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Patulin Induced Dominant Lethal Gene in Mature Male and/or Female Rats

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ABSTRACT

Many fungi including *Penicillium* and *Aspergillus* species produce patulin in the contaminated foods. Patulin is a heterocyclic unsaturated lactone that reacts with SH group of biological molecules causing harmful effects in human and animal tissues. Hence, the present investigation was designed to evaluate the possible teratogenic effect of patulin (0.002mg/kg b. wt) which was examined through the induction of dominant lethal gene and the alteration in number of live births in the female rats. Results and Conclusion: patulin is a dangerous teratogen in rats. This was confirmed by the significant increase in the percentage of maternal and embryo toxicities.

Keywords: Patulin, Pregnant rats; Dominant lethal gene; Mycotoxins; Fetal resorption; Defective embryo; Teratogenicity.

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1.Introduction

Patulin, a very toxic teratogenic, hetero- cyclic lactone, is synthesized by many fungi including *Aspergillus* and *Penicillium* . This toxin has been extracted from apple and its products and is commonly stable in grape juice, apple and dry corn (1).

The eating of patulin contaminated vegetables and fruits can cause many health problems, such as carcinogenesis, immune suppression, teratogenic effects and embryotoxicity. The genotoxic effects of patulin on human cells resulted from its ability to induce reactive oxygen species. The main storage locations of patulin are red blood cells and blood – rich organs as kidney, spleen, liver and lung. Moreover, Patulin able to induce disruption of plasma membrane, protein synthesis inhibition, disturbance of transcription and translation, suppression of DNA synthesis, increase sister chromatid exchange, the chromosomal gaps, chromosomal aberrations and micronuclei formations (2 , 3 & 4).

Patulin was lethal to the dam and caused weight reduction in one survivor due to maternal toxicity in mice, its lethal effects may be due to its ability to react with SH-containing molecules . Moreover, it induced a decrease in the average of fetal body weight and all of implanted embryos were completely resorbed in pregnant rats. It caused a reduction of spermatozoa production, infertility and reduce the chance of reproductive activity in the treated male rats (5, 6).

So, the present work was aimed to evaluate the possible teratogenic effect of patulin (0.002mg/kg b. wt.) through the applying of dominant lethal gene test on the albino rats.

2.Material and methods

2.1. Patulin

Patulin was obtained from Sigma- Aldrich (St. louis MO, USA). It was dissolved in distilled H₂O and was injected orally for thirty five successive days to the animals in a dose level

of 0.002mg/kg. b. wt. that equivalent to (1/60) of LD₅₀ (50mg/kg b.wt.) (7).

2.2. Experimental animals

In the present work, 48 white albino rats (*Rattus norvegicus*) were used (mature males and females) of about three months old. The body weight of range 130 ± 10 gm. The animals were obtained from Animal House of Faculty of Medicine, Zagazig University, Egypt. The rats were housed in metal cages, bedded with wood shavings, fed on a standard pellet diet and water *ad libitum*.

2.3. The experimental design

The experimental animals were randomly classified into four groups as follows:

I- Control group; Included 12 mature rats (3 males and 9 virgin females). The rats were orally administered with distilled water using a metallic gastric tube for 35 successive days, after that, each 3 females were caged with 1male for mating .

II- Males Treated group; Included 12 mature rats (3males and 9 virgin females). Each male rat was orally injected with patulin (0.002mg /kg b.wt. for 35 successive days using a metallic stomach tube). At the end of treatment period, each three of untreated females were caged with one of patulin treated males for mating .

III- Females Treated group; Included 12 mature rats (3 males and 9 virgin females). Each female rat was orally injected with patulin (0.002mg /kg b. wt. for 35 successive days) using a metallic stomach tube. At the end of the treatment period, each 3 of patulin treated females were caged with 1 untreated male for mating .

IV- Males and Females Treated group;Included 12 mature rats (3 males and 9 virgin females). All female and male rats were orally injected with patulin (0.002mg /kg b. wt. for 35 successive days) using a metallic stomach tube . At the end of treatment period, each 3 of patulin treated females were caged with 1 of treated males for mating .

2.4. Methods of investigation

The vaginal plugs as indicators of successful copulation were checked every day between 8 - 9 a.m. for 3 consecutive days.

At 18th day of pregnancy, the females were sacrificed and the uteri were examined. Corpora luteal, living and dead fetuses were

recorded for each group and the photographs were taken when necessary.

