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Histological and Teratogenic Effects of Favimol in Albino Mice

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Abstract

The current study was designed with the aim of evaluating the histological and teratogenic effects of favimol using Swiss albino mice Mus musculus. Histological effects in liver and kidneys were estimated for doses 1200, 2400, and 3200 mg. kg-1. Body weight of Favimol (FAV), as well as an estimate of teratogenic effects at the 1200 mg. kg-1 dose of the same drug, as there were histopathological changes in each of the tissues of the liver and kidneys of the mice treated with the previously mentioned doses, manifestations of different stages of programmed death, necrosis and degeneration were observed, while atrophy of the rows of hepatocytes was observed at the dose of 3200 mg. kg-1. Treatment with different doses of the drug caused epithelial cell sloughing in the lumen of the convoluted tubules and infiltration of blood cells as well as atrophy and degeneration of the epithelial cells of the glomeruli, extensive necrosis of many walls of the convoluted tubules was noted at the dose of 3200 mg. kg-1, and the resulting dose is 1200 mg. Kg-1 in causing a teratogenic effect on the embryos of female mice, as well as the effect of the drug on the weight of the embryos, It is clear from the results of the current study that the drug caused different histological changes in both the liver and kidneys of adult white mice as well as causing teratogenic effects in the embryos of Female albino mice which treated at days 9, 10, and 11 of pregnancy.

Keywords: *Favimol, Apoptosis, Necrosis, Teratogenic effect.*

Introduction

Coronavirus (COVID-19) has been a strange shock in the twenty-first century, as most people who contract the virus suffer from mild to severe symptoms and recover without treatment. After this virus became an epidemic all over the world, it became necessary to find an appropriate treatment for this virus (1). Several drugs have been suggested for use against viral infections, which could positively affect the treatment of COVID-19, such as chloroquine and hydroxychloroquine (3, 2). Favipiravir (FAV) (6-fluoro-3-hydroxy-2-pyrazinecarboxamide) (trade name Favimol, also known as T-705, Avigan or Favilavir) has been presented as an effective and successful drug candidate for the treatment of COVID-19 after the virus pandemic. coronavirus (4).FAV is an antiviral drug manufactured by the Japanese pharmaceutical company Fujifilm Toyama Chemical, and it is derived from pyrazine (Fig. 1).It was initially discovered to be active against influenza virus in vitro, and was approved in Japan in 2014 for storage for Epidemic preparedness only It has been marketed in China as a second line treatment for new or re-emerging influenza outbreaks (5).

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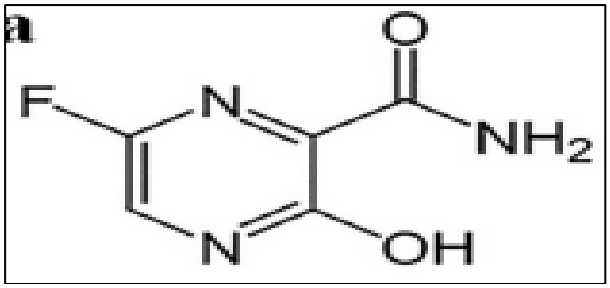

Figure (1) The chemical structure of Favimol (FAV) (5)

FAV is an antiviral compound that selectively and effectively inhibits the RNA-dependent RNA polymerase (RdRp) of influenza virus and many other RNA viruses (6). It has been found to inhibit all sterotypes and strains of influenza viruses A, B, and C that have been tested. It is also effective against West Nile virus, yellow fever virus, and foot and mouth virus, as well as other viruses, such as flaviviruses, arenaviruses, enteroviruses, and alphaviruses (7). The molecular formula of the drug is C5H4FN3O2, the molecular weight is 157.1 g/mol, and the melting point is 193℃ (8).

