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Assessment of Cytotoxicity and Genotoxicity Response of Zinc Sulphate on Eukaryotic Cells

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Abstract

Zinc Sulphate ($ZnSo_4$) is an inorganic compound. Zinc is used to treat and prevent zinc deficiency. Zinc is a naturally occurring mineral that is important for growth and the development and health of body tissues. In this study, specific concentrations of $ZnSo_4$ on cell viability were investigated by MTT method in hepatocellular carcinoma (HepG2), lung cancer (A549), and normal lung cell (Wi38). Cell cycle arrest and apoptosis were measured by flow cytometry assessment by PI Staining and Annexin V/PI Staining, respectively. Results showed that Zinc induced cytotoxicity in HepG2, A549, and Wi38 using different concentrations (IC50 = 308.11, 413.02, 463.15 µg/ml). These data indicated that $ZnSo_4$ decreased cell viability in malignant and non-malignant cells and confirmed the occurrence of their cytotoxic effects. Cell cycle and apoptosis by flow cytometry showed a significant increase in $ZnSo_4$ -damaged HepG2 cells by cell cycle arrest in the G2/M phase and increased apoptosis. In addition, the mRNA expression levels of p53 and casp3 increased while Bcl-2 decreased in HepG2 cell lines when treated with a high concentration of $ZnSo_4$. The effects of $ZnSo_4$ on different yeast haploid knockout strains were evaluated in this study (YKO). We used the Comet assay method of the three different concentrations of $ZnSo_4$ at which this set of $ZnSo_4$ could cause DNA damage. The comet assay exhibited a better sensitivity of yeast cells, which was undeniably confirmed. The genotypes of YKO were chosen based on the (Clustal Omega Multiple Sequence Alignment EMBL-EBI) alignments of human and yeast gene sequence homology.

Keywords: Zinc Sulphate, cell lines, flow cytometry, apoptosis, RT-pcr, Comet assay.

Introduction

Zinc is an essential trace element with important biological functions that control many processes in the cell, such as DNA synthesis, normal growth, brain development, behavioural response, fetal development and bone formation **(Yehy *et al.*, 2011)**, regulation of response to insulin, reproduction, antioxidant cellular defense systems **(Zodl *et al.*, 2003)** and protein synthesis **(Klug, 2010).**

Zinc is effective at very low concentrations and therefore its excessive amount in body fluids could be harmful **(Barbier *et al.*, 2005)** Zinc is a significant trace element required for many signalling pathways in the human body by acting as a cofactor of more than 300 enzymes. These enzymes are related to the proliferation, metabolism, and functions of cells **(Costello and Franklin, 2016).** Furthermore, high concentrations of zinc are toxic to cells, and also, it induces a number of intracellular pathways provoking reactive oxygen species (ROS) generation **(McCord and Aizenman, 2018**).

Different cell types have been exposed to Zn concentrations from 25 to 300 µM, which showed great variability in cytotoxicity and genotoxicity levels **(Sliwinski *et al.*, 2009 and Plum *et al.*, 2010).** Zinc deficiency results in an increased sensitivity to oxidative stress **(Nazıroğlu and Yürekli., 2013)** and may, in part, increase the risk for cancer development **(Silvera and Rohan., 2007)** Excess zinc, however, can induce chromosomal instability and DNA double-strand breaks in human lung cells **(Xie *et al.*, 2009).**

Zaman *et al.*, 2019 demonstrate that CK2 is involved in regulating zinc homeostasis in breast and prostate cancer cells as both TBB and CX-4945 substantially decreased cell viability upon zinc exposure. Cytotoxicity and programmed cell death (apoptosis) was tested on in vitro human cell growth.

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Cell cycle arrest and apoptosis-related genes of the human cell lines were also evaluated (**Rashad *et al.*, 2018**).

Rashad *et al.*, 2019 Indicated that additives decreased cell viability in malignant and non-malignant cells as well as confirmed the occurrence of their cytotoxic effects. Saccharomyces cerevisiae cells were shown to be more sensitive to the action of some additives. The effects of additives on several yeast haploid knockout strains were studied in the Comet test method to find the optimum amounts at which this set of dietary additives could cause DNA damage **(Rashad *et al.*, 2021).** AuNRs has a cytotoxic activity on human carcinoma and normal cells, Flow cytometric analysis demonstrated that AuNRs has a cytotoxic effect on human carcinoma cells (HepG2, CaCo2, A549, and CDD-19Lu) repeated through the increased G2/M phase cell cycle arrest (**Rashad *et al.*, 2022**).

Marcinčáková *et al.*, 2019 evaluated the *in vitro* nephrotoxicity of zinc Sulphate heptahydrate $ZnSo_4$ × 7H2O using rabbit epithelial kidney cells RK13 as the model cell line. They reported that the inhibition concentration IC50 value for xCELLigence monitoring was 101.8 mg/l, for MTT test.

$ZnSo_4$ at a high concentration (100 µM) inhibited cell viability **(Zhang *et al.*, 2017)**. influences of a specific concentration range of $ZnSo_4$ on cell cycle and apoptosis by flow cytometry, and cell viability by MTT method in MDAMB231, HepG2 and 293 T cell lines. It was found that the influence manners of $ZnSo_4$ on cell cycle, apoptosis and cell viability in various cell lines were different and corresponding to the changes of Zn2+ content of the three cell lines, respectively. The significant increase on intracellular zinc content of MDAMB231 cells resulted in cell death, G1 and G2/M cell cycle arrest and increased apoptotic fraction. Additionally, the mRNA expression levels of ZnT and ZIP families in the three cell lines, when treated with high concentration of $ZnSo_4$, increased and decreased corresponding to their functions, respectively. The objective of this study was to assess the potential cytotoxic and apoptotic effects of $ZnSo_4$ on human cell growth (**Wang *et al.*, 2013**).

Materials and methods

1. Cell lines

1.1. **Mammalian cell lines: HepG-2** cells (human Hepatocellular cancer cell line), **A-549** (human Lung Carcinoma) and **Wi38** cells (human lung fibroblast normal cells) were obtained from the American Type Culture Collection (ATCC, Rockville, MD).

Chemicals Used: Dimethyl sulfoxide (DMSO), MTT and trypan blue dye were purchased from Sigma (St. Louis, Mo., USA).

Fetal Bovine serum, RPMI-1640, HEPES buffer solution, L-glutamine, gentamycin and 0.25% Trypsin-EDTA were purchased from Lonza (Belgium).

1.2. Cell line Propagation:

The cells were grown on RPMI-1640 medium supplemented with 10% inactivated fetal calf serum and 50 µg/ml gentamycin. The cells were maintained at 37ºC in a humidified atmosphere with 5% CO2 and were subcultured two to three times a week.

1.3. Cytotoxicity evaluation using MTT assay:

For antitumor assays, the tumor cell lines were suspended in medium at concentration $5x10^4$ cell/well in Corning® 96-well tissue culture plates, and then incubated for 24 hr. The $ZnSo_4$ concentrations were then added into 96-well plates (three replicates). 0.5 % DMSO was run for each 96 well plate as a control. After incubating for 24 h, the numbers of viable cells were determined by the MTT test. Briefly, the media was removed from the 96 well plates and replaced with 100 µl of fresh culture RPMI 1640 medium without phenol red then 10 µl of the 12 mM MTT stock solution (5 mg of MTT in 1 mL of PBS) to each well including the untreated controls. The 96 well plates were then incubated at 37°C and 5% CO_2 for 4 hours. An 85 µl aliquot of the media was removed from the wells, and 50 µl of DMSO was added to each well and mixed thoroughly with the pipette and incubated at 37°C for 10 min. Then, the optical density was measured at 590 nm with the micro plate reader (Sun Rise, TECAN, Inc, USA) to determine the number of viable cells and the percentage of viability was calculated as [(ODt/ODc)] x100 where **ODt** is the mean optical density of wells treated with the tested sample **ODc** is the mean optical density of untreated cells

The relation between surviving cells and $ZnSo_4$ concentrations is plotted to get the survival curve of each tumor cell line after treatment with the specified compound.The 50% inhibitory concentration (IC_{50}), the concentration required to cause toxic effects in 50% of intact cells, was estimated from graphic plots of the dose response curve for each conc. using Graph pad Prism software (San Diego, CA. USA) (**Rashad *et al.*, 2022).**

2. Flow cytometry

2.1. Cell cycle analysis by PI assay using flow cytometry

The cells were digested with warm Trypsin-EDTA + warm Phosphate Buffered Saline

(PBS) -Ethylene diamin tetra acetate (EDTA) (0.25%) (500µl + 500µl) with incubation for 10 minutes at 37°C. The mixture was centrifuged 450 rpm for 5 min, and then supernatant was carefully removed. The mixture was washed twice in warm PBS and the cell pellet was re-suspended in 500 µl warm PBS, centrifuged and supernatant was removed. A volume of 150 µl PBS + 350 µl ice-cold 70% ethanol was added and incubated at 4°C for 1 hour to fix the cells. To remove ethanol, the mixture was centrifuged at 350 rpm for 10 minutes and then the supernatant was carefully removed. The mixture was washed twice in warm PBS and the cells were re-suspended in 500 µl warm PBS, then centrifuged and the supernatant was removed. The cells were re-suspended in 100 µl PBS and stored at 4°c for up to 4 days in darkness. The cells were stained with 100 µl of PI (Propidium Iodide) solution + 50 µl RNase A solution (100 µg/ml), and incubated in darkness for 30-60 min **(Rashad *et al*., 2022)**. The stained cells were read in Attune flow cytometry (**Applied Bio-system, USA**).

