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Genotoxic and Cytotoxic Effects of Favimol in White Mice

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Abstract

The current study aimed to assess the genotoxicity and cytotoxicity of favimol in Swiss albino mice (Mus musculus). The genotoxic and cytotoxic effects were examined at doses of 1200, 2400, and 3200 mg/kg. The evaluation was based on cytogenetic indicators such as chromosomal abnormalities in primary spermatocytes and morphological abnormalities in sperm. The results demonstrated a significant increase in mean differences for numerical chromosomal abnormalities during Diakinesis of the first metaphase in the treated groups. The highest value was observed at the 3200 mg/kg dose, with a value of 7.840 ± 0.52. Similarly, the mean values of structural chromosomal abnormalities reached their peak at the dose of 3200 mg/kg, with a value of 17.80 ± 0.54. The study also revealed the occurrence of morphological abnormalities in the sperm head after exposure to the drug. This led to a significant increase in mean differences for head abnormalities in the treated groups, with the highest value recorded at the 2400 mg/kg dose (256.60 ± 3.10). It is noteworthy that all animals administered the 3200 mg/kg dose died within 5 days, and this outcome was consistent across three repetitions of the experiment.

Keywords: *Genotoxic, Cytotoxic, Favimol, Primary Spermatocytes, Sperm shape abnormalities.*

Introduction

Genotoxicology investigates the potential effects of chemical agents on both somatic and germ cells. These agents have the ability to cause alterations in DNA, which can result in cell death or mutations in cells that are susceptible to such changes. When somatic cells are impacted, it can potentially lead to the development of conditions like cancer or neurodegenerative diseases. (1). If the damage occurs specifically in the germ cells, it can manifest as genetic alterations that have the potential to cause hereditary diseases. These changes in the genetic material of the germ cells can be passed on to future generations, resulting in a higher risk of developing inherited disorders. (2), In toxicology, the term genotoxicity refers to the property of a substance to cause damage to the genetic material (DNA) within a cell, thereby compromising the integrity of the cell. Genotoxicity is sometimes mistakenly conflated with mutagenicity. (3While all mutagens are also genetically poisonous, not all mutagenic compounds are also genetically toxic. (4). Also, genotoxins can be, depending on their effects, either carcinogens or cancer-causing agents, mutagens or mutagenic agents, or agents causing teratogenic effects (3), and cytotoxicity means the toxic effect of many chemical, physical and biological agents on the mitotic spindle system and on Enzymes acting in the cytoplasm, which may lead to changes in the functioning of the spindle apparatus and the function of cytoplasmic

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organelles (5). As the study of cytotoxicity is a useful initial step to determine the potential toxicity of the substance used in the test, including plant extracts and biological compounds isolated from plants or chemical and physical substances as well as viruses, through which it is possible to identify the ability of these substances to cause functional or genetic damage to cells(6).

Favimol, also known as Faviprevir, is an antiviral compound that selectively and effectively inhibits the RNA-dependent RNA polymerase (RdRp) of influenza virus and many other RNA viruses (7). It has been found to inhibit all sterotypes and strains of influenza viruses A, B, and C tested, and is effective against West Nile virus, yellow fever virus, and foot and mouth virus, as well as other viruses, such as flaviviruses, sandviruses, enteroviruses, and influenza viruses. Alpha (8). The molecular formula for the drug is C5H4FN3O2, the molecular weight is 157.1 g/mol, and the melting point is 193 C (9). With regard to germ cells, the purpose of testing for chromosomal abnormalities in vivo is to identify chemicals that cause structural or numerical chromosomal abnormalities in primary spermatocyte (1). Chromosomal abnormalities can be identified in the primary spermatocyte, by preparing mitotic spreads from the seminiferous tubules. The chromosomes were examined in the diaknasis of the first metaphase (10), and the clastogenic effects on primary spermatocytes could not be described by a single cytogenetic test, due to differences in response by primary spermatocytes to chemical mutagens (11), and indicated (Sadeq) in (2016), of the chromosomal abnormalities that occur in the primary spermatocytes in male albino mice, as in the normal case there are 19 autosomal bivalent and one sexual bivalent (X and Y), and that univalent and quadrilaterals are the most types of structural chromosomal abnormalities seen in In this type of studies, other structural chromosomal abnormalities such as deletions, fragments, translocations and types of numerical chromosomal abnormalities such as complete chromosomal polysomy and incomplete chromosomal polysomy can be seen(12).

