

Cytogenetic and Ultrastructural Studies of Effects of Antifungal Drug, Fluconazole on Liver of New-born Mice

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Abstract: As a potent antifungal drug, fluconazole clinically used to eradicate both systemic and superficial mycoses resulting in hepatotoxicity. The objective of the current study was to evaluate hepatotoxicity and genotoxicity in newborn male mice. Mice were treated orally with 0.5 ml fluconazole doses of (0, 25, 50, and 100 mg/kgbw) per day for five consecutive weeks. Micronucleus test, chromosomal aberrations in bone marrow cells, histopathological investigation and DNA fragmentation in the liver tissue was done. Micronuclei are significantly noticed in bone marrow cells of mice given 50 and 100 mg/kgbw fluconazole however, there is no effect on the genotoxicity induced by 25 mg/kgbw dose of fluconazole. A dose-dependent and significant increase in structural and numerical chromosomal aberrations were detected in the 50 and 100 mg/kgbw fluconazole-treated group but a 100 mg/kg was highly significant. The chromosomal aberrations were manifested in hypoploidy, deletion, centric fusion and stickiness. Besides, hepatocellular massive infiltration, cytoplasmic vacuolation, congestion and dilatation in the central veins were seen in 50 and 100 mg/kgbw fluconazole. Interestingly, 25mg/kgbw fluconazole-treated mice showed mild hepatocellular degeneration. Consequently, these findings confirmed that fluconazole to a greater extent was a potent hepatotoxic drug *in vivo* in newborn mice.

Keywords: Fluconazole, Genotoxicity, Micronucleus, Chromosomal Aberration, Hepatotoxicity

1. Introduction

Environmentally, many species of fungi are associated with human disease. Fungal infections cause a certain risk for many populations. Any person can expose to the fungal source such as spores on surfaces or in the air, soil, or bird droppings [1].

Fungi as a member of a large group of eukaryotic organisms could produce biologically active compounds called mycotoxins or secondary metabolites which can cause disease and death in human beings and other vertebrates [2]. During neonatal life, fungal infections may cause considerable morbidity and mortality; particularly in premature neonates [3]. It is found that a delay in antifungal therapy could increase mortality rates to a hundred percent.

Furthermore, inflammation and tissue damage are triggered by fungal infection [4].

Over the last few years, the number of antifungal drugs, azoles, has constantly been increasing [5], where they are used in therapy and management of superficial and systemic mycoses [6]. Fluconazole is one of azole drug group which has antifungal activity and may eradicate both superficial and invasive fungal infection [7]. Also, it has many other attributes like antiparasitic characters, initiates wound healing, and declines the development of organ dysfunction in human [8]. Besides, fluconazole can prevent the neonatal candidiasis in the newborn infants which have very low body weight [3]. Leonart *et al.* [9] estimated the safety and efficacy of different doses of fluconazole used for invasive prophylaxis of fungal infection in neonates. They found that

3 mg/kg of it could be recommended for *Candida* prophylaxis in neonates. Additionally, Lollis and Bradshaw [10] found that *Candida* spp may cause invasion, colonized and make few signs and symptoms, leading to death. Fluconazole could also estimate hepatotoxicity and cause an increased incidence of hepatocellular adenomas in male rats treated orally for 2 years at doses of 5 and 10 mg/kg/day [11]. Peffer *et al.* [12] found that some conazoles fungicide (as a class of *N*-substituted azole antifungal drugs) are carcinogens, reproductive toxins, and induce hepatomegaly and increase hepatic cell proliferation in mice. Acute liver injury was uncommon in treatment with fluconazole [13]. Hepatotoxicity caused by fluconazole is caused due to its ability to alter sterol synthesis, or it may be a potent inhibitor of a cytochrome P450 enzyme. This can lead to a higher increase in plasma levels that are metabolized by CYP3A4, particularly the statins and cyclosporine.