Essential for building, maintaining and repairing tissues is the ability to stimulate the suicide of senescent cells that are not needed by the tissue, or damaged by a phenomenon called programmed death, which is a natural phenomenon that occurs in cells of the body that have the ability to regenerate, such as in skin cells and digestive cells (9). Two phenotypically different types of cell death were observed in humans, apoptosis and necrosis. In necrosis, which occurs suddenly as a result of acute cellular injury, cells swell, plasma membranes rupture, and cellular components are released. In programmed death, the cell goes through many stages, including the fragmentation of the DNA strand into a number of separate pieces, the fragmentation of the nucleus and then the destruction of cytoplasmic organelles, such as mitochondria, the endoplasmic reticulum and others, and the formation of apoptosomes containing parts of the chromatin, then the cell shrinks and loses its connection with the neighboring cells, then begin to decay and are finally engulfed by macrophages (10). This process prevents the release of inflammatory factors and is therefore called clean cell death, since apoptosis occurs during embryonic development or in cells that die under physiological conditions (11). Cell death can be initiated by a variety of stimuli including DNA damage, nutrient deficiency, exposure to carcinogens, heat shock and binding of death receptors on the cell surface through activation of caspases (12). and the mechanism of cell death is complex because it involves a series of complex biological processes, as this process occurs through two pathways, the first is the extrinsic pathway that begins when interactions occur across the cell membrane by death receptors, that leads to stimulation of the enzyme Caspase 8, which carries out cell death (13,14). The second pathway Which is known as the mitochondrial pathway, as a group of stimuli leads to changes in the inner membrane of the mitochondria, which leads to the release of enzymes that bind to Caspase 9 to begin its work, and there is an additional pathway that includes cytotoxicity by T-cells and killing the target cell depending on Perforin / Granzyme (15) .

Congenital malformations are single or multiple defects in the formation of organs or body regions that can be identified at birth or during intrauterine life (16), and malformations in the offspring can lead to long-term disability, disease and death. The global prevalence of congenital malformations is about 2-3%. The most common severe congenital malformations are heart defects and neural tube defects, as well as genetic and socioeconomic factors that may increase the risk of congenital malformations in the embryo. Other possible causes include maternal exposure to alcohol, tobacco, radiation and drugs (17). The study of abnormalities in the structures of embryos and newborns for animals Laboratory is an important step in teratogenic screening processes, as teratogenic exposure has the potential to interfere with the functional and structural development of

embryos.Although teratogenic exposure increases the risk of major phenotypic malformations, it also causes risks such as spontaneous abortion, growth failure, and structural abnormalities and others (18).

Materials and Methods

Twenty-five mice were used for histological examination, and another 15 mice for the test of teratogenic effects. The groups of 5 females were divided as follows: the negative control was given distilled water, while the positive control mice were injected with cyclophosphamide at an amount of 10 mg. kg-1 in the peritoneum, and the remaining three groups were dosed orally with favimol as follows: the first group was given 1200 mg. kg-1, and the second group 2400 mg. kg-1, and the third group 3200 mg. kg-1. While the oral dose of the drug was done only in the first dose of 1200 mg. kg-1 when studying teratogenic effects.

Study of histological changes in the liver, kidneys and ovaries of female albino mice:

