterone, motility, viability, and epididymal sperm count, as well as a drop in malondialdehyde, because various studies have shown that the ingestion of ginger extract raises testosterone levels (Morakinyo et al., 2010). Zinger may have beneficial impacts on reproductive processes due to both its robust antioxidant and androgenic qualities. However, little is known regarding ginger extract's influence on rat testis treated with CP. Even though it is now widely acknowledged that ginger promotes fertility (Ali et al., 2020), this work aims to examine the potential of ginger extract to shield male rats against cyclophosphamide-induced genetic harm. The goal of the research lies in two points.

1. To assess the mutagenic effects of cyclophosphamide on Sperm cell mutations.
2. To estimate the potential anti-mutagenic and protective action of ginger extract on abnormalities in sperm.

Related Work

The findings showed that CPA altered the testis' histological characteristics, resulting in blood vessel congestion, cytoplasmic vacuolation, degradation of germ cells, and a reduction in the seminiferous tubules' diameter and height of the germinal epithelium; both the apoptotic marker caspase-3 and the cell growth marker were downregulated. The biochemical results showed that testosterone and LH levels had decreased, and a rise in the marker for lipid peroxidation, MDA, and a decrease in the blood activity of SOD and CAT antioxidant enzyme. The histological structure of the testis improved in rats given CPA and fennel oil. MDA levels dropped while LH, testosterone, SOD, and CAT levels increased. Giving fennel oil to male rats was found to protect against the reproductive toxicity produced by CPA. One or more of fennel oil's components may induce antioxidant defence systems, which could explain the oil's protective properties (Sakr et al., 2017; Bhattarai et al., 2001). They employed three doses with varying time intervals to ascertain the effects of small and big amounts of cyclophosphamide. The findings demonstrated that while high doses resulted in substantial tissue changes in the testes, low doses for extended periods led to a significant rise in the proportion of tertatospermia, or aberrant sperm heads, without appreciably changing tissue sections. When the dosage was 5 mg/kg, the proportion of aberrant sperm heads increased to 20.72% in the testes, but less than the 5 mg/kg dosage at 15 mg/kg and 10 mg/kg. The percentage of anomalies in the head sperm was 14.75 and 13.19, respectively (Wtwt et al., 2015).
In this work, mature male mice were administered 10 mg/kg of cyclophosphamide on the eleventh embryonic day to examine the consequences of one medication dosage on sperm parameters. Two groups of adult female NMRI mice who were pregnant were given saline as the control group and 10 mg/kg of cyclophosphamide on the eleventh day of gestation. Male mice were sacrificed 60 days after the babies were born, and sperm were taken from the cauda of the epididymis for a study on sperm parameters. The administration of cyclophosphamide produced an important reduction in motility, sperm count, viability, and body and testicular weight ($p < 0.05$) and an increase in abnormalities of the sperm head, neck, and tail ($p < 0.05$). It was determined that CP has a detrimental impact over time on sperm characteristics, effects that may last past puberty (Al-Niwehee, 2019). Exposure to cyclophosphamide or any of the following: bleomycin, etoposide, cisplatin, or other medications used as a testicular cancer treatment.
Four anticancer medications caused a notable rise in DNA damage. Therefore, etoposide did not directly affect telomeres, and only the pair of alkylating agents generated telomere dysfunction, 4OOH-CPA and cisplatin, even though all four anticancer medicines led to damage to the DNA in this spermatozonial cell line; this telomere malfunction may be linked to infertility and birth abnormalities in the progeny (Liu et al., 2014). Using conventional chemotherapy to deal with the growing number of cancer cases has given rise to several contradictory concerns, as the treatment has numerous unpleasant side effects in addition to its curative advantages (Toale et al., 2016). Men use cyclophosphamide annually to treat autoimmune disorders and different types of cancer. While this treatment frequently helps the tumour shrink, it also results in infertility and testicular apoptosis brought on by the drug's metabolite, acrolein (Fusco et al., 2021). Since ancient times, ginger has been used in medical systems around the world to treat conditions like the common cold, nausea, headaches, digestive disorders, upset stomach, diarrhoea, rheumatic conditions, asthma, parasitic infections, arthritis, and muscular soreness (El-Borm et al., 2023).
Gingerols and shagaols, which show anti-carcinogenic, anti-inflammatory, and antioxidant properties in "in vitro" and "in vivo" systems, are abundant in ginger extracts (Abd EL-Monem & Elwakeel, 2020). A study by (Aziz et al., 2020) demonstrates how plants with antioxidant-rich substances, such as flavonoids, can reduce the toxicity of cyclophosphamide to testicles. It was shown that ginger possesses both androgenic and antioxidant qualities in animal models that influence DNA damage