2.2. **Apoptosis analysis by Annexin V-FITC Assay using flow cytometry**

Collect 1-5 × 10^5 cells by centrifugation and supernatant was removed. Cells were then collected, washed twice with warm PBS buffer and the cells were re-suspended 500 µl of 1X Binding Buffer. Add 5µl of Annexin V-FITC and 5µl of propidium iodide (PI 50 mg/ml) and then incubate at room temperature for 5 min in the dark **(Vermes *et al*., 1995)**.Analyze Annexin V-FITC binding by flow cytometry (**Applied Bio-system, USA**).

3. Quantitative RT-PCR analysis

Total RNA was isolated from rat liver using Gene JET RNA Purification Kit (Thermo Scientific, # K0731, USA) according to the manufacturer's protocol. Total RNA (5µg) was reverse transcribed using Revert Aid H Minus Reverse Transcriptase (Thermo Scientific, #EP0451, USA) to produce cDNA as previously described (**Rashad *et al*., 2018)**. The cDNA was used as a template to determine the relative expression of the apoptosis-related genes using Step One Plus real time PCR system (Applied Bio system, USA). The primers were designed by Primer 5.0 software. Forward and reverse primer sequences for Casp3, Bcl-2, p53 and GAPDH inflowing table (1).

Table (1): Forward and reverse primer sequences for and *Casp3* genes.

Gene	Forward primer(/5 ------ /3)	Reverse primer(/3 ------ /5)
Casp3	TTCATTATTCAGGCCTGCCGAGG	TTCTGACAGGCCATGTCATCCTC
Bcl-2	CATGCAAGAGGGAAACACCAGA	GTGCTTTGCATTCTTGATGAGGG
p53	AGAGTCTATAGG CCACCCC	GCTCGACGCTAGGATCTG AC
GAPDH	TGCACCACCAACTGCTTAGC	GGCATGGACTGTGGTCATGAG

The housekeeping gene GAPDH was used as a reference to calculate fold change in target gene expression. A 25µL PCR mix was prepared by adding 12.5 µL of 2X Maxima SYBR Green/ROX qPCR MM (Thermo Scientific, # K0221, USA), 2 µL of cDNA template, 1 µL forward primer, 1 µL reverse primer, and 8.5 µL of nuclease free water. The thermal cycling conditions were as follows: initial denaturation at 95°C for 10 min, 40-45 cycles of amplification of DNA denaturation at 95°C for 15 s, annealing at 60°C for 30 s, extension at 72°C for 30 s. At the end of the last cycle, the temperature was increased from 63 to 95°C for melting curve analysis. The cycle threshold (Ct) values were calculated for target genes and the housekeeping gene, and relative gene expression was determined using $2^{-\Delta\Delta Ct}$ method **(Livak and Schmittgen, 2001)**.

4. Yeast Comet assay (YCA)

The in vitro Comet assay was performed using the first procedure published by (**Rashad *et al*., 2021**). We used yeast culture media with the concentrations of $ZnSo_4$ (50, 75, 100 µg/ml). A medium without chemical components was also employed as an untreated control. 1 g of cell pellets was placed in a one-cubic-centimetre container with cold PBS.

This suspension was filtered after five minutes of stirring. 100 µl of cell suspension was combined with 600 µl of low-melting agarose (0.8 percent in PBS). On pre-coated slides, one hundred percent of this mixture was spread out. For fifteen minutes, the coated slides were immersed in lyses buffer (0.045 M TBE, pH 8.4, containing a pair of 0.5% SDS). The slides were placed in an activity chamber with a same TBE buffer but no SDS. The coated slides were placed in the electrophoresis tank filled with electrophoresis buffer at 4 °C and an electric field of

2 V/cm for 15 min. neutralize the micro gels by neutralization buffer at room temperature for 10 min. drain neutralization buffer and samples in ethanol 76 % and subsequently in 96 % both for10 min at room temperature. Each slid stained by 50 µL of ethidium bromide (20 mg/m1) staining. The polymer fragment migration patterns of one hundred cells for each exposure level were analyzed with a visible radiation magnifier while the samples were still moist (With excitation filter 420-490nm [issue 510 nm]). The tail lengths of extraterrestrial objects were measured from the nucleus to the top of the tail with a 40x increase to count and measure the comet's size. Observations of Gel Red-stained polymer were done using a 40x objective on a fluorescence magnifier to visualize polymer damage. By measuring the length of polymer migration and the proportion of migrated polymer, an extraterrestrial object five image analysis code developed by Kinetic Imaging, Ltd. (Liverpoo1,UK) connected to a CCD camera was used to assess the quantitive and qualitative extent of polymer injury within the cells. The program then estimated the tail moment. In most cases, fifty to one hundred randomly selected cells were evaluated per sample according to (**Rashad *et al.*, 2021)**.

5.1. knockout yeast strains of choice

haploid knockout strains with completely distinct genotypes were used in this investigation, and the sequences of each strain were chosen and aligned with human sequence information in NCBI (The National Center for Biotechnology Information). Four genes aligned with cancer-related human genes were chosen to correspond with the yeast genes used in this investigation (Table 2).

Table (2): Selected yeast proteins which matched with cancer related human genes.

Selected strains	Selected genes of yeast strains (genotypes)	Homologous genes in human
YMR177W	MMT1	SLC30A9
YMR199W	CLN1	CCNA1
YMR224C	MRE11	MRE11
YMR243C	ZRC1	SLC30A10

5.2. Selection of yeast haploid strains deficient in genes similar to human cancer genes

The genotypes of yeast haploid (knockout) strains were chosen based on (Clustal Omega Multiple Sequence Alignment EMBL-EBI) alignment between human and yeast sequence similarity Table (2).

5.3. Protein-protein interaction prediction

In accordance with the sequence, the interaction network was used. GENEMANIA (http://www.genemania.org) is a flexible, user-friendly web interface for evaluating gene function hypotheses, examining sequence lists, and prioritizing genes for specific experiments.

Sources of information

Co-expression information from the organic phenomenon Omnibus (GEO); physical and genetic interaction information from Bio GRID; foretold macromolecule interaction information supported by orthology from I2D; and pathway and molecular interaction information from Pathway Commons, which includes data from Bio GRID, Memoria, and Pathway Commons. Yeast protein-protein interaction network, Human protein-protein interaction network

6. Statistical analysis

All the data were expressed as means ±S.E. The statistical significance was evaluated by one-way analysis of variance (ANOVA using SPSS, 18.0 software, 2011 and the individual comparisons were obtained by Duncan's multiple range test (DMRT).
Values were considered statistically significant when $p<0.05$.

Results

1. Cytotoxic effect by MTT assay

Zinc Sulphate revealed its cytotoxic activity at the different concentrations on the proliferation of HepG2, A549 and Wi38 cells in comparison to a positive control were determined using the MTT cytotoxic assay.
In general, the cell viability was decreased gradually as the concentrations of zinc Sulphate increased as illustrated in Table (3). The cytotoxicity and cell viability of zinc Sulphate. The viability of positive control was reduced as the concentration increased of tested zinc Sulphate. The Dose inducing 50% cell growth inhibition (IC50) against hepatoma cell line cells (HepG2) is presented in Table (3) and Dose-response curves for cell viability in Figure (1).

Table (3). Effect of different $ZnSo_4$ concentrations on hepatocellular carcinoma cells **(HepG2)**

$ZnSo_4$ conc. (µg/ml)	Viability %	Inhibitory %	S.D. (±)

500	**26.49**	**73.51**	**3.75**
250	**57.08**	**42.92**	**3.14**
125	**81.43**	**18.57**	**1.79**
62.5	**98.12**	**1.88**	**0.46**
31.25	**100**	**0**	
15.6	**100**	**0**	
7.8	**100**	**0**	
3.9	**100**	**0**	
2	**100**	**0**	
1	**100**	**0**	
0	**100**	**0**	

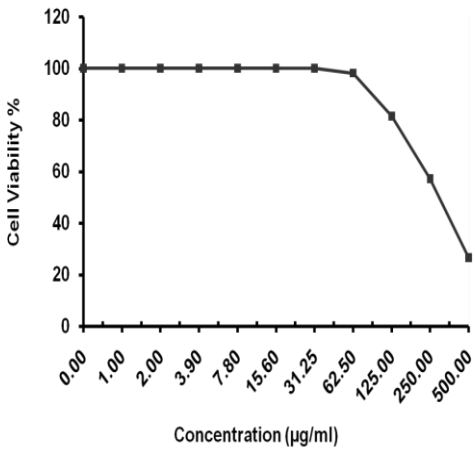


Fig. 1: Inhibitory activity of $ZnSo_4$ concentrations against Hepatocellular carcinoma cells **(HepG2)**

The cell viability was decreased gradually as the concentrations of zinc Sulphate increased as illustrated in Table (4). The cytotoxicity and cell viability of zinc Sulphate. The viability of positive control was reduced as the concentration increased of tested zinc Sulphate. The Dose inducing 50% cell growth inhibition (IC50) against lung cell line cells (**A549**) is presented in Table (4) and Dose-response curves for cell viability in Figure (2).