The animals were sacrificed by separating the cervical vertebrae 24 hours after the time of the last dose, as the duration of dosing was five days. The histological sections were prepared according to the method described in (19). The organs to be studied were immobilized immediately after dissecting the mice of the studied groups with formalin fixative for 24 hours (20), then the samples were washed with running water for an hour and a half to remove excess fixative from the tissue, then the samples were passed with an ascending series of concentrations of ethyl alcohol 70%-100%. For the purpose of drawing water from the samples for a period of 30 minutes for each concentration, then using xylene for the purpose of liquefaction because of its property in making it more transparent as the samples are placed in it for 25 minutes, the samples were placed in a mixture of xylene and paraffin wax in a ratio of 1:1 and the mixture was placed in an oven of the Marubeni Japan type at a temperature of 60 °C for 15 minutes, after that it was transferred to molten wax for half an hour each time with three passes, then the samples were buried with the same type of wax used by pouring the molten wax quietly into an iron mold in the shape of the letter L, then left to cool and harden, and then Separating them from the molds after making sure of their hardening by cooling the mould, then the wax molds containing the models were trimmed with a sharp knife, and the sections were cut with a thickness of (5-7) micrometers and spread over the surface of the water in the water bath device at a temperature of (37-40)℃ for the purpose of weaving textiles and prevent the accumulation of cells on top of each other, then the sections were loaded on a glass slide marked (the name of the group and the type of organ), after wiping it with albumin and placed on a hot plate at a temperature of 40 °C, and left to dry, then transferred to the glass Coplin jars containing xylene with heating the slide for a short period of time on a heat surface before being placed in xylene, and the slide is left in it for 30 minutes in order to remove the wax or its remaining traces from the tissue well. Then the slide is removed from the xylene and the process of giving water to the tissue section hydration begins by placing it in the descending alcohol concentrations (100%, 90%, 70%, 50%, 30%) and then to distilled water. Each concentration for a period of (1-2) minutes. Hematoxylin & Eosin stain was used according to the method (21), the sections were stained with hematoxylin stain for 5 minutes, and washed in running tap water for the purpose of color discrimination, then the sections were stained with eosin stain for (15-30) seconds, and washed in Coplin dyeing utensils glass jar in warm water for 5 seconds for the purpose of color discrimination. Then the slides are left on a hot plate at a temperature of 40 °C for the purpose of accelerating drying, and then kept in their respective boxes. The processed sections were examined using a light microscope, and then observations were recorded on them.

Study of teratogenic effects and skeletal abnormalities using dual staining of skeletons:

The animals were sacrificed on the 18th day of pregnancy, and the skeletons of the embryos were stained according to the Erdoğan (22) method. The embryos were extracted from the uterus and placed in 95% ethanol for 3-4 days, then removed from the fixation solution and their skins gently removed, then transferred to absolute acetone for a period of two days to melt the fat from it, then staining with alichian and alizarin stains. The embryos were placed in the coloring solution for 3-5 days, after which they were washed with running water for two hours. Then the embryos were placed in a 1% potassium hydroxide solution for a period of two days or more until the structure becomes transparent and clear, Then the embryos were transferred to the following concentrations:

20% glycerol / 80% [potassium hydroxide 0.5%] for 2 days.

50% glycerol / 50% [potassium hydroxide 0.5%] for 3 days.

80% glycerol / 20% [potassium hydroxide 0.5%] for 5 days. Embryos were preserved in 100% absolute glycerol, Skeletal abnormalities were recorded based on standard forms.

Results

Study of histological changes in the liver and kidney of female albino mice:

Figure (2) shows a histological section of mouse liver cells from the negative control group and the liver appears normal, while Figure (3) shows several histological sections of liver cells of mice treated with different doses of Favimol, in which the programmed death of the cells appears with the presence of vacuoles around the nuclei, of most hepatocytes, debris of some degenerated hepatocytes, infiltration of a number of white blood cells, necrosis of numbers of liver cells and transformation of their areas in the form of caverns filled with debris of damaged hepatocytes with narrowing in the rows of hepatocytes, as shown in Figure (4:a,b) a histological section of a mouse kidney from the negative control group, the cortex and the pulp region appear in their normal state, while Figure (5:a,b) shows several tissue sections of the kidneys of mice treated with different doses of Favimol, as it appears that the cells suffered from degeneration as well as being surrounded by the wide capsular space and degeneration of the cells lining the convoluted tubules, as well an acidic pigment glomerular infiltrate in the form of hyaline molds was found in the lumen of a number of tubules, infiltration of white blood cells on the surface of the renal glomeruli with hyperplasia of epithelial cells.