Genotoxicity test such as micronucleus assay is biomonitoring of human exposure to genotoxic agents [14]. When the chromosome is failed to be included into daughter nuclei after mitosis, micronuclei are arising as fragments of chromatin containing bodies. This provides an indirect measure of the induction of structural chromosomal aberrations [15]. The micronucleus test, *in vivo*, is a method devised primarily for screening chemicals for chromosome breakage, and it also detects the effects on the spindle apparatus [16]. In a dose-dependent, fluconazole was found to enhance micronuclei at a statistically significant level. Yuzbasioglu *et al.* [17] found that fluconazole initiates structural chromosomal aberrations such as sister chromatid union, chromatid, chromosome breaks, dicentric chromosomes, chromatid exchange and fragments. Chromosomal breakage may lead to abnormalities of chromosomal segregation at mitosis [18]. Also, it could induce numerical chromosomal aberration such as polyploidy. Holeckova *et al.* [19] detected chromosomal aberrations in cattle lymphocyte cells that were cultured *in vitro* after conazole fungicide treatment. There are many documented studies in the literature of azole-induced hepatotoxicity. Consequently, due to the adverse effect that fluconazole may cause, the present study is a continuation of hepatotoxicity with a focus on the genotoxicity and histopathological evaluation as a main role of fluconazole in hepatocellular alterations.

2. Experimental

2.1. Chemicals

Fluconazole 2-(2, 4-di fluorophenyl)-1, 3-bis (1H-1, 2, 4-triazole-1-yL)-2-propanol was purchased from the pharmacy under the international trade name: Diflucan capsule (50 mg fluconazole/ capsule). It was manufactured by Pfizer, Egypt, under Authority of Pfizer INC., U.S.A. Three stock solutions of different doses were well prepared according to the equivalent daily prescribed dose for a human. Fluconazole was liquefied in saline solution (0.9% NaCl).

2.2. Animals and Doses

One-hundred and sixty newly weaned male mice (three weeks old and weighing nearly 6-8g) were received from the Faculty of Agriculture, Alexandria University, Alexandria, Egypt. Mice were put in the usual type of stainless-steel cages (5 mice/cage), which were scribed day by day continuously. Mice were modified to the controlled environmental conditions at room temperature of $25\pm 2^{\circ}\text{C}$, relative humidity $60\pm 10\%$, and given free access of food (milk, wheat, bread) and tap water *ad libitum*. These acclimated male mice were divided into four groups of approximately equal in body weights (15 mice/each). They were assigned to three experimental groups and one control. Mice of each experimental group were administered orally at morning between 9 and 11 am with 0.5 ml fluconazole at doses of 25, 50, 100 mg/kgbw/day, for five consecutive weeks. The control mice were administered daily only with 0.5 ml saline solution in the same manner.

Experimental procedures were achieved in conformation with principles of laboratory animal care approved by the institutional animal care and use committee Alexandria University, Alexandria.

2.3. Clinical Examination

Carefully, all mice were screened for signs of clinical toxicity examinations daily for morbidity, mortality, stereotypy, or behavior throughout the experimental period.

2.4. Bone Marrow Processing for Genotoxicity Analysis

Twenty-four hours after administration of the last dose of fluconazole, and two hours before euthanasia and sacrifice, mice were administered intravenously 0.5 mg/kgbw Colchicines [20]. Surgically the femora of each mouse were aseptically anatomized. The contents of the bone marrow were collected from the proximal extremity of each sectioned femur by flushing in 0.075 M KCl, incubated at 37°C for 25 min., and centrifuged at 2000 rpm for 10 min. The homogenous suspension of bone marrow cells recentrifuged at 1,500 rpm for 10 min and then the cellular pellets were washed in saline solution. Thereafter, the cellular pellets of bone marrow were fixed in acetic acid: methanol (1:3 respectively). This process was repeated four times at an interval of 15 min each. The slides of best-spread metaphase cells were smeared, dried at 25°C , and stained with 5% Giemsa stain for 3-5 min followed by removing the excess stain in distilled water to distinguish polychromatic erythrocytes (PCEs). Nucleated PCEs were examined at 1000x under light microscopy in 180 metaphases for each animal to a total of 6000 metaphases for each treatment and control group and counted. The number and percentage of PCEs were determined. The type and frequency of structural and numerical chromosomal aberrations in the different groups were observed, photographed and tabulated. We estimated ten mice for both the experimental and control group. Three replicates of each endpoint were applied for all groups.

2.5. DNA Analysis

The genomic DNA was isolated from the liver tissue using spin column method Axy Prep Multisource Genomic DNA Mini-Prep Kit obtained from Axy gen Biosciences, Union City, CA94587 USA. Gel electrophoresis by Sub-Cell GT electrophoresis systems (BIO-RAD, USA) was stained by Ethidium bromide [21].