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brought on by H2O2; it might also be an oxygen radical scavenger and an antioxidant (Phan et al., 2005). Utilised to protective effects of red beetroot juice and ginger extract against testicular and cytogenetic damage produced by cisplatin. Sixty mature male albino rats were allocated at random to six groups; each group received one daily dose of the medicine for five weeks, four days a week; samples were collected after the experiment, catalase (CAT), and the amounts of reduced glutathione (GSH), and malondialdehyde (MDA). The animals given cisplatin demonstrated a substantial rise in MDA and a noteworthy decrease in catalase activity when the levels of MDA, reduced testosterone hormone, and glutathione (GSH) were measured.

A significant improvement was observed in animals receiving cis therapy when GE or BRJ were administered concurrently. In cis-treated mice, a substantial drop in blood testosterone levels was significantly enhanced by co-administering GE or BRJ. When GE or BRJ were co-administered, the cis-treated mice showed a considerable improvement in tail abnormalities and sperm head and a noteworthy reduction in sperm count and motility. When GE or BRJ were administered concurrently with cis treatment, the histopathological lesions in those animals ultimately improved compared to GE and BRJ. Previous research shows ginger improves sperm parameters and viability, including count, morphology, motility, and DNA integrity. It also reduces oxidative stress, breaks down oxidative chain reactions, and modifies gonadotropin hormones (FSH, LH), sex hormones, and free radical production (such as testosterone). Based on studies conducted on different species, ginger appears to possess potent antioxidant qualities (since it contains active phenolic components) and androgenic potential. The utilisation of ginger boosts every specific sperm fertility indicator and dramatically improves their biological parameters (total motility, normal morphology, middle, number, and survival rate).

A sperm's standard head and tail morphology and more compact chromatin are the result of ginger's androgenic and antioxidant qualities (Gholami et al., 2021). Numerous studies recommend ginger as a useful remedy for nausea during periods of pregnancy (Bryer, 2005; Abu Baker, 2013). To determine whether ginger could protect male rats receiving CP from the negative effects of cyclophosphamide on their testicular histopathology, thirty male albino rats weighing between 300 and 350 grams each were used in the investigation. The creatures were split up into the subsequent three groups: Group 2 (CP group; received a single intraperitoneal dosage of CP at 100 mg/kg-1 BW), Group 1 (control, untreated group), and Group 3 (CP+ginger, which for 35 days following CP injection got 500 mg/kg of oral ginger extract), and their testicular morphology and histology were compared, compared to the controls, the germinal epithelium in the CP-treated group was disorganized, the group given CP and ginger demonstrated a notable restoration of the cellular linkages and germinal epithelium organization. The CP group showed a substantially higher quantity of Caspase-3-positive cells, while the CP+ginger-treated group had remarkably reduced levels of these cells; rats exposed to CP-induced poisoning can retain their reproductive capacities thanks to ginger extract (Ali et al., 2020).

The CP-treated group showed a decrease in the seminiferous tubules' width and connective tissue breakdown. These alterations were improved in the (CP)+ginger-treated group, indicating that 500 and 1000 mg kg1 B.W. of the ginger extract positively impact rats' male reproductive systems (al-Yahya et al., 2022). Looking at Zingiber officinale extract's ability to shield rat testicles following chemotherapy using Histological and metabolic parameters evaluated in rats treated with cyclophosphamide with and without the ingestion of ginger extract. Ten male rats were randomly split into four groups; one intraperitoneal injection of one millilitre of isotonic saline was given to the control group. One Intraperitoneal dose of Cyclophosphamide (100 mg kg1 BW) was administered to the Cyclophosphamide (CP) group, and the CP + 300G mg and CP + 600G mg were administered orally for a duration of 6 weeks after the CP injection; their histology and testicular morphology were compared.