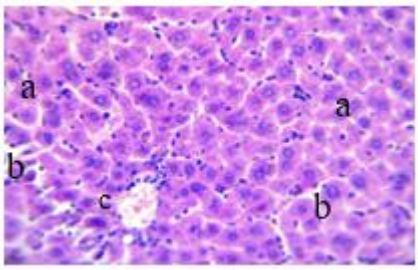

Figure (2) Histological section of mouse liver cells from the negative control group (H & E stain) 40x, a: polygonal hepatocytes with large nuclei, b: sinusoids, c: central vein

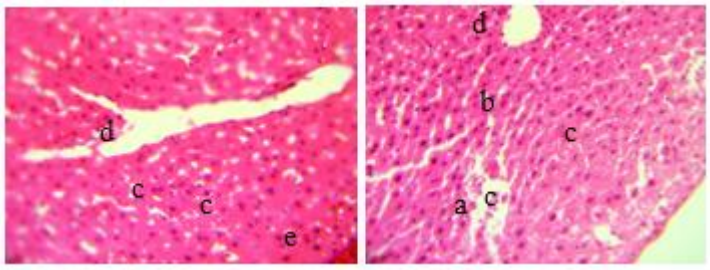

Figure (3) Histological section of a mouse liver treated with FAV (H&E) 40x, a: necrosis of hepatocytes into cavernous debris of damaged cells, b: atrophy of hepatocyte rows, c: programmed death, d: central vein , e: atrophy of hepatocyte

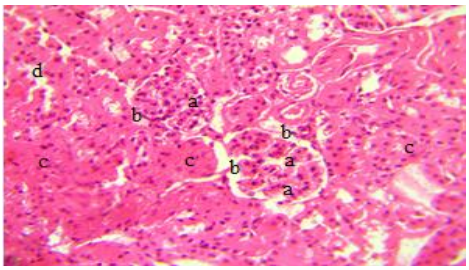

Figure (4:a) Histological section of a mouse kidney from the negative control group (H & E stain) 40x renal cortex: a: glomeruli, b: capsular space, c: proximal convoluted tubules, d: distal convoluted tubules

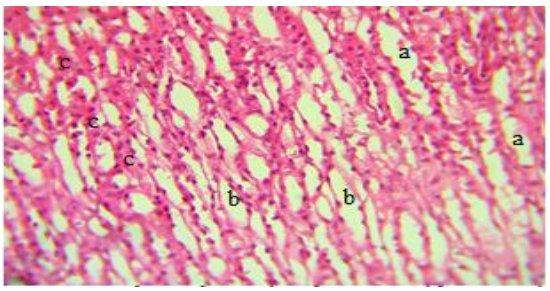

Figure (4;b) Histological section of a mouse kidney from the negative control group (H&E stain) 40x the pulp of the kidney a: the renal tubules, b: the thin cusps of the loops of Henle, c: the interstitium containing fibroblasts and white blood cells

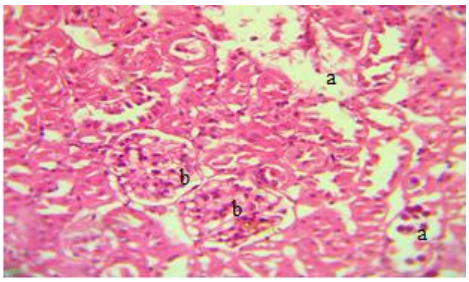

Figure (5:a) Histological section of a mouse kidney treated with FAV, (H&E) 40x renal cortex: a: extensive necrosis of several convoluted tubule walls, b: presence of dislocated cells in the lumen of tubules and white blood cells, c: proliferation of white blood cells on the surface of the glomerulus

