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The Morphometric Analysis of the Effects of Hydrogen Peroxide on the Off Springs of Helix Lymnaea Model

Ekhlas Sabah Hassan*[1], Fadhaa Abdulameer Ghafil[1], Sahar Abdulrudha[1], Hussein Abdulkadhim[1], Israa Ktab[2], Murooj Luai[1], Rana Talib[1]

[1]Department of pharmacology and therapeutics, Faculty of medicine, University of Kufa, Iraq.
[2] Department of microbiology, Faculty of medicine, Jaber Bin Hayan University, Iraq.
Corresponding author: Department of pharmacology and therapeutics, Faculty of medicine, university of Kufa, Iraq. Email ekhlass.khazaal@uokufa.edu.iq

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ABSTRACT

Background: Hydrogen peroxide free radical is a potent mutagen and teratogen that can obviously affects the color of the offspring's of the land snail Helix Lymnaea.
Aim: To assess the morphometric changes of the offspring's of the land snail Helix Lymnaea after parents exposure for H2O2 for three years.
Materials and Methods: A twenty four Helix Lymnaea snails were randomly divided into two groups (12 in each).Group A is the control whereas group B is the H2O2 test group. All were adults (4-6 weeks age) of the same size 5-7 mm length, all were hermaphrodites. The two groups were followed for three years for ten generations for different morphometric parameters
Results: There was a significant reduction in the movement score in the group treated by H2O2 in comparison with the control group 25% vs 8.3 % respectively p = 3.8, chi square = 0.8. The eating activity of the offspring's had a minute change noticed in between the two groups 95% in H2O2 exposed vs 92% in the control group. The morphometric outcomes mainly the albino offsprings were none in the control group that showed no teratogenicity in comparison with 60% in H2O2 group, $p < 0.05$. The rate of successful eggs hatching was significantly differed between the control and the H2O2 groups, p = 0.017. There was also an obvious difference in the mortality rate between the control and H2O2 group, however this difference was not significant p = 0.2.
Conclusion: There was a significant teratogenic effect of H2O2 on the offsprings of the snail Helix Lymnaea. This effects may extend to include different free radical producing substances.
Key words: Helix Lymnaea, H2O2, morphometry, reproductive rate, teratogenicity.
Correspondence:
Ekhlas Sabah Hassan
Department of Pharmacology and Therapeutics
University of Kufa
Iraq
E-mail: ekhlass.khazaal@uokufa.edu.iq
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INTRODUCTION

Mutagenesis, teratogenesis and carcinogenesis are the pathological processes that alter the genome wild integrity and hence initiate a real health challenges not only for human rather for all of the living species and the ecosystem (Tchounwou, et al, 2012).

Environmental teratogens are so many that originate from different sources in our ecosystem such as chemicals, radiation and viral origins (Parsa, 2012). Of the most potent chemical mutagen is the free radicals (Phaniendra, et al, 2015) of which oxygen radical species H2O2 are the most important (Wen, et al. 2013).

Many byproducts from household and factories are toxic to the biosystems like marshes, runnels and soil (Lobo, et al. 2010). Mutagenic byproduct for any biosystem is a potential toxin and mutagen for the remaining biosystems including the human being (DeVito, et al. 2017). H2O2 sharing the same mechanism of mutagenesis and teratogenesis with any reactive radical in that they react directly with nucleotides or facilitate their epigenetic reactions like methylation, oxidation or acetylation (Afanas'ev , 2015). The net result is an altered DNA base pairing and epigenetic structure (Afanas'ev, 2013). This mechanism is the commencement step of cell transformation and teratogenesis (Afanas'ev , 2010).