Table (4). Effect of different $ZnSo_4$ concentrations on lung carcinoma cells **(A549)**

$ZnSo_4$ conc. (µg/ml)	Viability %	Inhibitory %	S.D. (±)
500	**34.68**	**65.32**	**2.34**
250	**78.94**	**21.06**	**2.82**
125	**94.03**	**5.97**	**1.75**
62.5	**99.26**	**0.74**	**0.48**
31.25	**100**	**0**	
15.6	**100**	**0**	
7.8	**100**	**0**	
3.9	**100**	**0**	
2	**100**	**0**	
1	**100**	**0**	
0	**100**	**0**	

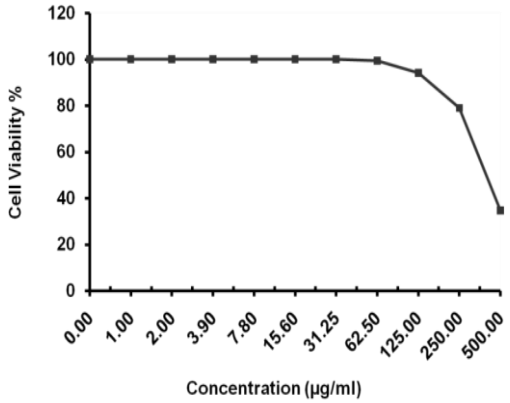


Fig. 2: Inhibitory activity of $ZnSo_4$ concentrations against lung carcinoma cells **(A549)**

The cell viability was decreased gradually as the concentrations of zinc Sulphate increased as illustrated in Table (5). The cytotoxicity and cell viability of zinc Sulphate. The viability of positive control was reduced as the concentration increased of tested zinc Sulphate. The Dose inducing 50% cell growth inhibition (IC50) against normal lung cell (**Wi38**) is presented in Table (5) and Dose-response curves for cell viability in Figure (3).

This results according to (**Rashad *et al*., 2019**) four different types of human cell lines; namely, colon carcinoma (Caco-3), breast carcinoma (MCF7), lung carcinoma (A549) and normal lung cell line (Wi38) were treated. Viability in shapes of the cells showed considerable variations between control and treatment and confirmed the carcinogenic effect of these components.

Table (5). Effect of different $ZnSo_4$ concentration son human lung fibroblast normal cells **(Wi38)**

$ZnSo_4$ conc. (µg/ml)	Viability %	Inhibitory %	S.D. (±)
500	**43.87**	**56.13**	**3.69**
250	**85.06**	**14.94**	**2.81**
125	**97.31**	**2.69**	**0.75**
62.5	**100**	**0**	
31.25	**100**	**0**	
15.6	**100**	**0**	
7.8	**100**	**0**	
3.9	**100**	**0**	
2	**100**	**0**	
1	**100**	**0**	
0	**100**	**0**	

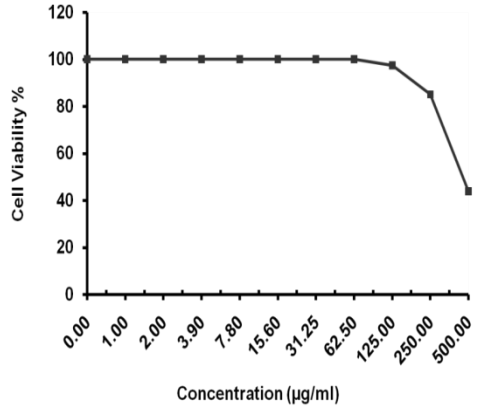


Fig. 3: Inhibitory activity of $ZnSo_4$ concentrations against human lung fibroblast normal cells **(Wi-38)**

2.1. Cell cycle analysis by PI assay using flow cytometry

$ZnSo_4$ at concentrations 75µg/ml affected the DNA content of HepG2 cells. The G0/G1 phase showed a decrease from 44.69% to 42.51% for control. Similarly, the S phase percentage also exhibited a decrease from 39.54% to 35.31% in the control and$ZnSo_4$. In the G2/M phase there was an increase in the DNA contents of the HepG2 cells when treated with $ZnSo_4$ (23.43%) comparing with the control (15.77%) as illustrated in **Table (6)**. These results showed significant accumulation of HepG2 cells in the G2/M phase, and confirmed that $ZnSo_4$ hasmarked cytotoxic effect via induction of G2/M phasearrest of the cell cycle as shown in **Figure (4)**.

Table (6): Average % of DNA content in each cell cycle phase using HepG2 cells treatment

Groups	Percentages of DNA content each in cell cycle phase			
	G0/G1 phase	**S phase**	**G2/M phase**	**Pre-G1**
HepG2-control	44.69	39.54	15.77	1.64
HepG2-treated with $ZnSo_4$	41.26	35.31	23.43	8.92

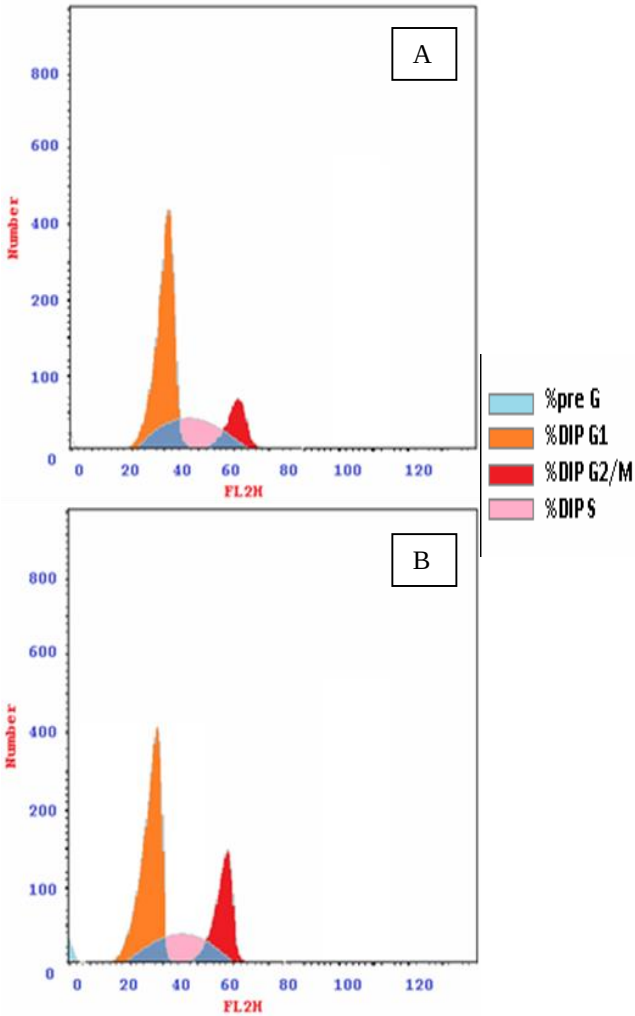


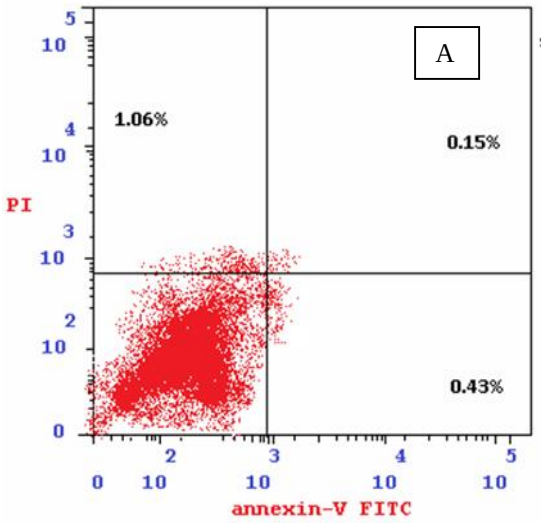
Fig. 4: **(A)** liver cancer cell line **(HepG2)** - untreated **(B)** Liver cancer cell line **(HepG2)** where treated with $ZnSo_4$ at concentration 75µg/ml and effect at **G2/M** cell cycle arrest.