Serum parameters included testosterone levels, antioxidant capacity, reactive oxygen species, and malondialdehyde. The results showed that serum levels of antioxidants and testosterone were significantly higher than in the CP group and that the CP + 300 and CP + 600 groups displayed histological changes. The ginger extract could not change testis weight, malondialdehyde (MDA), or reactive oxygen species. The ginger plant shields the rat testis from cyclophosphamide following treatment because it has a higher blood content of antioxidants. (Mohammadi et al.2014).

Materials and Methods

The current research will be completed at the zoology department of the Faculty of Science at Omar Al-Mukhtar University.

Chemicals

The medication cyclophosphamide was supplied as a 50 mg tablet by Baxter Corp. The medication was given orally to the animal after being dissolved in ultrapure distilled water. Eosin stain, Methanol, Ethanol, Sodium chloride, potassium chloride, and puffer phosphate.

Plant Material

The ginger plant utilised in this research was sourced from a local market in Albeda, Libya.

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Experimental Animals

The animal house yielded twenty-eight adult male albino rats for use in Tobruk City. After that, the rats were moved to the home for animals at the zoology department at the University of Omar Al Mukhtar. Their weights, ranging from 200 - 300 g, will be used to carry out the current work, and wood shavings will be used for bedding, which will be altered twice a week for the duration of the trial. The rats will be placed on a particular diet and given an adaptation time of two weeks. Fresh food and water supplies will be provided to the rats every day. Following the animals' acclimation, they will be split into four primary groups and kept in four cages, each holding seven rats. The rats will be randomly chosen, and their tails will be painted to allow for individual identification. After the rats are weighed, the dosage is determined based on their body weight, and they are then separated into four groups of seven animals, separately at random, as follows.

Group 1. For four weeks, the only food given to the negative control rats was the regular diet and distilled water.

Group 2. Rats got cyclophosphamide at an amount of 5 mg/kg body mass daily by oral route for 4 weeks (Wtwt et al. 2015)

Group 3. rats given ginger extract at a 200-mg dosage /kg body mass (Badawy et al. 2019; El-Borm et al. 2023), orally daily for 4 weeks

Group 4. protective group; this group will be divided into two steps: given that ginger extract at an amount of 200 mg/kg body weight, primarily followed by ginger extract, then given cyclophosphamide 2 hours later at an amount of 5 mg/kg body mass every day orally for 4 weeks. (Table 1) offers the experimental design.

Table 1. Experimental design

Groups	Types of treatment	Dose mg/Kg	Period of treatment
Control	Distilled water and standard diet	2 ml	4 weeks
Treatment	Cyclophosphamide	5 (Wtwt, et al. 2015)	4 weeks
G	Ginger	200 (Badawy,et al., 2019), (El-Borm,et al., 2023)	4 weeks
Protective	G then Cyclophosphamide	200 en 5	4 weeks

Preparation of Ginger Extract

The gathered plants will be properly cleaned with tap water, then cleaned with distilled water, dried, and ground . 200 g of ground ginger powder will then be placed in 400 millilitres of ethanol at a concentration of 95% by maceration. In an opaque bottle, it is hermetically sealed and rotated for 9 hours with continuous shaking every 6 hours. The solvent will be removed from samples by rotary evaporator, then filtered and done, and kept in the refrigerator for use (Abdul-hanif et al., 2005; Ajith et al., 2007).

Sperm Morphology Assay

Preparation of sex cells sperm percentage of rats: To obtain sex cells, the method of (Coles, 1980) was followed with some modifications, where the skin was cut below the abdominal cavity of the animal, and the testes and epididymis were extracted after removing the tunica albuginea of the testicle and cutting the testicles. It was mashed in a solution of phosphate buffer (saline-PBS) (8 g of NaCl, 0.015 g of Na2Hp04, 0.2 g of KH2PO4, and 0.2 g of KCl). A slide smear; after this, Eosin or Hematoxylin dye was used to stain the slide for 15 minutes. Then the slide was washed with ethanol to remove the dye, and the slide was examined microscopically for abnormalities in the sperm.