Different models are adopted to predict the level by which a test chemical exerts morphometric level of teratogenicity. Common in vitro models involve Ames test in typhimurium species, Escherichia mutagenic tests, karyotyping, cell culture models (Samiei, et al. ,2015) whereas mice, rat, rabbits and monkeys are common animal models (Badyal & Desai, 2014). Different snail species are suitable to be a reliable model of environmental pollution and its effects on offsprings. One of these helices is the Helix Lymnaea (Regoli, et al.,2006). This helix type has favourable features such as its high productive rate. Its continuous reproduction, high amount of offsprings per each generation, short period of maturation of offsprings to adults, its aqueous watery environment makes it accessible with test chemicals and easily monitored by morphometric image analyzer. Moreover Helix Lymnaea can sustain extreme environments which enables a prolonged years of monitoring (Regoli , et al. 2006; Abdel-Halim, et al. 2013& Gomot-de, 2002). The research hypotgesis of this current study which assume a potential effects of environmental oxygen radicals on the morphology of living organisms generations.

STUDY OBJECTIVES

To assess the morphometric outcomes for the offsprings of Helix Lymnaea after 3 years of exposure to environmental H2O2.

MATERIALS AND METHODS

Table 1: Materials used, vendors, dosage form and country of source

Materials	Vendor	Dosage form	Country	ISO
H2O2	Fisher	500 ml liquid	Germany	Yes
Distilled water	Local distilator	5 L	Local	-
Soil	Natural runnels	Semisolid	Local	-

Runnels water	Natural runnels	Liquid	Local	-
Formalin	Fisher	1 L Liquid	Germany	Iso

Table 2: Instruments and tools used, their company, models, and country of origin.

Instruments and lab tools	Company model	Country	Iso/ properties
Camera	Zennet	Russia	Yes
Light microscope	Ziets	Germany	Yes
Image processor for morphometry on PC	Matlab	-	-
Glass containers	Bayer	Germany	1 L / 3 containers
Sensitive balance	-	Germany	3 decimals
Volumetric flasks	Bayer	Germany	500 ml
Dropper	-	Germany	5 ml
Micropippet	Eppendorph	Germany	2 microml

Study design

The current research designed an experimental assessment of the morphological analysis of a three years H2O2 exposure in Helix Lymnaea model.

Twenty four Helix Lymnaea snails were divided into two groups (12 in each).Group A is the control whereas group B is the H2O2 test group.

All snails were adults (4-6 weeks age) of the same size 5-7 mm length. The sex was hermophroditism. The study was conducted in a private lab in Baghdad for three years.

Figure 1: Shows the species used of the snail Helix Lymnaea. It was aquatic and hermaphrodite.

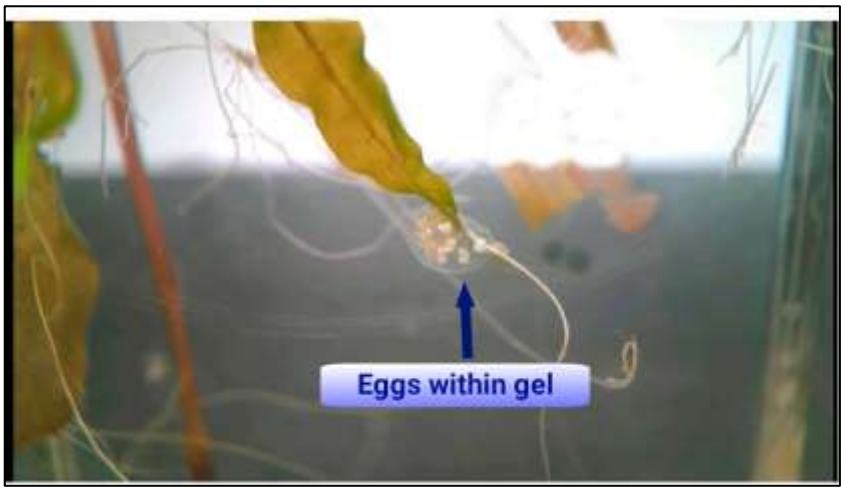

Figure 2: The culture prepared for breading Helix Lymnaea. It reveals the aggregates of eggs within a shell of gel. It was used to count eggs laying and hatching rate as an indicator of the reproductive activity.