2.2. Apoptosis analysis by Annexin V-FITC Assay using flow cytometry

Apoptosis is a tightly regulated process under the control of several signaling pathways, such as the mitochondrial pathway and caspase cascade. Effects of $ZnSo_4$ on HepG2 cells at the 75µg/ml concentration was applied to the cell culture to determine cell necrosis and apoptosis. Apoptosis and necrosis were measured with Annexin V-FITC/PI double-labeled flow cytometry **(Figure 5)**. Theapoptotic rate was calculated as the percentage of the early and late apoptotic cells. As shown in (**Table 7**), change in the apoptotic rate was observed in $ZnSo_4$ -treated HepG2 cells, as they were 0.84% and 1.83% for early and late apoptotic cells, respectively. While, control were 0.43% and 0.15% for early and late apoptotic cells, respectively. The necrotic effect was 6.25% for $ZnSo_4$ -treated HepG2 cells and 1.06% for control. These results showed $ZnSo_4$ had significant impact of apoptotic and necrotic effect on HepG2cells.

Table (7). Apoptotic and necrotic effect on **HepG2** when treated with $ZnSo_4$

Groups	Percentage of apoptosis		Percentage of necrosis
	early	**late**	
HepG2-control	0.43	0.15	1.06
HepG2-treated with $ZnSo_4$	0.84	1.83	6.25



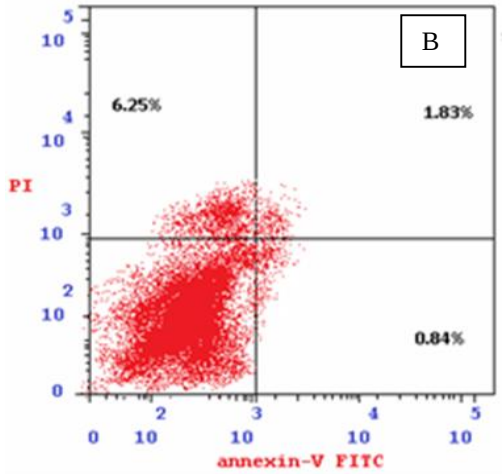


Fig. 5: **(A)** liver cancer cell line **(HepG2)** – **(B)** untreated liver cancer cell line **(HepG2)** where treated with $ZnSo_4$ at concentration 75µg/ml**. Nt** .Lower left (live cells) - lower right (early apoptosis) - upper left (necrotic cells) - upper right (late apoptosis)

4. Quantitative RT-PCR analysis

$ZnSo_4$ **induced genotoxicity of some related genes, *casp3 Bcl-2* and *p53* in HepG2 cells**

The role of apoptosis in $ZnSo_4$ induced cytotoxicity on liver cancer cell lines (HepG2) was studied. The expression levels of apoptosis-related genes such as casp3, p53 and Bcl-2 in HepG2 cells were estimated by real time PCR (qRT-PCR). *Casp3* increased by 3.12285797 than control **(Table 8),** also p53increased by 2.577512 than normal **(Table 8).** Bcl-2 decreased by 0.6682521than control (**Table 9**), **s**howed that, compared to the untreated group control **(Table 10)**, the expression levels of casp3 gene and p53 gene were increased, whereas that of Bcl-2 gene was decreased **(Figure 6)**. These results indicated that the $ZnSo_4$ killed HepG2 cells through apoptosis mechanism mainly via over expression of casp3 and p53 genes, while Bcl-2 down regulated.

Table (8): Effect of $ZnSo_4$ compound administration on the relative expression of *casp3* gene in HepG3 cells.

Groups	Casp3 Ct values	Δ Ct	ΔΔ Ct	Relative quantification
Control HepG2	**33.88**	**10.2**	**0.00**	**1.00**
Treated HepG2	**31.32**	**8.39**	**-1.78**	**3.122857**

Table (9): Effect of $ZnSo_4$ compound administration on the relative expression of *p53* gene in HepG3 cells.

Groups	*p53* Ct values	Δ Ct	ΔΔ Ct	Relative quantification
Untreated HepG2	**33.08**	**9.37**	**0.00**	**1.00**
Treated HepG2	**30.82**	**7.89**	**-1.48**	**2.577512**

Table (10): Effect of $ZnSo_4$ compound administration on the relative expression of *Bcl-2* gene in HepG2 cells.

Groups	Bcl2 Ct values	Δ Ct	ΔΔ Ct	Relative quantification
Untreated HepG2	**28.51**	**4.8**	**0.00**	**1.00**
Treated HepG2	**28.36**	**5.43**	**0.36**	**0.6682521**

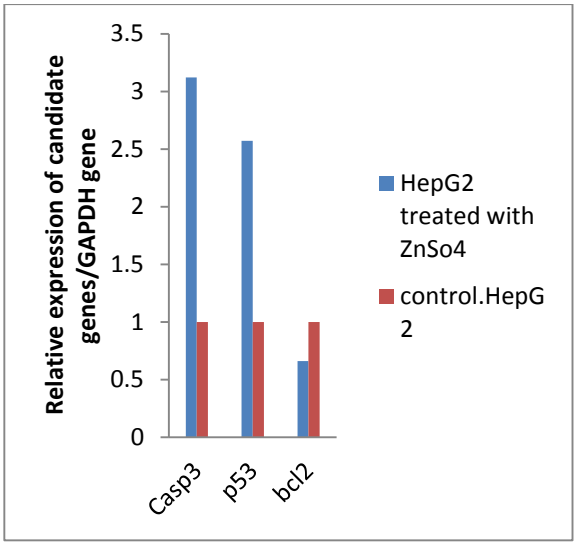


Fig. 6: Effects of $ZnSo_4$ on apoptosis-related genes after exposure to 75µg/ml, mRNA expression of *casp3*, *Bcl-2* and *p53* was assessed by quantitative RT-PCR *P < 0.05, compared to the control group

5. Toxicity to (YKO) strains tested with Zink sulphate by comet assay

Zink sulphate displayed varying degrees of yeast significant genotoxic effects on YKO in accordance with the comet assay. The concentrations of $ZnSo_4$ are (50, 75, 100 µg/ml) revealed its genotoxic effect.

The genotoxic effects of the MMT1, CLN1, MRE11 and ZRC1genes were severe, whereas the genotoxicity of the MMT1 gene was low than other genes. The distribution of the share of determined comets for Zinc sulphate was shown in **Table (11)**. It should be noticed that the yeast predicted significantly more comets than the control for each of the four tested genes **(Fig. 7)**, indicating that the tested $ZnSo_4$ caused a large number of identified deoxyribonucleic acid damages. The cells were clear that Zinc sulphate treatment caused significant damage to each of the four genes evaluated.

Table (11): Image analysis of comet assay parameters in cells of all groups after $ZnSo_4$ treatment.

Concentrations	Tail Length (px)	Tail DNA (%)	Tail Moment	Tail Olive Moment
Control MMT1 (A)	3.2	16.42885	0.856675	1.331495
50 µg/ml (A1)	5.84	18.56993	2.698397	2.569634
75 µg/ml (A2)	7.96	24.76888	3.678652	3.649392
100 µg/ml (A3)	10.9	26.52228	5.214025	4.659043
Control CLN1 (B)	4.14	17.84848	1.408786	2.093441
50 µg/ml (B1)	7.02	21.30251	2.494028	3.093563
75 µg/ml (B2)	9.7	30.84848	3.890823	4.417296
100 µg/ml (B3)	15.86275	38.18079	8.828677	6.79219
Control MRE11 (C)	3.7	11.32471	0.644259	1.424435
50 µg/ml (C1)	5.74	18.33586	1.895839	2.269366
75 µg/ml(C2)	9.66	26.86337	4.662948	4.175398
100 µg/ml (C3)	17.48	30.11288	9.030276	6.586223
Control ZRC1 (D)	4.510204	15.1704	0.988069	1.662803
50 µg/ml(D1)	7.627451	23.06511	3.322405	4.314785
75 µg/ml(D2)	12.08	25.41651	6.076189	5.287539
100 µg/ml(D3)	18.66	38.52412	10.73177	8.686483

Different superscript letters in the same column of tail length showed significance difference at P< 0.05.

