

# Treating cancer and viruses by dissolved oxygen in water and alkalinity increasing

Dr. Makhoulf Mahmoud Makhoulf

Alexandria university Cairo .Egypt

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**Abstract:** The global aim of the proposal is to evaluate the utility and effective of hyperoxygenation water against hepatocellular carcinoma ( HCC ) and introducing unique technique for treatment of hepatocellular carcinoma through reoxygenation of tumor by dissolved oxygen in water and increasing alkalinity and also testing against viruses specific corona virus and c virus

**Keywords:** hepatocellular carcinoma, oxygen, coronavirus, c virus.

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## 1. INTRODUCTION

The solid tumors contain region at very low concentrations of oxygen (hypoxia) often surrounding areas of necrosis .the presence of the in-se hypoxia regions in human tumors was postulated by Thomblinson and Gray some 50 years ago based on their observations of the distribution of necrosis relative to blood vessels it was known at that time hypoxic cells were resistant to killing by ionizing radiation and chemotherapy so increasing of oxygen supply can optimize the penetration of the conventional chemotherapeutic agents and hence improve its delivery to the tumor to tissue this makes the tumor much more sensitive to treatment with conventional chemotherapy and radiotherapy

### Aim of the work

The global aim of this proposal is to evaluate the utility and effective of hyper oxygenated water against hepatocellular carcinoma (HCC) in rats and introducing unique technique for treatment of hepatocellular carcinoma through reoxygenation of tumors by dissolved oxygen in water and increasing alkalinity and also testing against viruses specific Corona virus and C virus

## 2. MATERIALS AND METHODS

Starting propagation and storage of cell cultures from frozen cells. after 24 hours cells were examined by inverted microscope and sub-culture as necessary

Animals used. 80 of adult albino rats of wester strain of 130--150 g from animal house unit of National research center - Egypt and they were kept under the same hygienic conditions and fed with standard laboratory food and water

Available facilities

Equipments for cell cultures lab. 2 inverted microscope. Inverted microscopes with digital camera. Cooling centrifuge. Carbon dioxide incubator. Small liquid nitrogen tank . 3 laminars air flow type 2 .Autoclave. Oven up to 300 degree centigrade . 4 digit decimal balance. Ultra deep freeze (-70). Elisa reader variable wave length. Air curtain.

Diethyl Nitroso amine was used for induced hepatocellular carcinoma (HCC) in rats

Cell lines also done in National research center in Cairo, Egypt.

Figures, Graphs and Tables

Groups	Number of Rats	Response treated Rats	unresponsive treated rats	Percentage of efficiency
Group 1 Negative control group	20 Rats	20	-	100% No side effects
Group 2 treated group in stages 1 and 2	Stages 1 and 2 36 Rats	30 Rats completely response for treatment 6 rats only improved	-	84%- 90%
Group 3 treated group in stages 3 and 4	Stages 3 and 4 36 Rats late stages	5 Rats completely response for treatment 9 Rats only improved	22 Rats unresponsive	20% - 30%
Group 4 Positive control group untreated group	20 Rats	-	20 Rats unresponsive	0 % untreated
Group 5 toxicity control group	8 Rats	8 Rats	-	100% No side effects
Group 6 toxicity tested group over dose group	8 Rats	8 Rats	-	100% No side effects

National Research Centre-  
 Consulting Service for Virus  
 Researches and Bioassays

المركز القومي للبحوث  
 الوحدة الاستشارية لبحوث الفيروسات والأنتيكبات الحيوية

Head of Al-Azhar University sample

Antiviral Bioassay for HCVc genotype 4 for the non toxic dose of the tested sample:

Initial viral titre	Final viral titre	% of reduction
1X10 <sup>6</sup>	3X10 <sup>5</sup>	70%
1X10 <sup>6</sup>	4X10 <sup>5</sup>	60%
1X10 <sup>6</sup>	4X10 <sup>5</sup>	60%

Non toxic dose on Huh 7.5 cell line is: 7.35 mg/ml

Antiviral Bioassay for Human Coronavirus NL63 for the non toxic dose of the tested sample:

Initial viral titre	Final viral titre	% of reduction
1X10 <sup>6</sup>	4X10 <sup>5</sup>	60%
1X10 <sup>6</sup>	4X10 <sup>5</sup>	60%
1X10 <sup>6</sup>	5X10 <sup>5</sup>	50%