Groups division

The 24 Helix Lymnaea were divided into the following:

1. The control group (N = 12) were given runnels soil (5 grams every day) within a 1 liter dandom runnel water. The culture was washed and replaced with the next 5 grams soil every day.

2. H2O2 group (N = 12) were given 5% H2O2 in their culture. Otherwise similar preparation to the control group.

Sample selection

The snail sample selection;

A random selection of snails was followed from different areas of the runnels
The soil and runnels water selection
The random way of selecting a sample was also followed.

Parameters to be monitored
Monitoring and record registry were done at rate demonstrated per each parameter measured and along ten generations throughout 3 years of follow up. The snails reproductive activity was diminished during fall and winter and hence the monitoring was postponed untill the next spring when their activity was recruited.

1. Number of active, innert and died snail every weak along the course
2. Soil eating activity score (as indicated in waste fraction out of 5 grams of soil per day. This parametercan be indicated by image processing software)
3. Active movement score (1 = low, 2 = moderate, 3 = high activity)
4. Weekly culture image for morphometric assessment
5. Reproductive Rate (Number of hatching egg per month and number of egg laying per month)
7. Ratio of monthly successful egg hatching
8. Death rate monthly

RESULTS

Table 3: The number of active and innert snails (active is a continuously moving, reproducing and eating snail).

Groups	Active number	Inactive number	Statistics
Control	12	1	SD = 1 p = 0.38 Chi square = 0.8
H2O2	10	3	

Table 4: The soil eating activity (fraction of waste in relation to the 5 grams of soil introduced daily to the culture.

Groups	Eating activity (waste/food percentage)	Statistics
Control	95	SD = +/- 6
H2O2	92	SD = +/- 5

Culture morphometric analysis

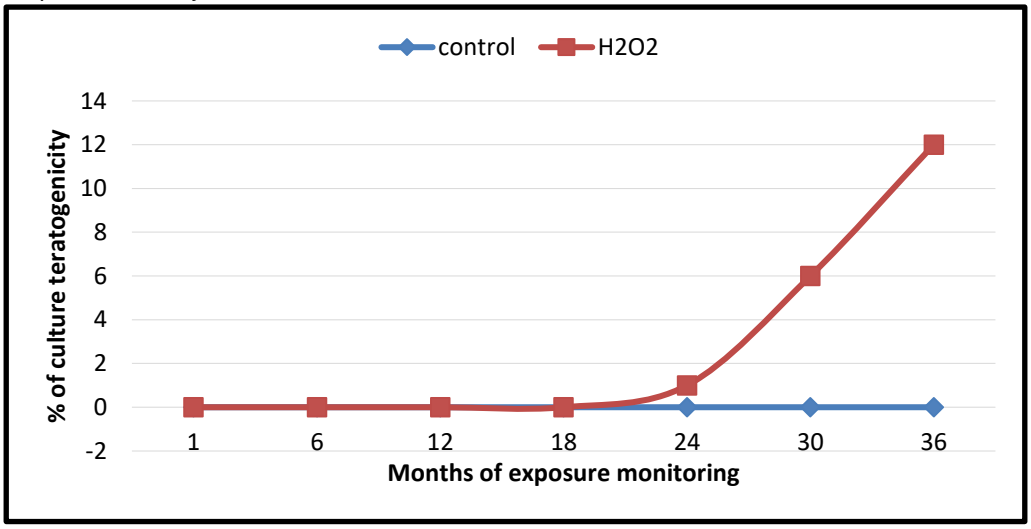

Figure 3: The percentage of teratogenicity recorded for the offsprings of Helix Lymnaea in form of albino generations.