Control	concentration of $ZnSo_4$ (50µg/ml	concentration of $ZnSo_4$ (75 µg/ml)	concentration of $ZnSo_4$ (100 µg/ml)

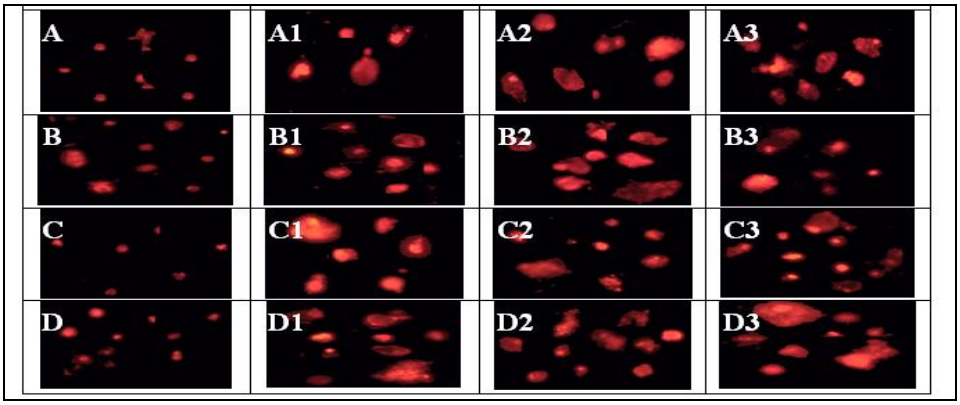
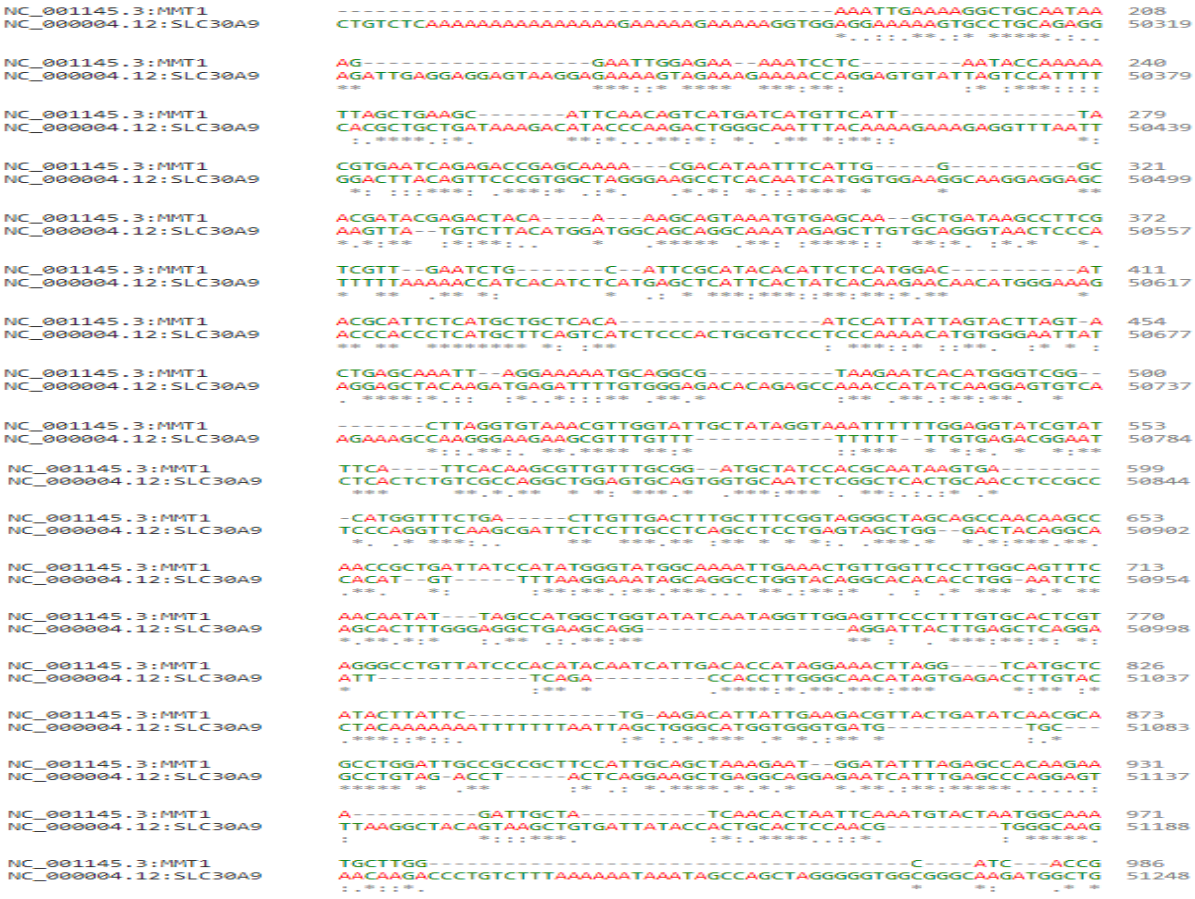


Fig. 7: Photomicrographs showing DNA damage in yeast strains using the Comet assay and Zink sulphate at a dose of (50, 75, 100 µg/ml). Control cells A: control MMT1 gene; A1, A2, A3: treated MMT1 gene; B: control CLN1 gene; B1, B2, B3: treated CLN1 gene; C: control MRE11 gene; C1, C2, C3: treated MRE11 gene; D: control ZRC1gene; D1, D2, D3: treated ZRC1 gene.

3.4. Selection of yeast haploid strains devoid of genes similar to specific human cancer genes in vitro.

Genotypes of yeast haploid (knockout) strains were determined based on sequence similarity results between human and yeast sequences. Figure (8) depicted the results of an alignment between the human SLC30A9 and the yeast MMT1sequences. MMT1 Putative metal transporter involved in mitochondrial iron accumulation; MMT1 has a paralog, MMT2 that arose from the whole genome duplication.



```
NC_001145.3:MMT1          TGTTGATTCATTAACTTCTCTTGTTGCTCTGGTTGCAATCAGTACTGGTTATTTGGTTAA  1046
NC_000004.12:SLC30A9      GATAGGAACAGATCCT-GT-CTGCAGCTCCCAGTGAGATCCATGCAGAAG--GTGGATAA  51304
                           .*:*.::** ::.**  *  ** :****  . **..***..*.*:*.:    ***:***

NC_001145.3:MMT1          T-------ATACAATCATTA------GACACGATTGGTGGTTTAATTGTTTCTGGTTTAA  1093
NC_000004.12:SLC30A9      CTTCTGCATTTCCAGCTGAGGTACCTGGCTCA--------TCTCATTGGGACTGGTCAGA  51356
                                :*:*.* *: :.       *.*:*.         * *.****  :***** :.*

NC_001145.3:MMT1          ----------TTATCAAGGCTGGTGGCG-----AGGGTATGTGCATCGCAATAAAGGAGT  1138
NC_000004.12:SLC30A9      CAGTGGGTGCAGCCCATGGAGGGTGACCCGAAGCAGGGTGGGGCATTGCCTCACCGGGGT  51416
                                   : . **:**. ****.*       ..** : * **** **.: *..**.**

NC_001145.3:MMT1          TAATCGATCAGTCAGTTTCTCGTGATGAT-CCACG-C-TACCTAGAGAT------AGAAA  1189
NC_000004.12:SLC30A9      AGTGCAAGGGGTC--------GGGGAACTCCCTCCCCTAGCCAAGGGAAGCCATGAGGGA  51468
                          :.: *.*  .***         * *.:..* **:*  * :.**:**.**:      **..*

NC_001145.3:MMT1          CTTTGGTTAAAGATACGTTGAACAAACTGATCTCTAATAATAATTCTCAGAAACCCTATG  1249
NC_000004.12:SLC30A9      CTGTGCTGTGAGGAACGGTGC--ATTCCAG-CCCAGA---TACTACGCTTT-TCCC-ATG  51520
                          ** ** * :.**.:*** **.  *::* .. * *:.*   **.*:* *: : :*** ***

NC_001145.3:MMT1          GATTGAAAGAACTGACGTTACTGTCCTCAGGACCGAATTTAC--GCGGA--CAT------  1299
NC_000004.12:SLC30A9      G-------------TCTTCACAACCCACAGACCAGGAGATTCCCTCGGGTGCCTACACCG  51567
                          *               :* * **:. **:***..*.*.* :*:*   ***.  *.*

NC_001145.3:MMT1          --TTAACCTTGGAAGTTCCTTTACAAAAATGGGGCAATATTTTAGG-TGTTAACGAGTTT  1356
NC_000004.12:SLC30A9      CCAGGGCCTTGGG-TTTCAAGCACAAAACTG-GGCGGCCATTTGGGCAGACACCAAGCTA  51625
                            : ..******.  ***.:  ******.** ***.. .:***.** :*: *.*.** *:

NC_001145.3:MMT1          GAAATTGTGACACATCATTTACGTAATGTGTTAACCAATGAAGTATCGAATTTGAG-AAG  1415
NC_000004.12:SLC30A9      GCT----AGACTAGTTTT------TTTTCATACTCCAGTGGTGCCTCGAATGCCAGTGAG  51675
                          *.:    :***:..* :*       ::*  .*:.:***.**.:* .******   ** .**

NC_001145.3:MMT1          ACTGGATATTG--AATACGTGGAA-----GAAAAAAATGGTGAGGAAAATG---------  1459
NC_000004.12:SLC30A9      ACAGAACCTTTTAATCCCTTGGAAAGGGGGCTGAAACCAGGGAGCTAAGTGGTCTAGCTC  51735
                          **:*.* .**    *: .* *****      *.:.***. .* *** :**.**

NC_001145.3:MMT1          ACTGGATATTG--AATACGTGGAA-----GAAAAAAATGGTGAGGAAAATG---------  1459
NC_000004.12:SLC30A9      ACAGAACCTTTTAATCCCTTGGAAAGGGGGCTGAAACCAGGGAGCTAAGTGGTCTAGCTC  51735
                          **:*.* .**    *: .* *****      *.:.***. .* *** :**.**

NC_001145.3:MMT1          AGCATATC----------------------------------------------------  1467
NC_000004.12:SLC30A9      AGCAGATCCCACCTCCAGAGAGCCCAGAAAGCTAAGATCCACTGGCTTGAAATTCTGGCT  51795
                          **** ***

NC_001145.3:MMT1          ------------------------------------------------------------  1467
NC_000004.12:SLC30A9      GCCAGCACAGCTGTCTGAAGTTGACATGGGATGCTTGAGTTTGGTGTGTGTTGGGGAGTT  51855

NC_001145.3:MMT1          ------AAGGGACA-ACAAAACTAC--------------------------A---------  1486
NC_000004.12:SLC30A9      GGGGGTTGGGGACCACCATTACTGAGGCTTGAGTAGGCAGTTTTCCCCTCACAGTGTAAA  51915
                                 :.*****. .**::***..                          *

NC_001145.3:MMT1          -------AAGAAGATGTTCTTATTAAGCACGACCATACGAATACTC--------------  1525
NC_000004.12:SLC30A9      CAAAGCCATCAGGAAGTTCGAACTGGACAGAACCCACCGT-AGCTCAGCAAAGCCACTGT  51974
                                 *: *.**:**** :* *...** .***.:.**: :.***

NC_001145.3:MMT1          ------------------------------------------------------------  1525
NC_000004.12:SLC30A9      AGCCAGACTGCCTCTCTAGATTTCTTCTCTCTGGGCAGGGCATCTCTGAAAGAAAGGCCC  52034

NC_001145.3:MMT1          ------------------------------------------------------------  1525
NC_000004.12:SLC30A9      AGCAGTCCCGGTCAGGGGCTTATAGATAAAACTCTCATCTCTCTGGGACAGAAAACTTGG  52094

NC_001145.3:MMT1          ------------------------------------------------------------  1525
NC_000004.12:SLC30A9      GGGTAGGGGCGGCTGTGGGCGCAGCTTCAGCAGACTTAAACGTTCCTGTCTGCTGGCTCT  52154

NC_001145.3:MMT1          ------------------------------------------------------------  1525
NC_000004.12:SLC30A9      GAAGAGAGCAGCGGATCTCCCAGCACAGCGCTCGAGGTCTGCTAAGGGACAGACTGCCTC  52214

NC_001145.3:MMT1          ------------------------------------------------------------  1525
NC_000004.12:SLC30A9      CTCAAGTGGGTCCTTGACCCCCATGCCTCCTGATGGGGAGATACCTCCCAGCAGGGATCA  52274
```