Statistical Analysis

With Minitab software (version 22.0), statistical analyses were performed, and a difference was deemed significant if $P < 0.0$. The three replicates' mean ± standard deviation is presented as the result. One-way ANOVA with a significance level of $P < 0.05$ and Tukey's test were used to assess statistically significant differences in the experiments conducted in the different tests.

Results

Mean of Sperm Defects of Male Rats Given CP Treatment (5 mg/kg)

Study of sperm defects of rats showed different mean values between groups. The ginger group showed the lowest mean (3.614±2.22) compared to other groups, followed by the control group (5.687±3.60), G+C group (6.232±2. 19) and cyclophosphamide group (16.65±5.94), respectively. Between the cyclophosphamide group and all other groups, there were significant differences at a significant level ($p<0.001$), with values of the difference between the levels ranging from 10-13 values (Table 2).

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Table 2. Mean of sperm defects of male rats given CP (5 mg/kg)

Groups	N	Mean ± SD
N.C	15	5.687±3.60[b]
CP (5 mg/kg)	30	16.65±5.94[a]
G (200 mg/kg)	35	3.614±2.22[b]
G 200 + CP 5(mg/kg)	25	6.232±2.19[b]

ANOVA of Sperm Defects of Male Rats Given CP Treatment (5 mg/kg)

The mean differences in sperm defects in rats were evaluated by measuring the significant differences between their different groups. Significant differences ($F = 65.48$, $p < 0.001$) between the means of sperm defects of rats of different groups were detected (Table 3). These significant differences explain the harmful effects of cyclophosphamide on the sperm of rats. (Figure 1) shows the interval plot of the mean sperm defects of the rats of each group, which were calculated using individual standard deviations.

Table 3. ANOVA of sperm defects of male rats given CP (5mg/kg)

Source	DF	AdjSS	AdjMS	F-Value	P-Value
Groups	3	3068	1022.54	65.48	0.001
Error	101	1577	15.62		
Total	104	4645			

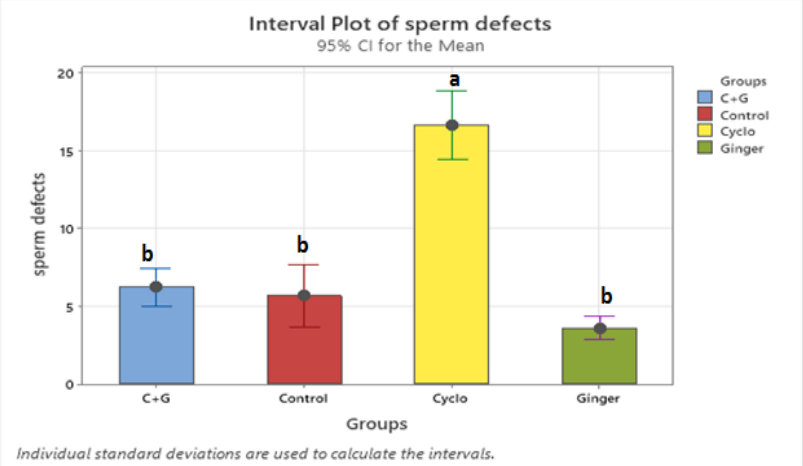

Figure 1. Sperm defects of male rats given CP (5mg/kg)

Abnormality of Sperm Morphology of Rats Given Cyclophosphamide and The Effects of Ginger Extract

The results obtained from this study to evaluate sperm morphology defects are given in (Figure 18), which show various sperm abnormalities in comparison to male control rats that were negative.

Control Group

The typical head of rat sperm has a less dense tip called the acrosome, and it resembles a hook with a dense nucleus. A sheath of mitochondrial material and centrioles wound in a spiral are located in the midpiece. When the spermatozoon reaches maturity, a long axial filament in the tail briefly becomes vibratile. It should be observed that occasionally, around halfway along the tail, the centrioles may be seen. This should be considered as something other than an anomaly. FP presents the regular sperm shape of the rats in this work compared to the CP group.