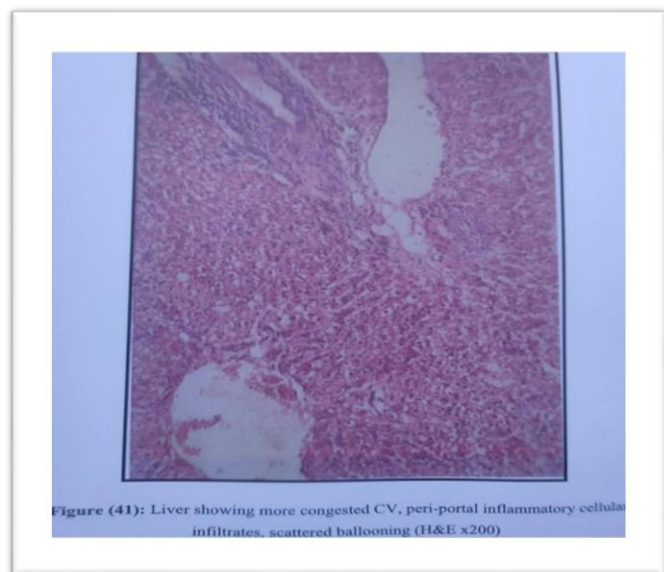
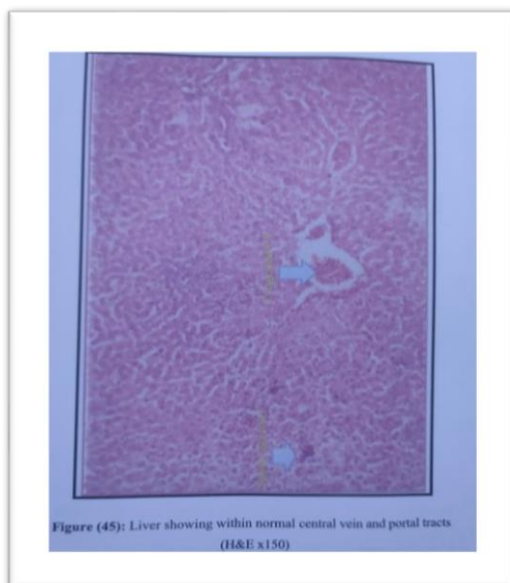
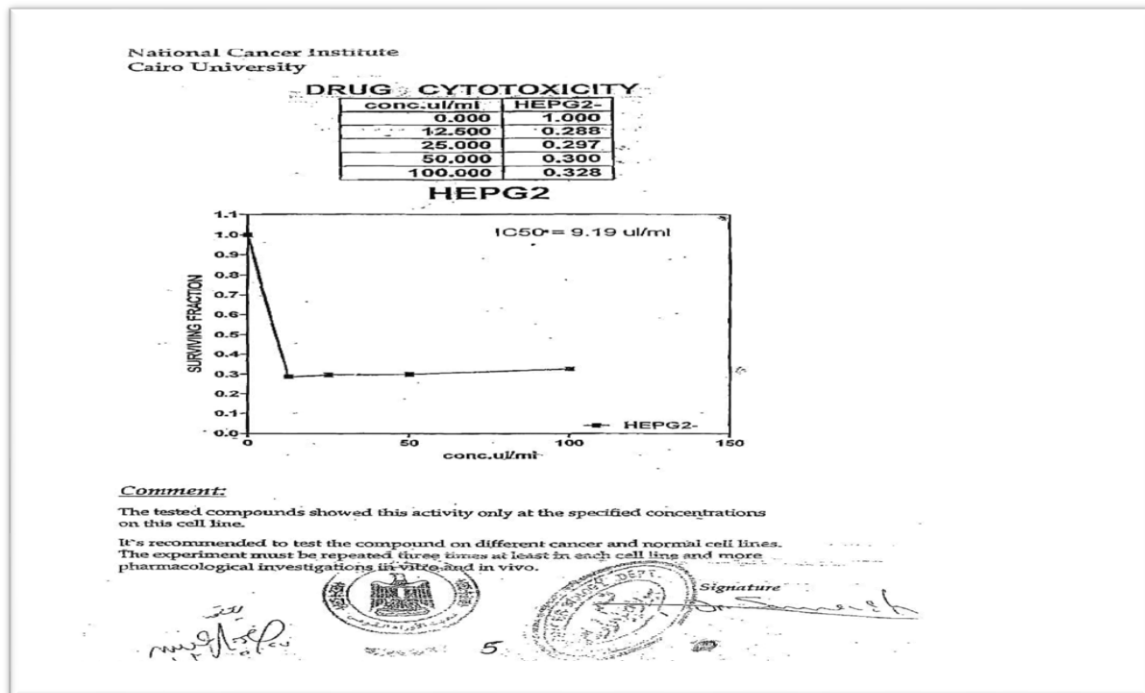
Non toxic dose on LLC-Mi2 cell line is: 7.35 mg/ml

Comment: Promising anti HCVc genotype 4 and human coronavirus NL63 *in vitro* for the non toxic dose of the tested sample.