Fig. 8: Gene alignment between human gene SLC30A9 and the yeast MMT1in the Clustal Omega web site ('*' indicates identical between two aligned, '-' indicates gaps missing of one) and ('.' indicates low similarity, ':' indicates more similarity used to denote the level of similarity that are not identical) at position.

Figure (9) depicted the results of an alignment between the human CCNA1 and yeast CLN1 sequences. G1 cycle in involved in regulation of the cell cycle; activates Cdc28p kinase to promote the G1 to S phase transition; late G1 specific expression depends on transcription factor complexes, MBF (Swi6p-Mbp1p) and SBF (Swi6p-Swi4p); CLN1 has a paralog, CLN2, that arose from the whole genome duplication; cell cycle arrest phenotype of the cln1 cln2 cln3 triple null mutant is complemented by any of human cyclins CCNA1, CCNA2, CCNB1, CCNC, CCND1 or CCNE1.

```
NC_001145.3    ------------------------------------------------------------ATGA    4
NC_060937.1    TACAGTGGCCCGAGGTCCCGATGCTTGTCAGATACTCACCAGAGCCCCGCTGGGCCAGGA    1200
                                                                          * **

NC_001145.3    -ACCACTCAGAAGTGAAAACTGGGTTA-----------------------------------    30
NC_060937.1    TCCCCCGCAGAGGACAGTGCTAGGGCTGCTAACTGCAAATGGGCAGTACAGGAGGACCTG    1260
                .**.* ****.*: *.:.**.**   :

NC_001145.3    ---------ATTGTCA----------------------------------CTGCAAAGCAGAC    50
NC_060937.1    TGGCCAGGTAATGACTCAGACGCATTGAGAATGATGCTTGTGGAGAACAGCTCTCCTGCT    1320
                            *:**:*:                                *:**:.: *:*.

NC_001145.3    ATATTACCCAATTGA------ATTGTCCAATGCAGAACTACTAACTCATTACGAAACCAT    104
NC_060937.1    TTGGTG-CCAGGTGCTTTTCTCTTGCCCTTGTACCTACAACTCCCCCTGAGTATTACAAC    1379
               :*. *. ***. **.       .*** **::   .. :**:***..* *: :. .::**.*

NC_001145.3    ACAGGAATAT---CACGAGGAA-----------------ATCTCTCAAAATG---TGC---    139
NC_060937.1    CCTGGAATCTGGACTACAGGAAAGTTGATTTATTTATTTTCTTTCTTTCTTTCTTTCTTT    1439
               .*:*****.*    *:. *****                  :*** **::::.*     * *

NC_001145.3    ---------------------------------------------TGGTC----CAATCTTC    152
NC_060937.1    CTTTCTTTCTTTCTTTCTTTCTTTCTTTCTTTCTTTCTTTCTTTCTTTCGTTCTTTCTTTCTTT    1499
                                                                 * **     *::****

NC_001145.3    CAAGACAAAACCAGACA-------------TAAAA----TTGATCGATCAGCAACCGGAGA    196
NC_060937.1    CTTTTCTTTCTCTTTCTTTCCTTCTTTCTTTTTCTTTGTTTCTCTTCCTTCTTCCTTTCC    1559
               *:: :*::::. *: :*:                *::::.     ** .** : *: *::**   : .

NC_001145.3    --------TGAATCCTCATCAAA----CT-------AGAGAAGCCATAGTAACATTTTTG    237
NC_060937.1    TTTTTTTTTCTTTCCTTTCCTTTCCTTCTTTTTTTTTTTTTGAGACGGAGTTTCGCTCTTG    1619
                        * ::**** : *:::     **        : : .**.*. ***::*. * ***

NC_001145.3    TATCAACTTTCAGTGATGACTAGAGTAA-GTAAT----------------GGTATCTTCTT    281
NC_060937.1    T--CGCCA---------GGCTGGAGTGCAGTGACGCGATCTCGGCTCACTGCAACCTCCA    1668
               *  *..*:          *.**.****.. **.*                  * *:* ** :

NC_001145.3    CCACGCTGTCAGGTTCTACGATCGCTATTGCTCTAAGAGAGTAGTGTTAAAGGACCAAGC    341
NC_060937.1    CCTCC----CGGGTTCAA---GCGATTCT--------------------CCTGCCTCAGC    1701
               **:*      *.*****:*     **.*: *                   .. *.* .***

NC_001145.3    TAAACTAGT---------TGTAGGCACCTGCCTTTGGTTAGCGGCCAAAACT--------    384
NC_060937.1    CTCCCGAGTAGCTGGGATTTCAGGCACCCACCACCA-CGCCCGGCTAATTTTTGTGTTTT    1760
                :..* ***          *  ******* .**:  .    . **** **:: *

NC_001145.3    ----TGGGGAGGGT-GCAACCATATTA-------TAAACAACGTCTCCATCCCCACAGGT    432
NC_060937.1    TAGTAGAGATGGGATTTCACCATGTTGGCCAGGCTGGTCACCAACTCCTGA-CCTCAGGT    1819
                   :*.*.:***:   .*****.**.        *.:**.*.:****: . **:*****

NC_001145.3    GGTAGGTTTTATGGTCCCAATCCTAGAGCTCGTATTCCACGCCTTTCTGAA---TTGGTT    489
NC_060937.1    GA---------------------------CCCGCCCACCTCGGCCTCCCAAAGTGTTGGGA    1853
               *.                          * **  . :**:** * * * .**    **** :

NC_001145.3    C-----------ATTATTGCGGCGGGTCCGATTTATTCGATGAATCAAT-------GTTC    531
NC_060937.1    TTACAGGCGTCAGCCACGGTGGCAGGCCAGTTGATTTCTTAAGATCACCTTGAGGGGTTC    1913
                             .   *   * ***.** *.*:* ::*** ::..****.          ****

NC_001145.3    A---TTCAAATGGAA-------AGACATATCTTGGATA-------CTCT-----------    563
NC_060937.1    GGTTTTCAGCTAGAAATAGTGATTAGTTTTCTTTGTTATTTTTTCCACTATCAAGGAAAT    1973
               .   ****..*.***         : * :*:**** *:**         *:**

NC_001145.3    -GAACTGGGACGTTTATGAGCCCATGATTAATGACTACATTTTAAACGTT-GAC------    615
NC_060937.1    AGGTCTAGGAACTGTTTGGGTT-ATGTATATTAAAGT-AATTTAAGGGCTGGGCTAAAAG    2031
                *.:**.***. * *:**.*    ***::**:*.*. : *:*****. * * *.*

NC_001145.3    GAAAATTGTTTGATACAATATGAACTTTACAAAAACCAGTTACAAAATAACAATAGCAAC    675
NC_060937.1    GAAATGTGAATGCTTCCAAAT--------------------------AAACTGTGGAACT    2065
               ****: **::**.*:*.*:**                            :***:.*.*.*.

NC_001145.3    GGCAAAGAATGGTCCT-GTAAGAGAAA--GTCACAATCTTCTGACGACAGTGA----TGC    728
NC_060937.1    G----GGGTTCCTCCAGGTTAGAGCAGTGTTCACAT--TGATGCAGGCTGTGCCTAAAGC    2119
               *     .*.:*   ***: **:****.*.    *****:   * .**..*.*:***.     :**

NC_001145.3    CACAGTGGAAGAACATATCAGTAGTTCACCGCA---------AAGTACTGGACTAG----    775