2.5. Statistical analysis

The obtained data were tabulated and the frequency of dominant lethal gene test was calculated according to (8) as following

Frequency of dominant lethal gene test =

$$\left[1 - \frac{\text{Living embryos per pregnant female in experimental group}}{\text{Living embryos per pregnant female in control group}} \right]$$

Percentage of frequency of dominant lethal gene test =

$$\left[1 - \frac{\text{Living embryos per pregnant female in experimental group}}{\text{Living embryos per pregnant female in control group}} \right] \times 100$$

3. Results

3.1 Dominant Lethal Gene Test in Control Group:

In the present work, a mating between untreated male and female resulted in

successful fertilization. The presence of a vaginal plug was always accompanied by pregnancy. All the 9 mated females were pregnant in the control group (Fig.1).



Fig. (1): Uterus of pregnant female rats on the 18th day of pregnancy showing 8 and 10 viable fetuses with Corpora lutea in the control group.

The number of corpora lutea in the ovary of pregnant females is equal to the number of viable fetuses (Table,1). The total number corpora lutea was 66 with an average 7.3%. The total number of viable fetuses was 66, the percentage of viable fetuses was 100%. There

is no dead fetuses was detected as shown in table (1). The number of implantations per female was always equal to the number of corpora lutea in the corresponding ovary of pregnant females.

Table (1): Comparison between the frequencies of dominant lethal gene after the treatment of rats (males, females and/or both) with 0.002 mg/kg b. wt. of patulin for 35 successive days.

Groups	No. of ♀ rats	Number of pregnant females			Number of corpora lutea / females		No. of implantation sites/ pregnant females			Viable fetuses / pregnant females			Dead fetuses / pregnant females			Dominant lethal gene	
		No	Mean	%	No	Mean	No	Mean	%	No	Mean	%	No	Mean	%	Frequency	%
Control group	9	9	1	100	66	7.3	66	7.3	100	66	7.3	100	0	0	0		
Males Treated group	9	5	0.5	55	65	7.2	31	6.2	47.6	31	3.4	47.6	0	0	0	0.54	54
Females Treated group	9	8	0.8	88	58	6.4	52	6.2	89.6	51	5.6	87.9	1	0.1	1.96	0.24	24
Male and Females Treated group	9	5	0.5	55	55	6.1	32	6.4	58.1	32	3.5	58.1	0	0	0	0.53	53

3.2. Dominant Lethal Gene Test in the Patulin Treated Groups:

Males, females and/or both were treated with patulin at a dose of 0.002 mg/kg b.w. for 35 successive days (treated males, treated females, treated males & females) respectively and were allowed to mate as mentioned above in the experimental design. The results of examination in the three groups were summarized in tables (1).

3.2.1 Males Treated Group:

When a group of male rats were treated with patulin and allowed to mate with untreated

females, some disturbances in the ratio between the number of corpora lutea and the number of viable fetuses were scored. Only 5 out of 9 mated females were pregnant depending upon the presence of a vaginal plug. The total number of 65 corpora lutea were observed in all mated females with an average of 7.2 per a female. The total number of viable fetuses was 31 with an average 3.4 and a percentage of 47.6%. No dead fetuses were detected (Table1 and Fig. 2 &3).



Fig. (2): Uterus of untreated pregnant females on the 18th day of pregnancy which were mated with patulin treated males showing 6 viable fetuses with Corpora lutea.

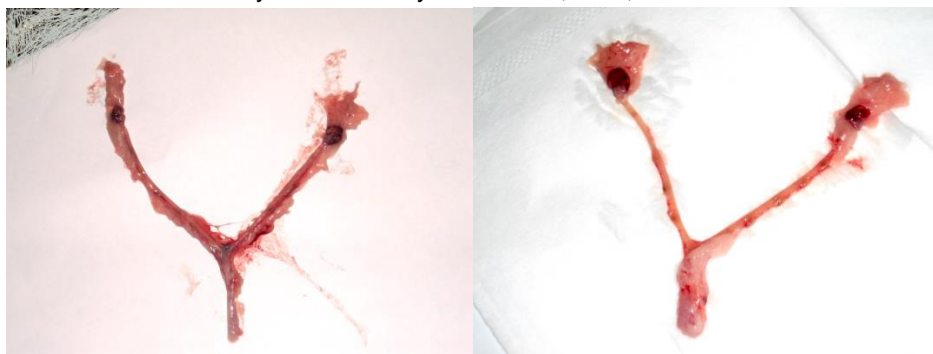


Fig. (3): Uterus with corpora lutea of untreated, non-pregnant females which were mated with patulin treated males

After the dissection, all of the 9 mated females were have corpora lutea but, 5 of which were had fetuses (47.6%). In this concept, the percentage of dominant lethal gene was 54%.