2.6. Light Microscopic Methods

Both control and experimental treated mice were killed by cervical dislocation under anaesthetic conditions. Macroscopically, the abdominal cavities were exposed and examined, and small pieces of the liver tissues were dissected out immediately from the peripheral areas of the two lobes and were rinsed overnight in Bouin's solution. Thereafter, fixed tissue was dehydrated and embedded in paraffin wax. Serial liver sections were cut at 5µm thick, stained by haematoxylin-eosin (H&E), cleared by xylene and were examined by the light microscopy for histopathological examination [22].

2.7. Electron Microscopic Methods

Very small slices from the periphery of livers of both control and fluconazole-treated mice were dissected out and fixed immediately in 2% 4F1G (4%formaldehyde and 1% glutaraldehyde), then put in 0.1M phosphate buffer, pH=7.4 at 4°C for around 1 h, then rinsed in 0.1 M phosphate buffer (pH 7.4). This was completed by post-fixation using 1% buffered OsO₄ (osmium tetroxide) for 1-2 h at 4°C, then the specimens were cleaned with phosphate buffer for several times for 30 min, dehydrated in ascending grades of ethanol concentration. The liver tissues were then treated with propylene oxide and embedded in a mixture of 1: 1 of Epon-Araldite. Specimens were put in pre-dried gelatine capsule (dried in the oven at 37°C for 1 h before use) [23]. Ultrathin sections of such specimens were cut in serial sections with a glass knife on LKB ultra-microtome, mounted on 200 mesh

Table 1. Showing% of polychromatic erythrocytes (PCE's) with micronuclei in 6000 bone marrow cells of mice, 24hr after the last oral treatment with different doses of fluconazole.

Treatment with fluconazole	Number of PCE's	% PCE's	Number of PCE's with micronuclei	% of PCE's with micronuclei
Control	978	16.30	14	1.43
25 mg/kg bw/day	886	14.77	23*	2.60*
50 mg/kg bw/day	700*	11.67*	65*	9.29*
100 mg/kg bw/day	660*	11.00*	85*	12.88**

*Significantly different from the control P<0.05.

3.2.2. Chromosomal Aberrations

Normally, the chromosomes in the mouse are acrocentric, and diploid (2n=40). After 5 weeks of treatment with 50 and 100 mg/kgbw of fluconazole, the incidence of numerical and structural chromosomal aberrations was significantly increased (Table 2, Figures 1-3). The most abundant type of numerical chromosomal aberrations because of the treatment of 100 mg/kgbw doses of fluconazole was hypoploidy and deletion (Table 2, P≤0.001; Figure 3). On the other hand,

naked copper grids, doubled stained with uranyl acetate and lead citrate.

2.8. Statistical Analysis

Statistical analysis was estimated using the SPSS software package version 17.0. All data were exhibited as the mean±Standard deviation (X±S.D.) and were analyzed statistically using one-way analysis of variance (ANOVA). All tests were performed at a significant level of probability value P≤0.05. The significance was determined by Fisher's exact probability test (F-test) and resulted in F-value which can be compared with the tabulated value.

3. Results

3.1. Oral Toxicity of Fluconazole

No scheduled mortality and all mice in the control and fluconazole-treated groups remained alive until the last experimental days of the study. However, mice were given 50 and 100 mg/kgbw of fluconazole showed signs of stereotypical behavior, hypoactivity, failed to respond to external stimuli in addition to hair loss. On the other hand, all animals in 25 mg/kgbw group exhibited a state of being free from illness or injury to the final point of the experiment compared to controls.

3.2. Genotoxicity Findings

3.2.1. Polychromatic Erythrocytes

The micronucleus results revealed significant increases in the percentage of polychromatic erythrocytes (PCEs) with micronuclei in the bone marrow cells of mice treated with 50 and 100 mg/kgbw/day fluconazole (Table 1). These findings suggest the presence of mutagenicity for fluconazole regarding the 50 and 100 mg/kgbw dose. In contrast, 25 mg/kgbw dose exhibited no mutagenic activity. So, these findings indicate that fluconazole can induce genotoxic effects in the bone marrow of the newborn mice.

fluconazole at a dose of 25 mg/kgbw showed insignificant numerical chromosomal aberrations. The frequency of structural chromosomal aberrations in bone marrow cells following treatment with 50 and 100 mg/kgbw dose levels of fluconazole also showed a significant increase compared with 25mg/kgbw and control group. The varieties of structural chromosomal aberrations that induced by the treatment with 50 and 100 mg/kgbw dose doses of fluconazole were deletion, centric fusion, and stickiness (Table 2, Figures 2, 3). In general, deletion and centric fusion

appeared to be more than stickiness.