NC_060937.1    AAACCTGGATAATCTTGGCTGT-GTTCATTGCGGATAGTGTTAAAATCAGGATTAGATCA    2178
               .*.. ****:.*:*:*. *:** *****  **.          **.::*:*** ***

NC_001145.3    ------ATGGCGATACAACTACCATGGATGAA--GATGAAGAACTAAATTCCAAAATTAA    827
NC_060937.1    GGCTAGATTAGATTACTCCA-AAATGGGTGCTTTGAAGGCAGACA----------------    2222
                     ** . .:***:.*: ..****.**.:  **:*....**:

NC_001145.3    ATTGATAAATTTGAAAAGATTCTTAATTGATCTGAGCTGTTGGCAATACAACTTGCTTAA    887
NC_060937.1    ---------------------TCTTCCCAGTACTGACTAAGCAGCTATGCTAACAGGTAAT    2262
                                   ****.. :*::****   :.   .**:**.*:*. :* *:*:

NC_001145.3    ATTCGAATTATACGAAATATGCAATGGTATGTTTTCTATAATAAACAAATT-CACTAATC    946
NC_060937.1    GGTC----CCTCAGATATTA-----GTTGTGTGAACTTAACTGATCATAGATACCTTAAC    2313
               . **      .*..**:**::      * *.*** ::**::*.*.*:**:* : ..**:*:*

NC_001145.3    AAGATCAAGGCCCTTTTCTCTCTATGCCCATTGG-------------TAATGATATAAACT    994
NC_060937.1    CAGACTGAAAACTATACCTTTCCTATTTCATAAGTGAGAAAATCTGAAAGGTTTAAGCCT    2373
               .***  .*...* :*: ** ** ::    ***:.*              :** *:*::*..**

NC_001145.3    CAAACACTCAAACGC--AGGTAT------------TCAGCATTATCATCAATGGCATAGTC    1041
NC_060937.1    CTGCCAAAAGAAAGCTATGGTAGAAACTAGATTAGAAGCC-TCTCATCTAC-----TGAC    2427
               *:..**.:..**.**   :****                .***. *.*****:*       :*:*

NC_001145.3    AATTCTCCCCCATCTTTAGTCGAAGTTTATAAGGAGCAATA----TGGTATAGTACCTTT    1097
NC_060937.1    T---CTTCACATGCTCTGTTCAAAGCTTT---GGAGAAAATTGAGTGGAAGTGGTTTTAA    2481
               :   ** *.*.: ** *. **.*** **:    ****.**::     ***:* :* :  *::

NC_001145.3    CATAT-------TACAAG----------------------TAAAAGATTATAAACTTGGAAT    1129
NC_060937.1    CAAATTTTAATTTGCTTAGCAATGTTTGCTTTTTAAAACTTAACGCCTGAGACTTGGTTC    2541
               **:**        *.*::.                      *:**.*. *.:.******::

NC_001145.3    T---------ACAAAAGAAACTGCA---ACTGGCCTCTACAATAGACCTAACCAGAAAAA    1177
NC_060937.1    TTATGAATTGTCAGTTGTGCCTGTGTTGAATGGTCGCATTTT---AAATTCCCAG-----    2593
               *          :**.::*:..*** .    *.*** * *:: ::    *..*:.****

NC_001145.3    TTGCTGTCAATTCTCGTTACTTTGACCAAAATGCCTCTTCATC-----------------    1220
NC_060937.1    TTGCTAACCTCTTATGTTA-------CCTGGTGCCACTTCTGCATTTCCCTGTACCTATC    2646
               *****.:*.: * : ****        *.:..****:****: *

NC_001145.3    ------------------------------------------------------------ATCAGTTT    1228
NC_060937.1    CTCTACAACCTGCTTAGAAATCTATTTTGTACCTTCCCTTCTCTGTTTAATCCTCAGTAA    2706
                                                                        .*****::
```

```
NC_001145.3      CT----TCTCCAAGCACATATTCTTCGGGAACCAATTAT------ACTCCA---------   1269
NC_060937.1      CCTCCTCCTTCATGCCCAGTCTCAGCTGATCACTTTGTATCTTATTTTCCTAAAAATATA   2766
                 *       ** **:**.** : **: * *.:..*::* ::        : ***:

NC_001145.3      ------------ATGCGAAACTTC----------AG--TGCAC---AATCAGACAACAGTGT   1304
NC_060937.1      GAAGCAAGCAGAAGAGAATCTCCACATGTCCCTGTGTGCCATATAATCTGCCATCTACAT   2826
                              *:*.***:** *          :*  ***..   ****:*.**:*:. .*

NC_001145.3      TTTCAGT--ACTACCAACATTGACCATTCA-----TCGCCGATCACC-----CCTCAC-A   1351
NC_060937.1      --CCTGTTATGTGACTGCTATGTCCCTGCTGTCATGCGAGGTGCCCCCCCCCCCCGCGCCA   2884
                    *:**  : *..*:.*::**:**.* *:       **. *: *.**      ** *.* *

NC_001145.3      T--GTACA-------CTTTTAATCAG-TTTAAAAACGAA----------------------   1380
NC_060937.1      TGCGTACAATATACTCGTGTCTTCTTGCTTCTTCAAGGATGTTACTCTAGCCACTTTTCT   2944
                 *  *****         * * *.:**:   **.::.*.*.*

NC_001145.3      --------AGTG------C-TTGTGACAGTGCCATAAGCGTAAGCAGTCTA---------   1416
NC_060937.1      TTCTCTCATGAGTTTTTTCTTTTCTACTGGGCCAAATCTGTAAGCATACAAATATATTGC   3004
                           :*:*       * **    **:* ****:*:   ******* :*:*

NC_001145.3      ------------------------------------------------------------   1416
NC_060937.1      CATTTTCCCATCTTAAAAACCTTTCAACTAAACGCTCCCTCTGAGTATTACAACCCTGGA   3064

NC_001145.3      ----------------------------------------CCTAATCAAACCC--------   1429
NC_060937.1      ATCTGGACCACAGGGAAGAAGTTGACTTTGTAAAATCACCTTGACCGGTCCCTTTTTCAG   3124
                                                          * *.* *..:***

NC_001145.3      ---AAAATGGTAAC------------------ATGCCATTATCAAGCAATTA---------   1460
NC_060937.1      CTAGAAATGGTGACGAATTTTCTTTGTTAGTTTTCCAATATCAAGGAAATAGGTCTAGGA   3184
                    .*******.**                          :* ***:*******  **:**

NC_001145.3      -------TCAGA-----------------ATATGATGCTAGAAGAAAGGAA--TAAA--GA   1493
NC_060937.1      GCTGTTTGCATTATGTCGATTAAAGTAATTTAAGTGCTTAAAGAAATTTAAGTAATGGGA   3244
                           ** :                 :*:..****:.******  :*  ***:  **

NC_001145.3      GAATAGAATTCCCAA-------------------------------------------   1508
NC_060937.1      CAGAAGCTATCCCATTTAAAGCCACATATCTTGAGAGTTTGTCGTATCTTGACATATATA   3304
                  *.:**.::*****:
```

Fig. 9: Gene alignment between human gene CCNA1 and yeast CLN1 gene in the Clustal Omega web site ('*' indicates identical between two aligned, '-' indicates gaps missing of one) and ('.' indicates low similarity, ':' indicates more similarity used to denote the level of similarity that are not identical) at position.