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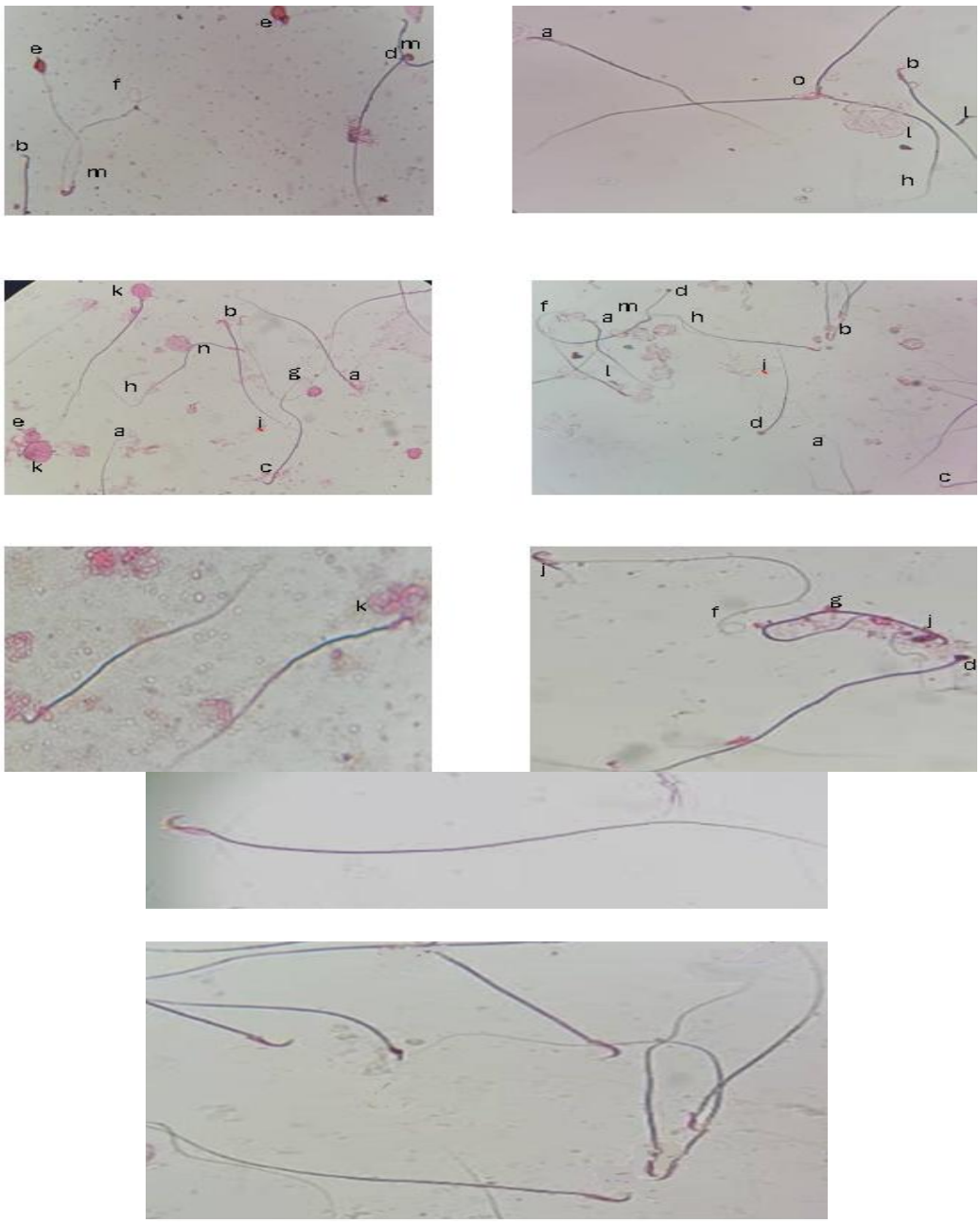

Figure 2. Photograph of typical sperm head and tail of control male rats (Eosin stain, 1000x)
Figure 3. Photograph of abnormal sperm head and tail of male rats given CP 5(mg/kg) (Eosin stain,1000X).