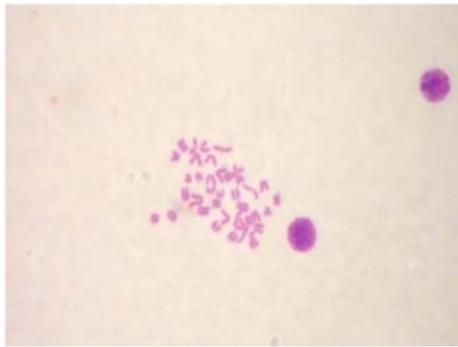


Figure 1. Showing the normal chromosomal appearance in the bone marrow cells of normal mice, X 1000.

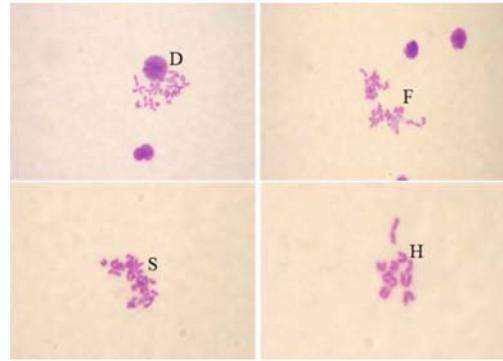


Figure 2. Showing structural chromosomal aberration in mice administered with 50 mg/kgbw/day fluconazole, Note: D: deletion (D); F: fusion; S: stickiness; H: hypoploidy, X 1000.

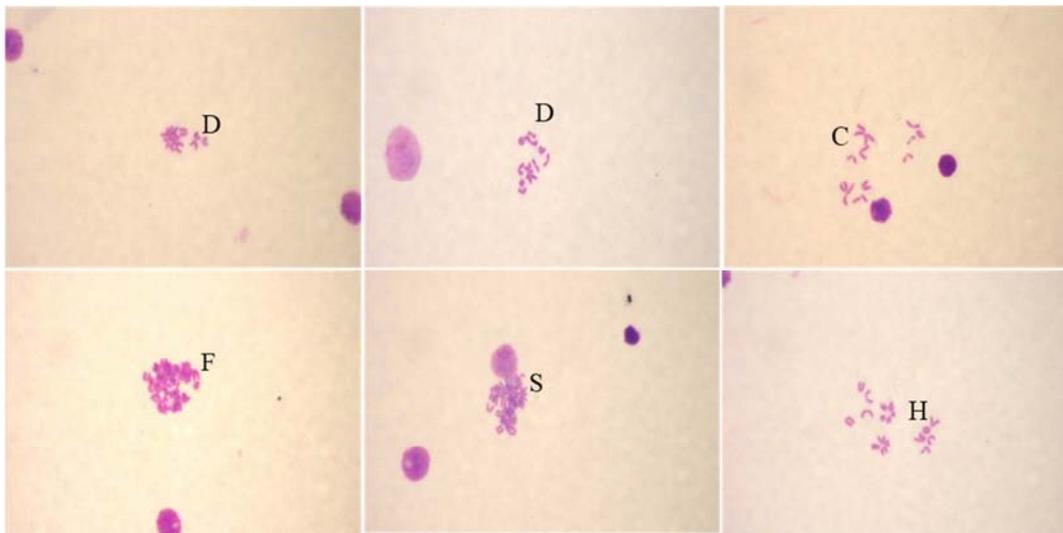


Figure 3. Showing structural chromosomal aberration in mice administered with 100 mg/kgbw/day fluconazole, showing: D: deletion (D); cf: centric fusion; S: stickiness; H: hypoploidy, X 1000.

Table 2. Showing% of chromosomal aberrations in 180 bone marrow cells of mice 24hr after the last oral treatment with different doses of fluconazole.

Treatment with fluconazole	No. of abnormal metaphases	Number of metaphases with				% of total aberrant metaphases
		Deletion	Centric fusion	Stickiness	Hypoploidy	
Control	6	1	2	-	3	3.3
25 mg/kg bw/day	12	3	4	-	5	6.7
50 mg/kg bw/day	57	17	15	10	15	31.7
100 mg/kg bw/day	74	22	18	11	23	41.1

*Significantly different from the control P<0.05.