REPRODUCTIVE RATE

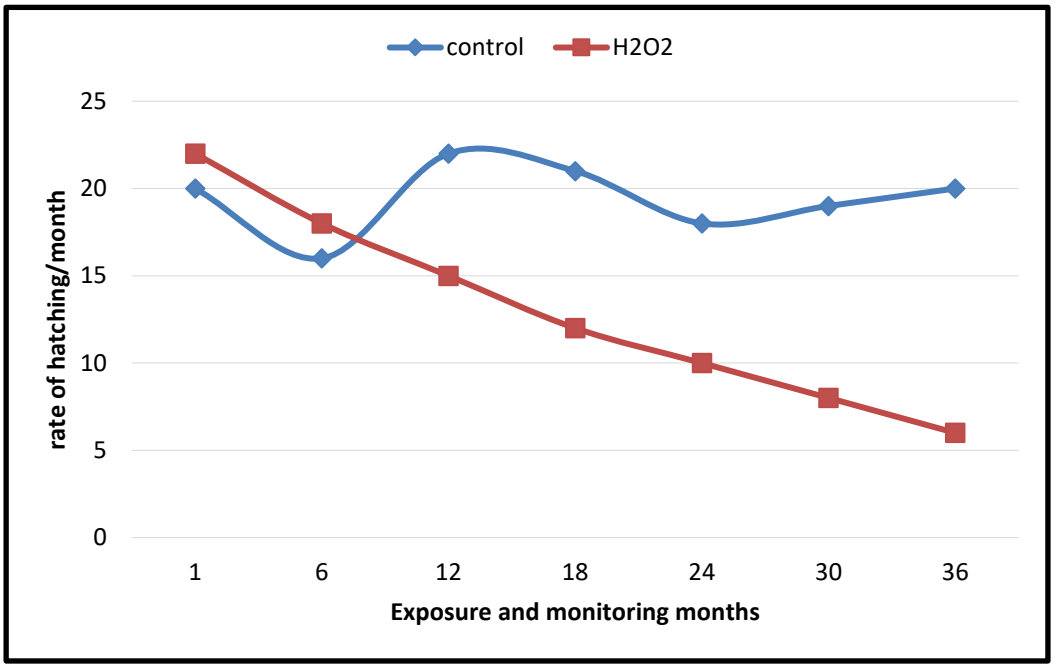

Figure 4: Changes in eggs hatching rate as a response to exposure to H2O2 throughout 36 months.