Cyclophosphamide Group

Male rats were administered a daily dosage of CP (5 mg/kg) orally for four weeks. Upon examining the sperm treated with CP. It was noted that CP resulted in morphological alterations in contrast to the control animals. Under a microscope, examination of the sperm treated with CP reveals several anomalies in the sperm head and tail, including hook-free, amorphous,banana-type, and hummer-shaped sperm after cyclophosphamide is administered. These findings showed that cyclophosphamide caused anomalies in the head & tail of the sperm in this group, while the control group showed no abnormalities at all. Various sperm abnormalities, including acrosome defects, decapitated heads, hook-less heads, microcephalic heads, and malformed heads. Coiled tail, curved tail, short tail, broken head, swollen detached head, mid-piece defects tail, bent neck and amorphous were demonstrated in (Figure 3), decreased hook or, on occasion, a head with varied degrees of reduced curvature or no curvature at all. (Figure 4) showed double head, double tail, mid-piece defects, curved tail, misshapen head, blunt hook, bent neck, detached head that was seen free head in the sperm smear nearby tail and decapitated head that appears to be without a tail. *a. decapitated head, b. hookless, c. microcephalic head, d. misshapen*

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head, e. acrosome defect, f. coiled tail, g. looped tail, h. curved tail, i. short tail, j. broken head, k. swollen head, l. detached head, m. mid-piece defects, n. bent neck, o.amorphous.

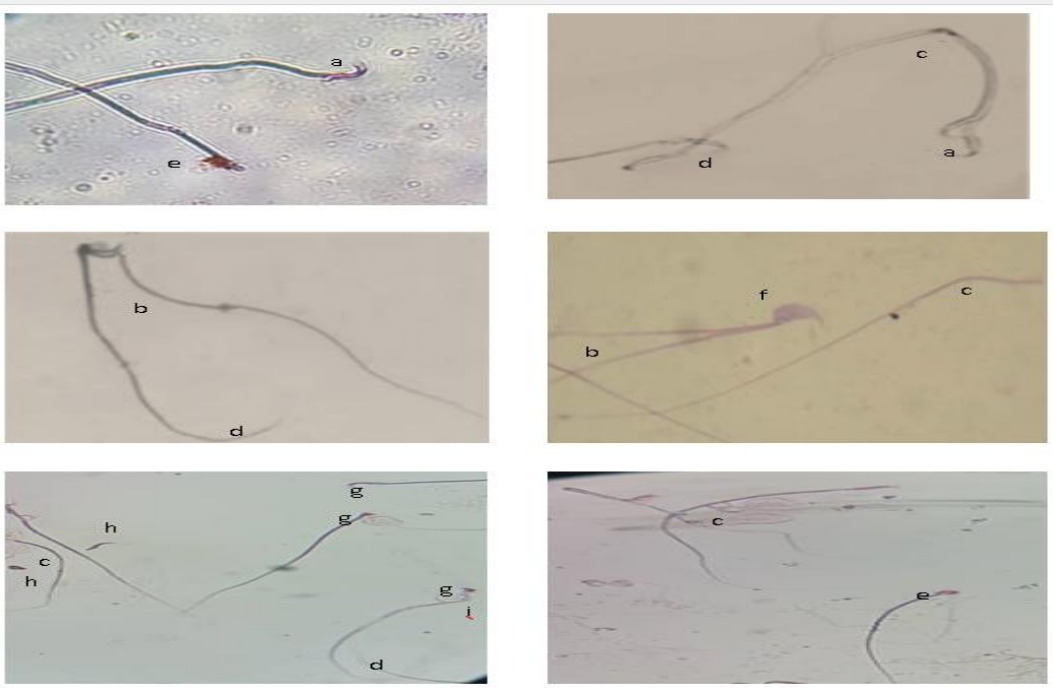

Figure 4. Photograph of abnormal sperm head and tail of male rats given CP (Eosin stain, 1000×)

a. double head, b. double tail, c. mid-piece defects, d. curved tail, e.Misshapen head, f. blunt hook, g. decapitated head, h. detached head, i. bent neck.

Ginger Extract Group

This group administered a daily oral dosage of ginger extract (200 mg/kg) for four weeks, assessing any irregularities in the head, neck, or tail using slides magnified 40X and 100X by a light microscope. The assessment, as shown in Figure 5, revealed that most of the sperm were in good condition, nearly identical to the outcomes reported by the control group.