3.3. Histopathology

3.3.1. Light Microscopic Findings

Light microscopic examination of sections of the liver of control mice showed the normal lobulation. The hepatocytes were closely adjacent and compactly arranged in branching cords. They usually single cell layer thick and radiated from the central vein to the periphery. The hepatic cords were separated from each other by large vascular spaces called hepatic sinusoids. The hepatocytes were polygonal in shape, having rounded-shaped nuclei and contain one or two prominent nucleoli. The binucleated hepatocytes and the Kupffer cells are clearly shown. Evidence of mild damage

was noticed after treatment with 25 mg/kgbw/day fluconazole for 5 consecutive weeks. Most hepatocytes appeared in the normal morphological appearance as in the control, however, disorganized hepatocytes, binucleated hepatocytes, dilated sinusoids and activated Kupffer cells were observed. Administration with 50 mg/kgbw/day fluconazole showed that the hepatic architecture had lost their usual arrangement of hepatic strands, and congestion in the central vein was observed. Also, cytoplasmic vacuolation in most hepatocytes was observed. However, extensive hepatocellular necrosis, degeneration in peri-acinar zona, hyperplasia of bile ducts including biliary cirrhosis and granuloma of binucleated and giant cells were noticed in the

hepatic tissues of mice taken 100 mg/kgbw/day fluconazole. Severe congestion in the central veins, dilatation in the

central veins, and a massive densely packed lymphocytic infiltration were also observed (Figure 4).

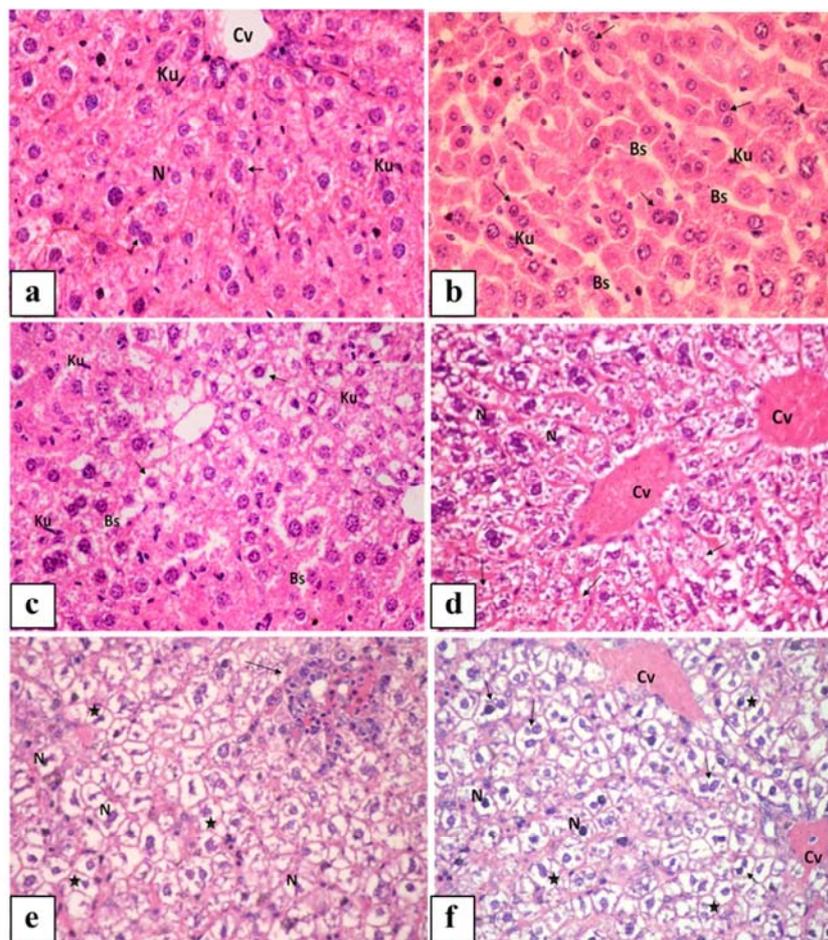


Figure 4. (a-f): Sections of liver mice, showing; a: in control mice showing hepatocytes are radiated from the central veins (Cv); the hepatocytes have centrally-located basophilic nuclei (N); binucleated cells (arrows); Kupffer cells (Ku); b: in mice treated with 25 mg/kgbw/day fluconazole, showing mildly disorganized hepatocytes, binucleated hepatocytes (arrows), dilated sinusoids (Bs), Kupffer cells (Ku); c & d: in mice treated with 50 mg/kgbw/day fluconazole showing vacuolation in the cytoplasm of hepatocytes (arrows), pyknotic hepatocyte nuclei (N); congestion of the in central veins (Cv); e & f: in mice treated with 100 mg/kgbw/day fluconazole showing severe vacuolation in hepatocytes, pyknotic hepatocyte nuclei (*); massive lymphatic infiltration (arrow). H&E, X400.