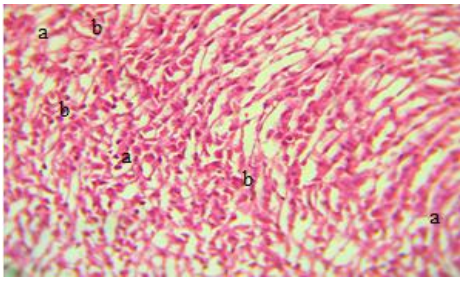

Figure (5:b) A tissue section of a mouse kidney treated with FAV (H&E 40x) kidney core: a Presence of epithelial cell debris in lumen of renal tubules, b: Infiltration of leukocytes and macrophages in the interstitial tissue of the pulp

Study of the teratogenic effects of Favimol in female albino rat embryos:

Table (1) shows results of the teratogenic effects of the drug Favimol at a dose of 1200 mg. kg-1 in embryos of albino mice. Figure (6) shows the teratogenic effects of the drug in embryos of pregnant female mice.

Table (1) the values of the mean differences for the total number of embryos, dead embryos, abnormal embryos, and weight in groups treated with a dose of 1200 mg. kg-1 of favimol and positive control CP.

treatment dose mg.kg^{1-}.b.wt	**The total number of embryos M±S.D**	**The total number of embryos MD±S.E**	**dead embryos MD±S.E**	**abnormal embryos MD±S.E**	**the weight M±S.D**	**the weight MD ± S.E**
DW	3.600±0.89	-	-	-	1.466±0.08	**-**
CP/10	-	2.600±0.47*	1.600±0.20*	3.200±0.36*	-	0.35±0.01*
FAV/1200	-	1.200±0.47	0.00±0.20	2.400±0.36*	-	0.65±0.01*

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

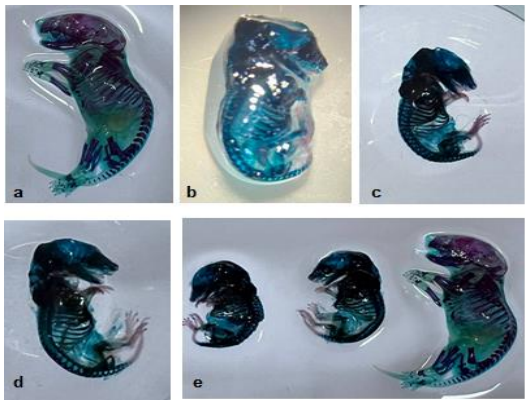

Figure (6) Embryos of albino mice exposed to favimol, (Alizarin + Alcian) 10x, a: Skeleton of an embryo from the negative control group, b, c, d, e: Skeletons of embryos from the group treated with favimol at the dose of 1200 mg. kg-1

The results of current study showed a significant increase in the mean differences values for abnormal embryos, as it reached 2.400 ± 0.36 at the dose of 1200 mg. kg-1, results of the study showed, a significant increase in the mean differences values for embryo weight, which amounted to 0.65 ± 0.01 at the dose of 1200 mg. kg-1, and this indicates that this drug may has a teratogenic effect on the embryos, in addition to its effect on the weight of the embryo, while no effect of the drug was recorded on both the total number of embryos and dead embryos, and no resorption was recorded (i.e. The appearance of the effects of the implantation process, but the embryonic growth process stopped as a result of devouring the components of the embryo by the cells of the wall of the uterine horn, so it appears in the form of a recessed vacuole at the implantation site) in the drug-treated

group, and this leads to the assumption that the drug did not have an effect on the cell cycle during menstruation embryonic.

Discussion

Studies conducted on the FAV drug confirmed that it has the ability to increase oxidative stress, damage organs and cause damage to both liver and kidneys of rats (23), as the drug turns into an inactive oxidative metabolite (M1,6-fluoro-3,5dihydroxy-2-pyrazinecarbox amid) in the liver cytosol of human males and females, monkeys, rats, and mice, M1 was discovered to be an oxidative metabolite of FAV catalyzed by aldehyde oxidase or by xanthine oxidase (24), since M1 is excreted by the kidneys, and thus exposure to the drug and doses high levels lead to the accumulation of M1, causing damage to liver and kidneys (25,26) due to oxidative stress, many pathogenic processes may occur when this balanced function is disrupted. Oxidative stress and the formation of reactive oxygen species (ROS) contribute to the development of liver and kidney injury. By activating the inflammatory response through the release of pro-inflammatory cytokines and the accumulation of inflammatory cells in tissues (27,28).