المشرف على التحليل  
 والبريد الإلكتروني  
 2016/18/31  
 أ.د.وليد مرسى السقوي  
 وحدة الفيروسات  
 بحوث تلوث المياه

رئيس مجلس إدارة الوحدة  
 د.محمود الطويل  
 أ.د.جميلة السيد الطويل

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### 3. DISCUSSION

Chronic hypoxia postulated by Tomlinson and Gray. acute hypoxia could also occurs by temporary obstruction or variable blood flow in the tumor vessels since that several investigators have demonstrated unequivocally that the extent of tumor hypoxia has a negative impact on the ability of radiotherapy and chemotherapy to locally control tumors because of the resistance of hypoxic cells to killing by radiation or chemotherapy. Hypoxic cells are also considered to be resistant to most anticancer drugs for several reasons first hypoxic cells are distant from blood vessels and as a result are not adequately exposed to some types of anti cancer drugs. Second cellular proliferation decrease as a function of distance from blood vessels an effect that is at least partially due to hypoxia.

Third hypoxia selects for cells that have lost sensitivity to P53 - mediated apoptosis which might lessen sensitivity to some anti cancer agents. Fourth. the action of anti cancer agents resembles that of radiation of in that oxygen increases that cytotoxicity of the DNA lesions they cause. Fifth. hypoxia upregulated genes involved in drugs resistance including genes encoding P - glycoprotein these cleare links between hypoxia and intrinsic resistance to chemotherapy and

radiotherapy treatments also hypoxia can compromise cure ability of tumors by surgery. Therefore hypoxia has a key negative role in tumor prognosis both because it causes resistance to standard therapies and because it promotes a more malignant phenotypes for these reasons. Tumor hypoxia could be potentially exploitable in cancer therapy up to date. Accordingly we tried in these work to reduce the hypoxic environment inside the tumor tissues using certain concentrations of oxygen dissolved in water this dissolved oxygen when reached to the tumor cells will result in oxygenation of the hypoxic areas in the tumor core and therefore promotes Cancer cells death. additional. These increase oxygen supply can optimize the penetration of conventional chemotherapy and radiotherapy agents and hence improve its delivery to the tumor tissues these make the tumor much more sensitive to treatment with conventional chemotherapy and radiotherapy. and also cancer cells increasing and spread by increasing acidity so by increasing alkalinity by using carbonate or bicarbonate we could obtain more positive results to destroy Cancer cells

#### **4. THE RESULTS**

The results which obtained on cells lines and animals experiments (Rats) invitro and invivo experiments. Confirmed that percentage of treatment hepatocellular carcinoma (HCC) in between 84% to 90% in stage 1 and 2 but in between 30% to 50% in stage 3 and 4 for 40 days treatment and toxicological studies confirmed that no gross acute or chronic toxicological signs were observed and no side effects

#### **5. CONCLUSION**

It has been clinically demonstrated that the spread or metastasis of cancer cells is inversely proportional to the amount of oxygen around cancer cells the more oxygen the slower Cancer cells spread. the less oxygen the faster cancer cells spread. If cancer cells get enough oxygen they will die because cancer cells are an aerobic cells. also cancer cells spread in acidic medium so by providing cancer cells by oxygen dissolved in water and increasing alkalinity by carbonate or bicarbonate that not only slow down the spread of cancer cells but also also kills cancer cells and also the results obtained for destroyed C virus about 60% to 70% and destroyed Corona virus from about 50% to 60%

#### **REFERENCES**

- [1] Alfarouk, K.O., Stock, CM., Taylor, S. et al. Resistance to cancer chemotherapy: failure in drug response from ADME to P-gp. *Cancer Cell Int* 15, 71 (2015). <https://doi.org/10.1186/s12935-015-0221-1>.
- [2] Brown, J., Wilson, W. Exploiting tumour hypoxia in cancer treatment. *Nat Rev Cancer* 4, 437–447 (2004). <https://doi.org/10.1038/nrc1367>.
- [3] Milane L, Ganesh S, Shah S, Duan ZF, Amiji M. Multi-modal strategies for overcoming tumor drug resistance: hypoxia, the Warburg effect, stem cells, and multifunctional nanotechnology. *J Control Release*. 2011 Oct 30;155(2):237-47. doi: 10.1016/j.jconrel.2011.03.032. Epub 2011 Apr 8. PMID: 21497176; PMCID: PMC3146561..
- [4] Muz B, de la Puente P, Azab F, Azab AK. The role of hypoxia in cancer progression, angiogenesis, metastasis, and resistance to therapy. *Hypoxia (Auckl)*. 2015;3:83-92. Published 2015 Dec 11. doi:10.2147/HP.S93413
- [5] Rockwell S, Dobrucki IT, Kim EY, Marrison ST, Vu VT. Hypoxia and radiation therapy: past history, ongoing research, and future promise. *Curr Mol Med*. 2009;9(4):442-458. doi:10.2174/156652409788167087.
- [6] Warren DR, Partridge M. The role of necrosis, acute hypoxia and chronic hypoxia in 18F-FMISO PET image contrast: a computational modelling study. *Phys Med Biol*. 2016;61(24):8596-8624. doi:10.1088/1361-6560/61/24/8596.