3.3.2. Electron Microscopic Findings

Under the electron microscope, the hepatocytes of the liver tissue of control mice showed the appearance of large centrally placed nuclei with the distinct nuclear envelope and prominent nucleoli. In the cytoplasm, the mitochondria were of different shapes and sizes, they were mostly bounded by inconspicuous mitochondrial membranes and their cristae were not prominent. Few scattered primary lysosomes and few small lipid droplets could be seen. Space of Disse was recognized as a narrow space between the endothelial cells and the surface of hepatocytes.

The electron micrographs of liver sections of mice treated with 25 mg/kgbw/day fluconazole showed an abundant amount of electron-lucent lipid droplets was observed in the cytoplasm of most hepatocytes. The mitochondria appeared swollen, and short profiles of the interrupted rough endoplasmic reticulum were seen with no close relationship with the mitochondria. In addition to a marked increase in the

proliferative amount of smooth endoplasmic reticulum was noticed, indicating the toxic effect of fluconazole, and few short micro-villous projections in the dilated sinusoidal space were seen in, while others devoid them.

While the electron micrographs of liver cells of mice treated with 50 mg/kgbw/day fluconazole revealed nuclear pyknosis. Few cisternal disruptions of the rough endoplasmic reticulum (rER) and no close association between the mitochondria and rER were observed. The mitochondria exhibited an irregular configuration; possessed dense matrices, and some showed bizarre forms. Furthermore, a few numbers of small light lipid droplets could be seen.

The electron micrographs of hepatocytes of mice administered with 100 mg/kgbw/day of fluconazole exhibited an appearance of many pyknotic nuclei of hepatocytes, and in others contained more than one proliferating nucleoli, indicating their active changes. The mitochondria showed a marked loss in their matrix and revealed a large degree of polymorphism. The rER was greatly reduced into few short

irregularly placed cisternae, partially surrounding the nuclei. Many small dense lysosomal particles and an increase in the

number and size of light electron density lipid droplets were observed (Figure 5).