3.2.2. Females Treated Group:

When a group of females were treated with patulin then, allowed to mate with untreated males. Eight out of 9 mated females were pregnant depending upon presence of a vaginal plug (a female rat was not pregnant). The total number of 58 corpora lutea were observed in all mated females with an average of 6.4 per female. The total number of viable fetuses was

51 with an average 5.6 and a percentage 87.9% .

It can be seen that successful mating and pregnancy occur only in 8 out of 9 females. In one case, the mating resulted in a complete failure of pregnancy despite of the presence of corpora lutea could be seen in the ovaries

In one of pregnant females, one of its fetuses was died at about mid time of the pregnancy, by a mean of 0.1 of 1.96 % of the fetuses. The percentage of dominant lethal gene was 24% (Table1; Fig.4&5).

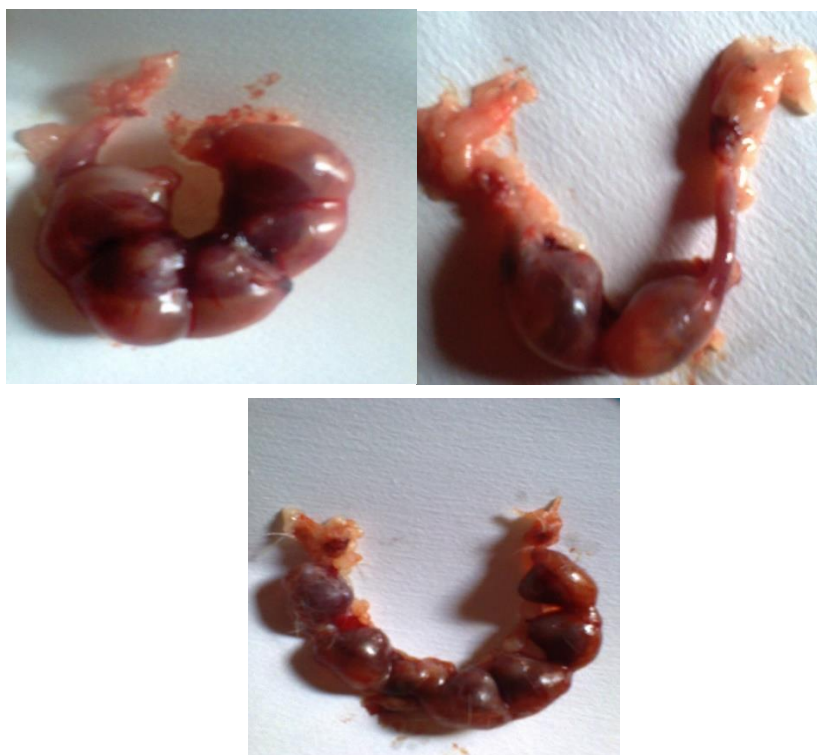


Fig. (4): Uterus of pregnant females on the 18th days of pregnancy which were previously treated with patulin then, mated with untreated males showing 2, 5 and 6 viable fetuses with Corpora lutea.



Fig. (5): Uterus with corpora lutea of non- pregnant females which were previously treated with patulin then, mated with untreated males

3.2.3. Males and Females Treated Group:

When both parents (males & females) were treated with patulin and then allowed to mate with each other, only 5 out of 9 mated females were pregnant depending on the presence of vaginal plug. Also the number of corpora lutea

was 55 with an average 6.1 per female and the number of viable fetuses was 32 with an average 3.5 and a percentage of 58.1 %, the dead fetuses were not seen in the uteri. The percentage of dominant lethal gene was 53%(Table1 and Figs. 6 &7).



Fig. (6): Uterus of pregnant females on the 18th days of pregnancy which were previously treated with patulin then, mated with patulin treated males also, showing 2 and 8 viable feotuses and corpora lutea .



Fig. (7): Uterus with corpora lutea of non-pregnant females which were previously treated with patulin then, mated with patulin treated males also.