In the current study, we found that different doses of FAV cause liver and kidney tissue damage in mice, as our findings indicated that organ damage caused by the drug was associated with the induction of oxidative imbalance and cytokines leading to inflammation, and the damage caused by the drug was more severe when the high dose of it at the low dose, this study confirmed the occurrence of degeneration in liver cells, which may be caused by excessive production of oxidants and cytokines that led to inflammation, therefore, given the harmful effects of the drug on the liver. The careful and frequent follow-up of the vital parameters of liver during treatment will be useful To predict the occurrence of complications, therefore, elevated liver function tests and/or liver damage are critical side effects of the drug that have been observed frequently in clinical studies (29,30). And although the exact mechanism of liver and kidney damage that occurs is unknown, the damage may be due to a specific interaction with the drug or its metabolites.

The teratogenic effects can lead to fetal death, or various malformations, which are caused by the effect of any substance capable of crossing the placental barrier in sufficient concentration to have a teratogenic effect at a specific time in pregnancy (31). Oral administration of FAV has been shown during organogenesis, decrease in diet consumption, water intake, and weight of pregnant mice, fetal growth retardation was noted, fetal toxicity as evidenced by low fetal weight, and exposure led to structural deformities (cranial) in embryos. A significant decrease was also observed in the weight of pregnant mothers in the group, this weight loss could be associated with reduced diet consumption and water intake (32). However, this alone cannot contribute to a decrease in maternal weight, as it could also be due to increased breakdown of fats and proteins leading to lower Organ weight as a result of drug toxicity (33).

It is forbidden to use the drug during pregnancy, according to what was stated in the drug leaflet of the drug. Some studies indicated that the drug has toxic teratogenic effects, as early fetal death was observed in mice (34). And another study on the emergence and development of embryos, which was conducted on mice, rats, rabbits, and monkeys have teratogenic effects, as well as a decrease in the number of live embryos, a decrease in birth weight, and the appearance of deformities in the embryos (35). So because of the very limited data on the safety of using the drug in human pregnancies, it is not recommended to use it for women during pregnancy, or women who They plan to become pregnant (36). A study conducted in Turkey on a number of pregnant women, as they were given the treatment during pregnancy, led to live births, with a slight heart anomaly (foramen ovale), as well as early births. In the case of the foramen ovale, it was noted that the mother was suffering from a lack of amniotic fluid in the thirty-fifth week, despite the

fact that the birth was on schedule. limited to reach a specific result (35). And in another study, the results of pregnancies with exposure to the drug were revealed, that among the births a child suffering from an expansion of the kidney pelvis and a deformity in the heart, while another birth resulted in a child suffering from birth distress that requires admission to the neonatal intensive care unit (37). Since the drug increases deformities and reduces the survival of the embryo in laboratory animals, therefore, the most effective contraceptives must be used by both men and women, during and after the treatment period, because the treatment can reach the sperm (38).

Conclusion

The current study showed that the doses 1200, 2400 and 3200 mg. kg-1 of Favimol has a toxic effect on the liver and kidneys, as it causes hyperplasia and enlargement of hepatocytes as well as necrosis and apoptosis of its cells, as well as acute atrophy in most renal glomeruli with expansion of their capsular space and sloughing of numbers of epithelial cells in the lumen of the tubules twisted, and the dose is 1200 mg. kg-1 has a teratogenic effect on the embryos of female albino mice, as well as its effect on the weight of the embryo.

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