The test for sperm phenotypes, one of the most widely used tests to detect genotoxicity to male reproductive cells, is a reliable short-term biomarker for estimating the toxicity of chemical, physical, and biological substances (13), as morphologic abnormalities in sperm are used routinely to determine specific damage to germ cells (14). GMOs can affect spermatogenesis, and congenital anomalies and nonviable pregnancies are associated with problems during spermatogenesis, including DNA damage and increased chromosomal abnormalities of human sperm (15). Testing morphologic abnormalities in the sperm of mice can identify substances and factors that cause disruption in sperm function, genetic mutations as a result of a defect in sperm differentiation or a defect during the process of cell division(16,17).

Materials and Methods

Twenty-five mice were used to analysis metaphase chromosomes in primary spermatocytes, and another 25 mice were used to test morphologic abnormalities of sperm. The groups of 5 males were divided as follows: The experimental groups consisted of a negative control receiving distilled water, a positive control group receiving an intraperitoneal injection of cyclophosphamide at a dose of 10 mg/kg, and three additional groups treated orally with different doses of favimol. The doses of favimol administered were 1200 mg/kg, 2400 mg/kg, and 3200 mg/kg.

Analysis of anaphase chromosomes in primary spermatocytes:

The experimental procedure followed the methodology described in reference (18). After the designated time of dosing, the animals in the negative and positive control groups were sacrificed by cervical dislocation 24 hours later. In the case of the groups treated with favimol, the animals received the drug for five consecutive days, and they were

sacrificed 24 hours after the final dose. Prior to sacrifice, the animals were injected with Colchicine in the calf region 2.5 hours before the procedure. The male gonads were then extracted and placed in a 2.2% sodium citrate solution. The outer layer of the tunica was removed, and the seminiferous tubules were transferred to a small petri dish containing 2.2% sodium citrate solution. Using curved forceps and a fine wire clip, the seminiferous tubules were carefully torn apart. The cell suspension obtained from the torn seminiferous tubules was transferred to a 3 mL test tube and centrifuged at 1000 rpm for 10 minutes. After discarding the liquid, the precipitate was then suspended drop by drop in 7 ml of 1.1% sodium citrate solution. The tubes were left at room temperature for 20-25 minutes. Subsequently, the tubes were centrifuged again for ten minutes at 1000 rpm, and the clear liquid was discarded. To fix the cells, Carnoy's fixative was added drop by drop while continuously shaking the tubes using a Vortex device. A total of 7 ml of fixative was added, and then the tubes were centrifuged at a speed of 1000 cycles/min. The fixation process was repeated twice more by adding 5 ml of fixative each time. Finally, the tubes were placed in the refrigerator overnight. After discarding the clear liquid by centrifugation at 1000 cycles/min, the precipitate was mixed using a Pasteur tube. The fixative was replaced with a fresh solution before preparing the slides. The cells were suspended in a small amount of the new fixative and mixed thoroughly. Then, 2-3 drops of the cell suspension were placed onto clean slides. To fix the cells on the slides, they were passed three times through the flame of an alcohol lamp. The fixed cells were then stained with a 10% Giemsa solution for a duration of 20-25 minutes. After staining, the slides were washed twice with sorenson buffer. Finally, the slides were dried and examined under a microscope to identify and document any abnormalities or anomalies observed.