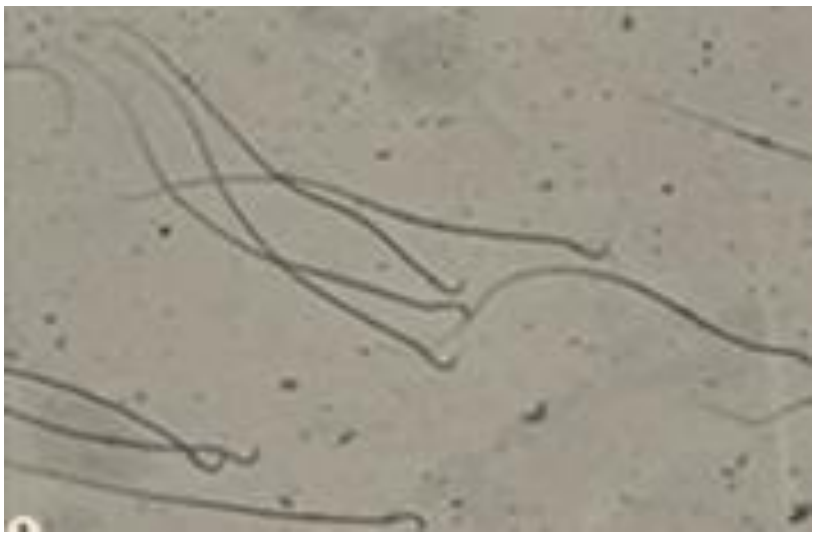

Figure 5. Photograph of typical sperm head and tail of male rats treated with ginger (200 mg/kg) of male rats (Eosin stain, 1000 X)

Protective Group

The animals in this group were given oral (GE) extract (200 mg/kg), and then took cyclophosphamide (5mg/kg) every day for 4weeks. (Figure 6) showed that the sperm morphology was roughly normal in comparison to the group that received cyclophosphamide treatment; rats in the protective group demonstrated that sperm morphology has nearly uniform traits. A few shape alterations occurred; the head and tail partially acquired traits close to normal.

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Figure 6. Photograph of normal sperm head and tail of male treated with ginger (200 mg/kg) + CP (5 mg/kg) (Eosin stain, 1000X)

Discussion

Spermatogenic lineage cells are particularly vulnerable to the damaging effects of cyclophosphamide (CP) due to their continuous turnover from the germline cell pool and the impaired maturation of new Leydig cells (Colvin, 1999; Jahnukainen et al., 2011). The findings of the present study demonstrated a highly significant difference ($p < 0.001$) in the mean sperm defects between rats treated with cyclophosphamide (16.65) and the control group (5.68). However, pretreatment with ginger extract prior to cyclophosphamide administration reduced the mean sperm defects to 6.23, whereas treatment with ginger extract alone further decreased the mean sperm defects to 3.61.

These results align with previous human studies reporting long-term male gonadal damage following cyclophosphamide chemotherapy, characterized by reduced hormone production and infertility secondary to spermatogonia depletion (Ridola et al., 2009; Nurmio et al., 2009). This damage is largely attributed to CP-induced atrophy of the testes and epididymis, ultimately leading to infertility (Johari et al., 2011). Furthermore, cyclophosphamide reduces germ cell production, which consequently decreases testicular weight (Comish et al., 2014).

The toxic effects of cyclophosphamide on the testes are primarily driven by oxidative stress within the seminiferous tubules and Sertoli cells, which impairs spermatogenesis and androgenesis while inducing germ cell apoptosis (Rezvanfar et al., 2008; Turk et al., 2010). Additionally, cyclophosphamide treatment reduces testicular and epididymal weights, decreases the number of spermatogonia within the seminiferous tubules, lowers testosterone levels, and culminates in reproductive failure (Rezvanfar et al., 2008). Previous investigations have established that administering cyclophosphamide to adult male animals decreases reproductive organ weight and reduces overall reproductive capacity (Trasler et al., 1986; Ichikawa et al., 1999). Because testicular weight directly correlates with the quantity of germ cells produced, weight loss reflects a reduction in germ cell production (Katoh et al., 2008).