It became clear that the percentage of male and female group . Moreover, this dominant lethal gene for fetuses of the treated female group increased and reached 24%. Meanwhile, it was increased to 53% in treated

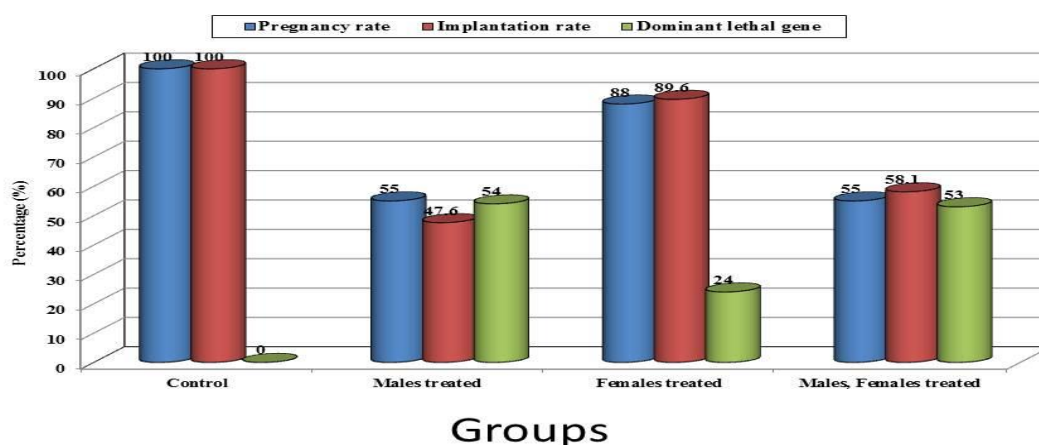


Fig (A):Comparison between the percentage of pregnancy rate, implantation rate and dominant lethal gene test after the treatment of male and/or female parent by patulin (0.002 mg/kg b.wt.) for 35 successive days .

In spite of this, development of the viable & dead embryos showed a wide range of variations, even these implanted well in the same uterus sometimes, the embryos were larger in size and presented in a more advanced phase of development than the other. The dead fetuses were also at variable phase of deterioration. Some of them seemed to have dried only recently while others presented as dry flakes. The placenta attached to the same uterine wall showed up also in variable size probably presenting different developmental stages. Where, some of them comparably large while others were fairly rudimentary. Others were fairly dry and had the appearance of a large mass of clotted blood as in all figures. Another point of interest seems necessary to be added. It concerns the morphological appearance of the placenta, as well as the size and phases of development of both the live and dead fetuses. In this connection it is necessary to repeat that the treated animals were housed in a ratio of one male to three females, mating was always successful by the

presence of vaginal plug and all females were dissected at the same mid gestation period.

4. Discussion

The present work is an attempt to find out the effects of patulin on the germ cell development through counting of the living embryos in relation to the number of fertilized ova resulting from mating preceded by treatment of one or both parents with patulin. Under regular conditions, the dissection of a normal pregnant female that was mated with a normal male, 18 days post mating, usually resulted in a number of live embryos that was equal to the number of corpora lutea in the ovary with an average 7.3. The fetuses spotted in a given female always had the same size and same phases of development.

In the present investigation, one and/or both parents were treated with patulin (0.002 mg/kg. b. wt. for 35 successive days) then allowed to mate as described previously in the experimental design and the observations were scored as following: in males treated group, when , male rats were treated with patulin and allowed to mate with untreated females , it was

found that, only 55 % of mated females were pregnant in spite of examination the vaginal plug for each female . These data denoted to un successful fertilization in 45 % in spite of presence of corpora lutea , these data denoted to side effect of patulin on sperm cell .

These data were concerned with the data concluded by (9,10) who concluded that, patulin influanced the morphology of sperm in male rats. Tail abnormalities as coiled and/or bent tails and sticked sperm tails were also observed. Patulin reduced the count of sperms and destroy the sperm normal shape in the rat. Also, it induced abortions in mice and rats after injection. Moreover, Teratogenicity and embryotoxicity were recorded in chick embryo

Also, (11) studied that the number of abnormal sperms increased and the amount of live sperms decreased with a clear reduction in weight of body and epididymal weights in mice treated with mycotoxins. These toxins may cause disturbances in testosterone hormone secretion and hence affecting animal fertility . It may reduce progesterone secretion by inhibition of the follicle stimulating hormone.

On the other hand, the results of the present work were on disagreement with those of (12) who reported that patulin did not induce any dominant lethal dangerous effects. Also, (6) who added that the mating of patulin treated male rats could not induce any dominant lethal gene mutations. In addition to (13) who stated that, the generations of Swiss mice treated with patulin, showed no transplacental carcinogenicity.