Evaluation of morphologic abnormalities in mature sperm of albino mice:

The experimental procedure followed the methodology outlined in reference (19). The animals in the experimental groups were sacrificed 42 days after the initial dosing. The epididymides were carefully removed and placed in a centrifuge tube containing 3 ml of 0.9% normal physiological solution. The epididymides were then transferred to a Petri dish and cut into small pieces using a scalpel. The resulting slurry was filtered through a wire mesh to remove any tissue fragments.

Next, 0.5 ml of the filtrate was transferred to a microcentrifuge tube, and 0.05 ml of a 1% eosin-spermin Y staining solution was added. The solution in the tubes was shaken gently, and the tubes were covered with moist cotton gauze to maintain the viability of the sperm. The tubes were then incubated at 37 °C for ten minutes.

To prepare the slides, a drop of the mature sperm suspension was placed on a clean glass slide. The suspension was spread evenly using three cover slides. The slides were then left to dry in the air. Subsequently, the examination process was carried out, and 1000 sperm were observed and recorded for each slide.

Results

Studying the effect of favimol in inducing numerical and structural chromosomal abnormalities of metaphase 1 chromosomes in primary spermatocytes of male albino mice:

Figure (1) shows the chromosomes of the diakinesis of the metaphase 1 of the meiosis of a mouse from the negative control group, the groups treated with favimol and the positive control group CP, as the types of chromosomal abnormalities were recorded in the primary spermatocytes in the diaknasis of the metaphase 1, as shown in tables (1) and (2).

Table (1) Mean values of differences for numerical abnormalities in chromosomal litters with chromosomal abnormalities in primary spermatocytes in male albino mice treated with different doses of favimol and positive control CP.

treatment dose mg. kg^{-1}.b.wt	Total numerical abnormalities MD ± S.E	aneuploidy %	polyploidy %
DW	0.00 ± 0.00	0	0
CP/10	10.60 ± 0.52*	3.12	29.68
FAV/1200	4.20 ± 0.52*	0.55	24.62
2400	6.20±0.52*	1.12	26.22
3200	7.80±0.52*	2.58	27.81

*Significant at P<0.05 (HSD tukey's test) , MD mean difference, S.E standard error, cyclophosphamide CP, FAV favimol.

Table (2) Percentages and mean values of differences for structural chromosomal abnormalities in primary spermatocytes in male albino mice treated with different doses of Favimol and the positive control CP.

treatment dose Mg.kg^{-1}.b.wt	Structural abnormalities M ± S.D	Structural abnormalities MD ± S.E	Autosomal univalent %	X-Y univalent %	Autosomal X-Y univalent %
DW	1.40 ± 0.45	-	12.5	18.6	3.5
10/CP	-	23.80 ± 0.45*	26.6	38.2	13.4
1200/FAV	-	7.20 ± 0.45*	20.8	31.1	5.8
2400	-	11.80 ± 0.45*	23.7	34.2	7.2
3200	-	17.80 ± 0.45*	24.1	36.2	9.6

Significant at P<0.05 (HSD tukey's test) , M mean, S.D standard deviation, MD mean difference, S.E standard error, cyclophosphamide CP, FAV favimol.