In agreement with previous reports by Johari et al. (2011), Nayak et al. (2015), Shabanian et al. (2017), and Comish et al. (2014), the present findings confirmed a decline in sperm viability in CP-exposed adult male rats. The observed reductions in sperm viability and motility can be explained by the capacity of cyclophosphamide to suppress serum levels of testosterone, luteinizing hormone (LH), and follicle-stimulating hormone (FSH) (Zhao et al., 2015). Furthermore, cyclophosphamide administration increases the incidence of sperm flagellar abnormalities (Trasler et al., 1986). The reduction in sperm motility may also stem from CP altering mitochondrial membrane permeability to calcium ions, a critical regulator of cellular motility (Nayak et al., 2015).

Sperm Morphological Abnormalities and the Protective Effects of Ginger Extract

Morphological evaluation revealed that cyclophosphamide induced structural alterations in sperm head and tail anatomy compared to the control group. Observed head and tail abnormalities included hook-free, banana-type, amorphous, and hammer-shaped heads. A comprehensive spectrum of defects was noted, including decapitated, hookless, microcephalic, misshapen, and acrosome-defective heads, as well as coiled, curved, short, and double tails, double heads, broken heads, swollen detached heads, mid-piece defects, bent necks, and varying degrees of reduced or absent head curvature.

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These observations confirm that cyclophosphamide induces marked morphological abnormalities in rat spermatozoa. In line with these findings, Tripathi and Jena (2008) demonstrated that cyclophosphamide induces sperm head abnormalities. Similarly, Selvakumar et al. (2006) and Ilbey et al. (2009) reported increased sperm abnormalities and mortality following CP administration. Furthermore, Çeribaşi et al. (2010) reported CP-induced tail defects in adult male rats, and Nayak et al. (2015) confirmed sperm head abnormalities in prepubertal exposure models.

The Role of Ginger (Zingiber officinale)

Medicinal plants represent valuable economic and therapeutic resources globally. Traditionally used in Ayurvedic and Chinese medicine, ginger treats cardiovascular issues, gastrointestinal upset, diarrhea, nausea, joint pain from arthritis, and respiratory ailments, while also serving as a flavor-masking agent and promoting bile secretion (Colvin, 1999; Jahnukainen et al., 2011; Ridola et al., 2009; Nurmio et al., 2009; Rezvanfar et al., 2008).

Pharmacologically, ginger exerts anti-inflammatory action by inhibiting cyclooxygenase-1 (COX-1) and cyclooxygenase-2 (COX-2) enzymes. It harbors diverse bioactive phytochemicals responsible for its cardioprotective, anti-inflammatory, antimicrobial, antioxidant, and antineoplastic properties.

The findings of this study confirm that ginger extract effectively mitigates cyclophosphamide-induced damage, providing significant protection for bone marrow chromosomes and rat spermatozoa against genetic mutations and DNA breakdown. These protective qualities are mainly attributed to the robust antioxidant activity of ginger, which efficiently scavenges the free radicals generated by cyclophosphamide exposure. This aligns with previous research demonstrating that ginger essential oil safeguards against hydrogen peroxide-induced DNA damage through its powerful action as an oxygen radical scavenger (Phan et al., 2005; Zahedi et al., 2010). Furthermore, ginger contains abundant antioxidant constituents, including β-carotene, ascorbic acid, terpenoids, alkaloids, and polyphenols such as flavonoids, flavone glycosides, and rutin (Ghasemzadeh et al., 2010). Phenolic compounds in ginger actively scavenge superoxide anions and inhibit lipid peroxidation triggered by reactive oxygen species (al-Yahya et al., 2022).

Conclusion

This study concluded by assessing the mutagenicity of CP in experimental animals and the potential anti-mutagenic action of ginger extract. In summary, this study confirmed that CP has genotoxic effects on sperm cell morphology in male rats. This study proved that ginger extract exhibited anti-mutagenic effects at selected doses, making it an important plant to protect against and treat the harmful effects of chemical materials such as CP. Using CP as a cancer treatment exposes human life to danger because it causes DNA damage. According to the current investigation, when administered at a dose of 200 mg/kg before CP, the ginger extract showed an antimutagenic effect. Because it has a protective effect against the mutagenic effects of CP, it can be employed as a therapeutic and protective treatment agent.

Conflict of interest. Nil

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