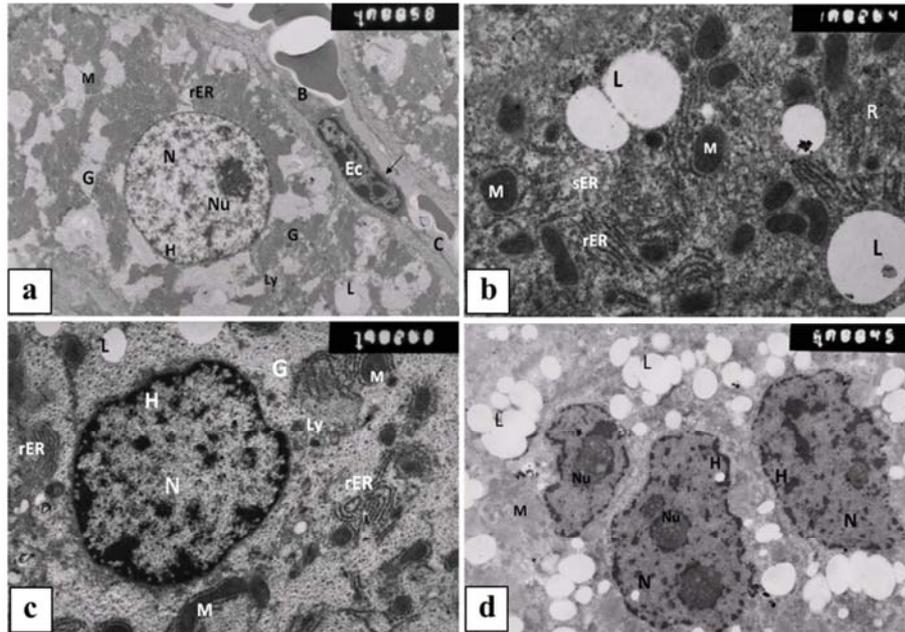


Figure 5. (a-d): Electron micrographs of the liver: a: in control mice showing the hepatocyte nucleus (N); nucleolus (Nu), heterochromatin (H), mitochondria (M); short arrays of the rough endoplasmic reticulum (rER), β -glycogen particles (G); space of Disse (arrow); capillary (C) and blood cell (B). X 4000; b: in mice treated with 25 mg/kgbw/day fluconazole showing swollen mitochondria (M); rER, lipid droplets (L), ribosomal particles (R), smooth endoplasmic reticulum (sER) X10000; c: in mice treated with 50 mg/kgbw/day fluconazole showing an irregular-shaped hepatocyte nucleus (N); mitochondria (M); disruption cisternae of rER, primary lysosomes (Ly); accumulation of β -granules of glycogen (G); few lipid droplets (L). X 7500; d: in mice treated with 100 mg/kgbw/day fluconazole showing pyknotic hepatocyte nuclei (N) containing enlarge nucleoli, mitochondria with indistinct matrix (M), lipid droplets (L). X 5000.

3.3.3. DNA Fragmentation

By applying polyacrylamide gel electrophoresis for analysis of DNA in the liver tissue, the results showed no difference in the DNA bands between control and different doses of fluconazole-treated mice (Figure 6).

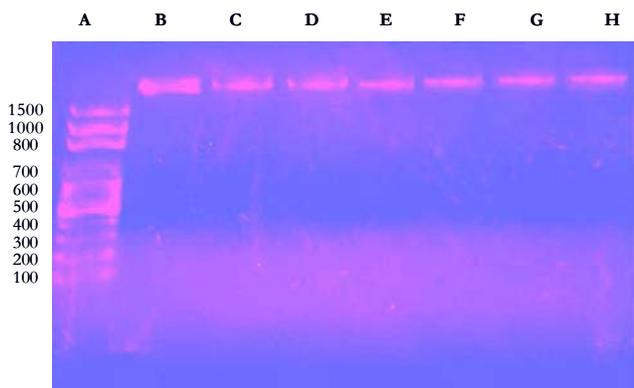


Figure 6. Gel electrophoresis photograph shows the genomic DNA of liver tissue from control and treated groups, showing Lane A: DNA marker; Lane B: Control; Lane C, D: Dose 1 (25 mg/kgbw); Lane E, F: Dose 2 (50 mg/kgbw); Lane G, H: Dose 3 (100 mg/kgbw).

4. Discussion

As a potent antifungal drug, fluconazole has been clinically used in the treatment of mycoses causing

hepatotoxicity, therefore, the objective of the current study was to evaluate hepatotoxicity and genotoxicity in newborn mice. Based on genotoxicity levels observed in the present findings, fluconazole had the highest hazard in prompting micronucleus and chromosomal aberrations by a dose of 50mg/kg and 100 mg/kg fluconazole for five consecutive weeks. Moreover, as it was reported by Sun *et al.* [24], fluconazole did not affect the body weight of mice compared with the control mice.

Concerning the micronucleus test, the results revealed an increase in the percentage of polychromatic erythrocytes of mice bone marrow cells taken 50 mg/kgbw and 100 mg/kgbw fluconazole, in comparison with 25 mg/kgbw and control group. This indicates that fluconazole could interfere with the nuclear division of the bone marrow cells in such a way that chromatin fragments or whole chromosome had lagged at anaphase and did not incorporate into one of the daughter nuclei at the time of the cell division [25]. Hypoploidy, deletion, centric fusion, and stickiness was the manifestation of structural and numerical aberrations at doses of 50mg/kgbw and 100mg/kgbw while 25 mg/kgbw dose of fluconazole was not harmful to bone marrow cells. These findings indicate the absence of incidence of systemic toxicity at a dose of 25mg/kgbw. Yuzbasioglu *et al.* [17] declared that fluconazole could induce structural chromosomal abnormalities as in a state of chromatid and chromosome breaks as well as sister chromatid union. While

Biswas *et al.* [26] reported that breaking in the chromosomal aberration may attribute possibly to that the chemicals acted after chromosome duplication at the G2 phase of the cell cycle. Stickiness might be attributed to an action on the proteins of chromosomes [27]. The mechanism of chromosomal aberrations has been shown by Michailova *et al.* [28], who explained that the chromosomal aberrations might be due to the consequence of processes which operate on damage induced by different factors in chromosomal DNA.