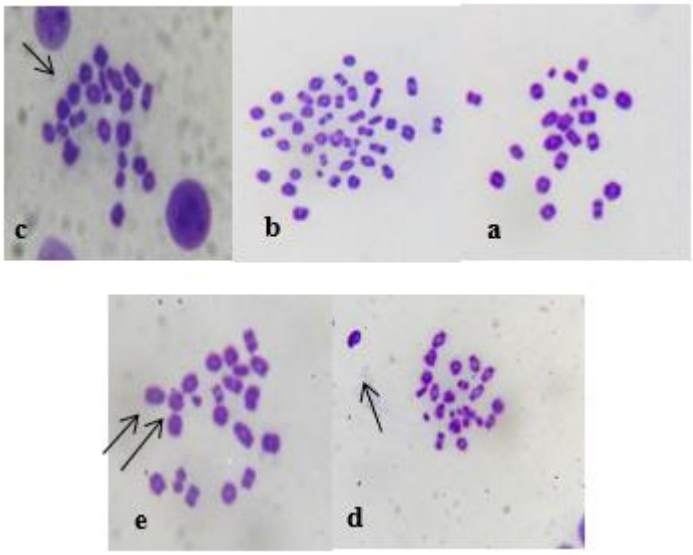

Figure (1) Chromosomal spreads of the Diakinesis of metaphase 1 of meiosis of male albino mice treated with doses (1200, 2400, 3200) mg. kg-1 favimol: a: normal chromosome duplications n=20, b: complete polyploidy, c: x-y univalents, d: x-y univalents, e: autosomal univalents and x-y univalents (black arrow), Giemsa, 100x

The results of the current study showed a clear significant increase in the values of the mean differences for numerical chromosomal abnormalities in the groups treated with FAV drug, as it reached its highest value at the dose of 3200 mg.kg-1 and was 7.80 ± 0.52, while it reached in both doses of 1200 and 2400 mg.kg-14.20 ±0.52 and 6.20 ± 0.52, respectively, as the complete systemic chromosomal polyploidy recorded the

highest percentage among the numerical abnormalities as shown in Table (1), and the types of structural abnormalities were observed, which were induced in primary spermatocytes by doses 1200, 2400, 3200 mg.kg-1 of the drug under study after five days of oral dosing, as among the types of abnormalities that were induced were autosomal univalents, as well as X-Y univalents, and some chromosomal spreads containing each of the autosomal univalents were recorded and X-Y univalents, as the highest value of structural abnormalities at the dose of 3200 mg.kg-1 was 17.80 ± 0.54, followed by the dose of 2400 mg.kg-1, which amounted to 11.80 ± 0.54, in the dose of 1200 mg. kg-1 has the lowest abnormality value, which amounted to 7.20 ± 0.54, and it can be seen from figure (1:a) the Diakinesis of the mouse from the negative control, as the examination of the chromosomes of meiosis at the Diakinesis of metaphase 1 showed that the somatic chromosomes were paired, consisting of 19 heterogenous diploids, while the two sex chromosomes join together at the end of each, forming an heterogenous pairing (20).

Astudy of the effect of Favimol in inducing morphologic abnormalities of mature sperm in male albino mice:

Table (3) shows the types of morphologic abnormalities of sperm exuded in the head and tail, while Table (3) shows the values of the average differences for the abnormalities observed in the head and tail with percentages

treatment dose mg.kg^{-1}.b.wt	Head abnormality M ± S.D	tail abnormality M±S.D	Head abnormality MD±S.E	tail abnormality MD±S.E	without a hook %	a big head %	a small head %	Triangle head %	Banana head%	irregular head %
DW	8.20±1.30	1.80±1.09	-	-	8.9	2.8	2.8	11.8	2.1	12.2
10/CP	-	-	467.60±3.10*	108.60±10.04*	.292	11.1	10.7	27.0	12.8	38.9
1200/FAV	-	-	190.40±3.10*	61.80±10.04*	21.8	4.7	5.8	21.9	5.2	29.8
2400	-	-	256.60±3.10*	86.80±10.04*	24.07	6.4	7.7	24.2	8.4	32.8

*Significant at P<0.05 (HSD (tukey, M mean, S.D standard deviation, MD mean difference, S.E (standard error), cyclophosphamide CP, FAV favimol.