When female parents treated with patulin followed by mating with untreated males it was noted that, the percentage of successful fertilization reached to 88 %. That is to say, 8 out of 9 females were pregnant , but it showed disturbances between the number of corpora lutea and the number of viable fetuses . The percentage of dead fetuses was appeared by 1.96 % . In our opinion, these data referred to that the germ cell (ovum) in specific lethal gene may or may not affected by patulin and/or

patulin toxin may interfere with ovarian hormones .

These results were concurrent with (1) who discussed that, patulin affect embryonic development *in vitro*. It was recorded to be lethal to embryos, affects protein synthesis and DNA. It is a dangerous toxin effects on mRNA and protein synthesis. The long term exposure to patulin may cause immunotoxicity ,embryotoxicity, genotoxicity ,mutagenicity, neurotoxicity and immunosuppression (14,15) .

In addition to (16) who illustrated that patulin caused reduction in DNA and protein content in rat embryo, length of crown rump , count of somite number and diameter of yolk sac . It induced an increase in the ratio of the defective embryos, abnormalities such as mesencephalon hypoplasia, growth retardation, hyperplasia and telencephalon.

When both parents (male & female) were treated with patulin and then allowed to mate together, the percentage of successful fertilization reached to 55 %, similar to data obtained in case of treatment of only male parent.

These data denoted to there is a deviation in germ cell (sperm or ova) which may be due to the interaction between patulin and DNA during meiosis division, interaction between patulin and protein of spindle fiber or due to its interaction with sulfhydryl group inhibiting the activity of some vital enzymes.

Similar data were concluded (17)who supported that patulin induce damage in DNA and affects the distribution of cell cycle. It has a strong ability for interacting with sulfhydryl group, affecting the action of many enzymes. Patulin recorded to be a reprotoxic, genotoxice, immunosuppressive and embryotoxic compound (18).

In addition, most of females which were dissected contained corpora lutea and without fetuses and 45 % of mated females were loss the spontaneous pregnancy. In our opinion, it

may be due to hormonal disturbances in treated female and/ or due to failure of sperms to penetrate through the plasma membrane surrounding the ovum.

This assumption is supported by several previous authors such as (19) who demonstrated that, the risk of patulin may include , immunotoxic, neurotoxic , genotoxic, immunosuppressive , carcinogenic and teratogenic effects. It may induce disruption of plasma membrane and protein synthesis inhibition . The patulin toxicity is due to modification of sulfhydryl of membrane proteins effecting cellular glutathione, plasma membrane, function of mitochondria and affecting the rat embryos.

Similarly, (20) added that, Patulin able to cause toxicity and teratogenicity in reproduction . The rates of fetal resorption were higher in patulin treated pregnant mice than in control group. The weight of embryos was clearly reduced in all patulin treated groups. A lot of malformed fetuses with cleft palate , scoliosis or exencephaly were scored. These data demonstrated the teratogenicity of patulin in mammals (21) .

Also, (22) confirmed that the mycotoxin cause disturbances in the hormonal system which coordinating fertility such as luetinizing hormone , follicle stimulating hormone , thyroxin 3&4 and testosterone . It can induce abnormal development of fetus in the farm animals and affect the function of sexual organs and the animal productivity .

Moreover, (23) supported that patulin caused maternal toxicities and significant increase in the percentage of fetal resorption in rats and mice. It was recorded to be teratogenic and embryotoxic in chick eggs .Also, in the pregnant female mice, patulin induced an increase in the frequency of malformation and cleft palates in both kidneys of the developing embryos .

In addition to (24) who demonstrated that , mycotoxins damage the tissue by oxidizing the proteins and its immune suppressive effect.

These toxins may causes severe toxicity leading to genetic disorders or inducing deformities in the developing embryo. The higher doses of patulin were toxic to all pregnant female mice.

On the contrary, (25) recorded that the ability of patulin to induce gene mutation in cells of mammals is still unclear .It induced no effect on the implantation number , delivered fetuses, resorption number, fetal death, weight of fetuses or malformation of the organs and skeleton (26).

5. Conclusion:

The results of the present work indicated that, patulin was maternal toxic , embryotoxic and significantly induced dominant lethal gene when it was administered orally to male and /or female rats with a dose of 0.002mg/kg b.wt. then allowed to mate as described above in the experimental design. Patulin toxicity was confirmed by the reduction in fertility rate, implantation number and the number of viable fetuses / pregnant female.

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