MORTALITY PERCENTAGES

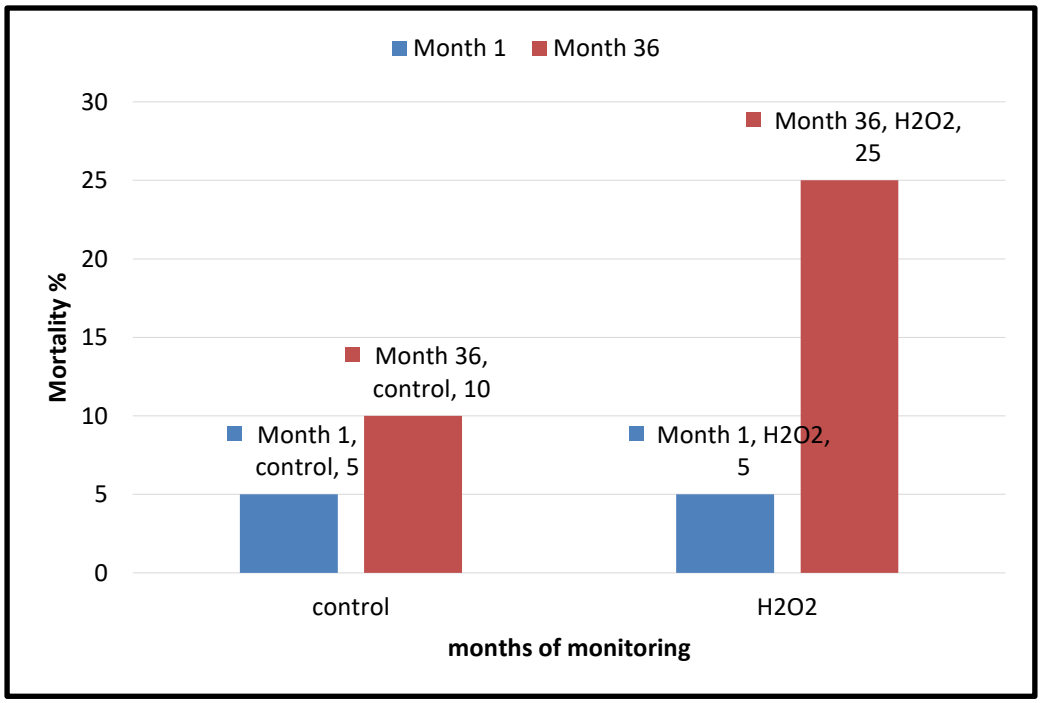

Figure 5: The mortality rate in response to a 36 months exposure to H2O2 as compared with the control group.

DISCUSSION

Any model of assessing the impact of a substance on offsprings and teratogenicity has to be characterized by a high reproductive rate (Forray & Foster, 2015) in form of high egg laying rate and high successful rate of hatching (Janssen & Baur , 2015). Moreover, the aqueous media animals are more prone for the teratogenic effects of environmental xenobiotics (Brent, 2004) since water and organic soluble substances are readily diffused into the organs (Brent,2004). The model of Helix Lymnaea is adequately fulfilling these criteria.

The results showed that there was a significant reduction in the movement score in the group treated by H2O2 in comparison with the control group 25% vs 8.3 % respectively p = 3.8, chi square = 0.8. This reduction in the movement of the Helix may be attributed to the direct impact of the free radicals on the muscles (Ji et al, 2009) or it may be due to the mutagenic effect of H2O2 on different cell components genes (Netto & Antunes,2016) . Many other environmental factors like besticides and organophosphorous may also contribute to change in aquatic animals movements (Jayaraj et al 2016 &Katagi , 2010).

Another property had been assessed as a general parameter for the impact of H2O2 on the generations of the Helix Lymnaea was the eating activity of the offsprings as indicated by the amount of waste produced over the amount of food introduced into the culture. There was a minute change noticed in between the two groups 95% in H2O2 exposed vs 92% in the control group.

The morphometric outcomes mainly referred to as the albino offsprings since those were the most prominent teratogenic effect of mutagenic mechanism since the exposure was to the parent snails. The control group showed no change in teratogenicity in comparison with 60% in H2O2 group, $p < 0.05$. Researches had verified color change in different species induced by several xenobiotic agents (Raunio et al. 2015; Moon et al. 2006).

The rate of successful eggs hatching was another important parameter measured. This indicate the healthy sperms, eggs and zygotes that were unaffected by the xenobiotic. There was a significant difference between the control and the H2O2 groups, $p = 0.017$. Studies that verified the influence of free radicals on the rate of successful egg hatching for different species include (Wong et al. 2008; Wong et al. 2004 & Rehkopf et al. 2017).

There was obvious difference in the mortality rate between the control and H2O2 group, however this difference was not significant $p = 0.2$. There are many environmental factors can cause an increase in mortality rate like pH, temperature, and chemical contamination (Bighiu et al. 2017). Of these chemical contaminants are the bisphenols (Corrales et al. 2015) and metals (Notten et al. 2005).

CONCLUSION

There was a significant teratogenic effect of H2O2 on the offsprings of the snail Helix Lymnaea. This effect may extend to include different free radical producing substances.

RECOMMENDATION

We recommend toconduct researches of free radicals producing drugs in similar models like tissue cultures and amphibians to assess the genetic and epigentic alterations as a result of such exposure.

CONFLECT OF INTEREST

None of the authors have any conflicts of interest relevant to this research subject.

ETHICAL CLEARENCE

The study was conducted in accordance with ethical principles that have their origin in the Declaration of Helsinki. The study protocol, care of animals and subject information were reviewed and approved by a local Ethics Committee.

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