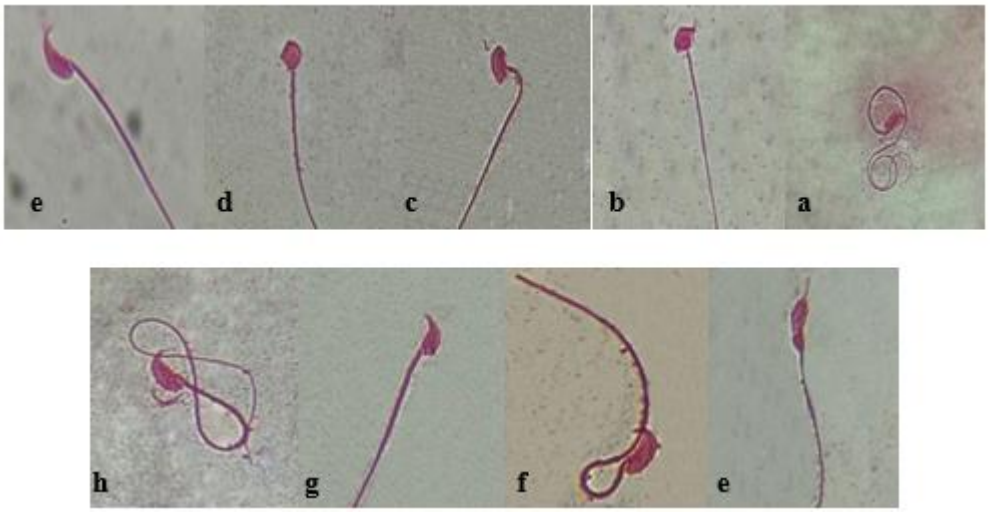

Figure 2: Sperm abnormalities induced after treatment with doses (1200 and 2400) mg. kg-1 Favimol, (Eiosin)100x, a: normal, b: spherical, c: without hock, d: irregular, e: curled tail, f: small head, g: large head, h: banana head

The results of the current study showed the occurrence of many morphologic abnormalities in the sperm head after exposure to the drug used in the study, as this exposure led to a significant increase in the values of the mean differences for the head abnormality in the groups treated with the drug, as it reached its highest value at the dose of 2400 mg. kg-1 and it was 256.60 ± 3.10, while it was at the dose of 1200 mg. kg-1 190.40 ± 3.10, and it is worth mentioning that all animals died 5 days after giving them the dose of 3200 mg. kg-1, while repeating the process 3 times for the same dose, and obtaining the same result, which is the death of all animals. It was also observed a significant increase in the values of the mean differences for tail anomalies, in the groups treated with the drug, as it reached its highest value at the dose of 2400 mg. kg-1 and it was 86.80 ± 10.04, while it was at the dose of 1200 mg. kg-1 61.80 ± 10.04.compared negative control 1.80 ± 1.09.

Discussion

To the best of our knowledge, there has been no documented research on the effect of Favimol on the primary spermatocytes, and the discovery of the effects of such drugs on the reproductive organs during the use of treatment, so we used some research on antiviral drugs, especially the nucleotide analogues to which the drug under study belongs. Among them is the drug Ribavirin, as many studies have proven its toxicity to the male reproductive system, as it was noted that testicular cells were affected by the drug (21), as many changes appeared within the testis, Sertoli cell alopecia, as it directly affects the differentiation and development of germ cells, as well as a decrease in the maturation and differentiation of sperm cells, with a decrease in secondary spermatocytes, and the presence of inflammatory cells (22), and may be the reason for this damage in germ cells, is related to the presence of Glutathione (GSH), which acts as an enzyme co-enzyme, and an important antioxidant to protect cells from free radical damage, as it is important for the integrity of cells and the functioning of proteins, lipid membranes, and others, as the treatment with ribavirin depletes the amount of GSH in these cells and thus increases their susceptibility to toxicity and the occurrence of genetic damage in them, and induces the inhibition of sperm formation, and therefore the human intake of such drugs has effects dangerous to the testicles (23, 24) Also, the drug is transmitted to the testicles through the peritoneum, and then reaches the germ cells and accumulates in the masses of these cells, causing them many damages, as it can cause changes in the shapes of sperm through point mutations in Sperm-forming cells (25), and may lead to the generation of free radicals, causing various damages to vital molecules within germ cells, including DNA (26), since it is clear that exposure to chemical toxic agents can stimulate DNA damage in male germ cells, Therefore, nucleotide analogues are toxic to cells, affecting cell division and thus acting as cytotoxins in the testes (22).