The antifungal drug, fluconazole caused dose-dependent micronucleus aspects for bone marrow polychromatic erythrocytes (PCEs). The PCEs is an indicator of the acceleration or inhibition of erythropoiesis and varies with the scoring interval. The increase in the PCEs percentage may indicate the suppression of cell division, the damage of erythroblasts, the addition of damaged cells, or exceed of the existing cell pool with newly deformed cells [29].

Various mechanistic interpretations may contribute to the genotoxicity and cytotoxicity of fluconazole induction [30], including of fluconazole in cellular DNA [31], the stability of the topoisomerase II-DNA complex [32] free radical-mediated toxicity caused by fluconazole [33], or the generation of reactive oxygen species (ROS) by the fluconazole [34].

50 and 100 mg/kgbw fluconazole showed certain histopathological alterations in the hepatic tissues of mice, where there was marked disorganization of the parenchymal cells and the presence of vacuoles might be the starting point of the cellular autolytic process. Moreover, 100 mg/kgbw/day fluconazole revealed severe dilatation and congestion of central veins and hepatic sinusoids in most of the hepatic tissue, and there were hydropic hepatocytic and lymphocytic infiltrations.

Indeed, the incidence of the hepatotoxicity during treatment with fluconazole has been described in several types of research followed by a compilation of interpreted reports on the mechanisms of hepatotoxicity induced by antifungal agents [35]. Shubin *et al.* [36] explained that the vacuolation of hepatocytes was most probably due to the retention of fluid inside the hepatocytes. Furthermore, it was reported that the swelling of hepatocytes results in hydropic degeneration which was due to reduction of the energy necessary for regulation of ion concentration of the cells or short term anoxia or metabolic stress [37, 38]. Jensen *et al.* [39] suggested that such cellular infiltration might be due to the presence of necrotic cells which act as an irritant substance attracting the inflammatory cells. In this context, the current results are consistent with the suggestion of Chen *et al.* [40] who stated that the patches of aggregation of inflammatory cells infiltration could be a defence mechanism of the liver against the toxic effect of the drug.

About the ultrastructure of hepatotoxicity, the accumulation of an abnormal amount of fat in the hepatic parenchymal cells is a sign of injury and could be attributed to impaired protein synthesis as a result of rER damage and accordingly inhibition of lipoprotein manufacture [41].

Consistent with the present results the hepatic alterations after antifungal drug fluconazole administration may be correlated with defects on all cell organelles and membranes in addition to the marked proliferation of smooth endoplasmic reticulum and presence of few microbodies and numerous defects on mitochondria in the hepatocytes [42]. Further, in the present results, 100 mg/kgbw/day dose of fluconazole showed mitochondrial swelling, displayed electron less dense matrices and a large degree of polymorphism suggesting sensitive cell injury. As such results could be attributed to the accumulation of fluid in the mitochondrial matrix which in turn destroyed the cristae. Since the mitochondria are the site of the main energy production of the cell, their damage may result in lowered energy output [13]. Somchit *et al.* [43] reported that disruption of the whole hepatocytes rather than inhibition of mitochondrial respiration.

Furthermore, the dilatation and destruction of rough endoplasmic reticulum and loss of adhering ribosomes are considered as the rER in liver cells the main target of many chemical agents and due to it is also participating in the metabolism of various drugs and poisons. Again, rough endoplasmic reticulum and its damage represent the main site of protein synthesis in the cells; such damage could be reflected in the impaired capacities of such cells in protein synthesis [44].

In the present results, DNA laddering electrophoresis revealed that fluconazole did not affect the quality of DNA in any dose levels compared to the control. This was confirmed by the results of [45] who found that fluconazole had no observable damage in the genome of adult animals (8weeks old), and causes a high increase in micronuclei frequency in young animals (3 weeks old), and in newborn pups.

5. Conclusion

The current assessment indicates that fluconazole toxicity mainly hepatotoxicity occurring in two diagnostic forms, that is, genotoxicity and histopathology, distinctly promotes micronuclei formation, chromosomal aberrations, and hepatocellular deterioration *in vivo* in newborn male mice. Indeed, Micronucleus, chromosomal aberrations (numerical and/or structural) is certainly a key factor in the genotoxicity progression of organ disorders.

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Conflicts of Interest

The authors declare that there is no conflict of interests.

Authors' Contributions

Azza Attia, Elhenshery Z. M. conceived and designed the study and also performed the experiments, photomicrographs (light and electron microscopy) and collected the data. Reda ElMazoudy wrote the primary manuscript. Matta C. A. provided her expertise and reviewed the manuscript.

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