Testing hmorphologic abnormalities in sperm can provide data through which it is possible to know the percentage of damage to germ cells and the percentage of genetic toxicity that occurs when exposure to chemicals and drugs and threatens the reproductive health of individuals, as infertility is an important concern for public health, as it affects approximately 15% of couples all over the world, and male infertility accounts for nearly half of all infertility cases, which may result from various diseases, or may be caused by external deformities in the head of the sperm, including external deformities in the tail, in addition to movement problems, this results from the influence of a wide range of factors, which affect the stages of sperm formation and its quality (27, 28), as it is believed that the apparent shape of the sperm is a strong indicator of the health of the individual's testicles, which is related to the physiological and environmental stresses that affect the physiology of the body, without the effect on the general health of the individual (29, 30). it is known that the process of human spermatogenesis, is a regulatory and dynamic process of cell differentiation, which is maintained through self-renewal and differentiation of sperm stem cells, as this process is precisely controlled by the

environment dynamic minutes in the seminiferous tubules of the testis (31). and that any abnormalities that occur in the germ cells of males will lead to failure of spermatogenesis and impaired male fertility (32). Adverse and potentially fatal effects of FAV have been reported, which occur more frequently in men those over 65 years old (33). as a study on Ribavirin (a drug used to combat viruses) confirmed that the drug caused abnormalities in the sperm of mice, the drug greatly affects the process of sperm formation, so it is a cause of infertility. In males, moreover, the drug causes genetic damage to male germ cells (34, 35). Sperms are sensitive to chemicals, which may negatively affect the process of DNA replication, since these cells, during their formation process, pass through many divisions, so induced sperm abnormalities may, indicate the occurrence of point mutations in germ cells, which may cause structural changes in the cell organelles involved in the formation of the head and tail, which leads to abnormalities in the shape of the sperm. Sperm abnormalities often indicate testicular toxicity, resulting from exposure to chemicals, as it is known, that the testosterone hormone in adults supports the formation and maturation of spermatozoa. Therefore, the disruption of the biosynthesis of this hormone in Leydig cells may negatively affect the process of spermatogenesis, and thus the occurrence of abnormalities in it (26, 36). While another study of another viral drug, Molnupiravir, confirmed that it has a negative effect on the host's DNA (37), so men should be careful when using this drug, because it has a potential genetic toxic effect on the stages of sperm formation, thus causing various abnormalities in the sperm. Also, the drug may cause tissue damage resulting from oxidative stress in the testicular tissues (38), and another study reported that both Lopinavir and Ritonavir have a negative effect on the process of spermatogenesis in mice, which may be caused by damage. The oxidative stress of the drug in testicular cells (32), as the resulting oxidative damage may cause excessive formation of reactive oxygen species (ROS), which leads to cellular death (39), moreover, the excessive production of proinflammatory cytokines may affect negatively on the formation of sperm, by increasing the autoimmune response and infiltration of white blood cells into the testicle (40), and thus may affect the testis and lead to harmful effects on the sperm (41), and ribavirin also causes abnormal shape of the middle piece and tail, through its effect on the sperm membrane, as it may be the reason for its effect on the membrane, Due to its formation of free radicals, especially reactive oxygen species ROS, which work to attack the double carbon bonds, which are found within the structures of polyunsaturated fatty acids, which enter into the formation of the outer membranes of the sperms, which leads to the collapse of the outer membrane of the sperms and the conversion of unsaturated fats into lipid peroxide, thus increasing the concentration of Malondialdehyde (MDA), which affects the vitality of the sperms and thus the appearance of abnormalities (22, 42).

Conclusions

The current study showed that the dose is 12002400, 3200 mg. 1- kg of Favimol has cytotoxicity and genotoxicity, through its effect on the chromosomes of metaphase 1 in the primary spermatocytes of male albino mice, as well as the clear effect of the drug on the external appearance of the sperms by inducing many morphologic abnormalities in the mature sperms of males White mice.

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