Figure (10) shows the results of alignment between human and yeast MRE11 sequences. Nuclease subunit of the MRX complex with Rad50p and Xrs2p; complex functions in repair of DNA double-strand breaks and in telomere stability; Mre11p associates with Ser/Thr-rich ORFs in premeiotic phase; nuclease activity required for MRX function; widely conserved; forms nuclear foci upon DNA replication stress.

```
NC_001145.3:c720653-718575      ----------ATGGACTATCCTGATCCAGACACAATAAGGATTTTAATTACTACAGATAA      50
NC_000011.10:c94512412-94415570 TTGTTCCTTTAACTGCAGTGTAAGTCGAGTATAGTCAACTGGCCTTGTTTCTGGGTGCTT   88080
                                          *:  .*:.*  :..** **:.:.: **  .   *:.**:**. . . ::

NC_001145.3:c720653-718575      TCATGTGGGTTACAACGAAAATGATCCCATTACTG-GCGATGATTCTTG---GAAAACTT      106
NC_000011.10:c94512412-94415570 TCA-GAGGGCCACAGCTCTATATAGATCTTTACTGTGCCTAGATTCTTGCCCTGGGTGTC   88139
                                *** *:***  ***.* .:*:: * . *:****** ** ::********    ...: *

NC_001145.3:c720653-718575      TCCATGAAGTCATGATGCTGGCCAAAAATAACAACGTAGACATGGTTGTACAGTCCGGTG      166
NC_000011.10:c94512412-94415570 ACAGAGATGTATATTGGCAGAATTATTTTGGTGTTGTAATTTTGGCTGT--GATCCAGTA   88197
                                :*..:**:**.:: : **:*.. :*:::*.. .: ***.: :*** ***  ..***.**.

NC_001145.3:c720653-718575      ATCTTTTTCACGTGAATAAGCCTTCCAAGAAGTCACTCTACCAAGTACTGAAGAC-TTTG      225
NC_000011.10:c94512412-94415570 GCTGGCATTTAACATTAGTGTCCAGTGGATAGGCTCTTACTCAGCTGCATAGTTCTTTTG   88257
                                .     :* :.. .::::.:* * :  ...:** *:** :. **. *.*: *. :* ****

NC_001145.3:c720653-718575      AGATTATGTTGCATGGGTGACAAGCCTTGCGAGTTAGAATTATTGAGCGAT-CCCTCACA      284
NC_000011.10:c94512412-94415570 TATTTCTGTGTGTTCACAGCCGTGCTCTGTGGTGGGGTTGGGGAGAGAGATGACCACTCA   88317
                                :.:**.***   :* . :*.*.:**  ** *.   .*::  . :***.*** .**:*:**

NC_001145.3:c720653-718575      AGTTTTTCACTACGATGAATTTACCAACGTTAACTATGAGGACCCCAACTTTAATATTTC      344
NC_000011.10:c94512412-94415570 TAAGGGCCACTCC------TGGACCATGG------GTGAGGTCCCTCCTATCACTGGCAC   88365
                                :.:    ****.*      *  ****: *      .*****:*** .. :* *.*.  :*

NC_001145.3:c720653-718575      TATTCCCGTATTCGGCATATCAGGTAATCATGATGATGCGTCGGGGGACTCACTGTTGTG      404
NC_000011.10:c94512412-94415570 TGTACCTGCATTACTGTTGTTTGGTGTCTTGG-----GTTGCAGGGCTCCCTTAGACAGA   88420
                                *.*:** * ***.   :*.* :***.:  : *     *   *.*** :* *: :*: . .

NC_001145.3:c720653-718575      TCCTATGGATATACTTCATGCGACTGGTCTAATAAATCATTTC-GGGAAAGTCATCGAAT      463
NC_000011.10:c94512412-94415570 GGCCATGGCTGCCAGACAGGCCACACCCTTCCCAGACCAGCACTGTGGAGGAAGGCATGT   88480
                                  * ****.*. .. :** ** **:    *.. *.* **  :* * *.*.*:.. *.:.*

NC_001145.3:c720653-718575      CTGATAAAATAAAAGTCGTGCCATTATTATTTCAGAAAGGGTCCACTAAGTTAGCATTGT      523
NC_000011.10:c94512412-94415570 CCCATTCCTGCACTGGC---CCACGAACCCACGTGTCTCACTCCTTTCAGTGTTCTGAAA   88537
                                *  **:..: .*.:* *   ***  *: . :  :*:.: . ***: *.*** : *: :.:
```

```
NC_001145.3:c720653-718575      ACGGATTAGCCGCTGTTCGTGATGAAAGGTTATTTAGAACTTTTAAGGATGGTGGTGTCA     583
NC_000011.10:c94512412-94415570 GTGGGATTTCTCCCCTACTTGAGTGCCAGCCC--CAGCTCTTAGCTCGACAG------CC  88589
                                . **.:*: *  *  *:* ***  ....*  .   **.:***: .: ** .*      *.

NC_001145.3:c720653-718575      CTTTTGAAGTACCGACTATGCGAGAAGGTGAATGGTTTAATTTAATGTGCGTCCATCAAA     643
NC_000011.10:c94512412-94415570 CCAGCTGTGTGCTG---TAGCCCTGTAGCACTGGGACCAGCCTGTGGCTCCATCGTCTGG  88646
                                * :   .:**.* *   ::** . .:.* ..: **:  *.  *.: *  * : *.**:..

NC_001145.3:c720653-718575      ATCATACAGGTCACACGAATAC--TGCATTTTTACCTGAACAGTTCTTGCCAGATTTCCT     701
NC_000011.10:c94512412-94415570 ACCCTCAGGGTCAGGCACCAGCTGTGCTGGGGAATCTGAAATGCTC---CCAGGCTGCCA  88703
                                * *.*...***** .*...:.*  ***:    :* *****.:* **   ****. * **:

NC_001145.3:c720653-718575      GGATATGGTGATATGGGGTCATGAACATGAGTGTATTCCGAATCTCGTACACAATCCAAT     761
NC_000011.10:c94512412-94415570 GGAAGGTACTCAGGTGGAACCACCAGCAAAAAACAAGGCTAGGCAGCAGAGGCTGCCCTG  88763
                                ***:.  .  .:.  **.:*.: .* .:.*.:. *:  * *. *:  :... .: **.:

NC_001145.3:c720653-718575      TAAAAATTTTGATGTATTACAACCAGGTTCATCTGTAGCTACTTCACTTTGTGAGGCTGA     821
NC_000011.10:c94512412-94415570 TGCACACATTCCTGCAGGGCAGCTTGG-----CAGGGGCCCTGGAAGGGGCTGTAGGGGA  88818
                                *..*.* :** .** *  .**.* :**     *:* .** .   .*      **:.*  **

NC_001145.3:c720653-718575      GGCACAACCCAAGTATGTCTTTATCCTTGACATAAAGTATGGAGAAGCAC---CAAAAAT     878
NC_000011.10:c94512412-94415570 GGAGGGCCTGCAGAACAGATGTTCCTCTGTCCCACAGGGAAGCTGGCCCCAGGCTGGCCT  88878
                                **.. ..*  .**:* . .* *: *  **:*. *.** .:.*. .. *.*   *:....*

NC_001145.3:c720653-718575      GACACCTATTCCTCTTGAGACTATACGGACATTCAAAATGAAATCCATTTCGTTACAAGA     938
NC_000011.10:c94512412-94415570 GCCCCAGCATTCAGGTGGGGCTAGTCCTAC--TCCAGGGGAAATAAGAAGTCTTAGGGGA  88936
                                *.*.*. .:* *:  **.*.*** :*  **  **.*.. *****...::   *** ..**

NC_001145.3:c720653-718575      TGTTCCCCATTTGAGGCCTCACGATAAAGATGCTACGTCTAAGTATCTTATTGAACAAGT     998
NC_000011.10:c94512412-94415570 TGGGCGCCTATGGCCACCTTCTGCTGCAGCTGCCTTAGATATGAAAACCCCTGGGCTCCA  88996
                                **  * **::* *. .*** . *.*..**.*** : . .**:*:*:.  . **..*:. :

NC_001145.3:c720653-718575      TGAAGAAATGATCCGCGACGCTAATGAGGAAACTAAACAAAAATTAGCGGACGATGGTGA    1058
NC_000011.10:c94512412-94415570 TGTATTCTGGAGCTCTGTCTCTGCCTACTGTTTGGGCCAATCCCC--CTGCCAGTTGAAA  89054
                                **:* :.: ** *   *:* **..  *  .::  ...***:..     * *.*..* *:.*

NC_001145.3:c720653-718575      AGGTGACATGGTTGCGGAATTACCGAAACCATTGATCAGATTACGTGTTGATTATAGTGC    1118
NC_000011.10:c94512412-94415570 CGTC-TCCAGGGGGCATGGAATCTTGTAGCTAGGATCTCTGAGGTCCATAGTGAGAGTGT  89113
                                .*   :*.:**  **. ..:::*  .:* *:: ****: : :.    :*..* * ****

NC_001145.3:c720653-718575      ACCCTCCAATACACAATCCCCAATAGATTACCAAGTTGAAAACCC--GCGTAGATTTAGC    1176
NC_000011.10:c94512412-94415570 GCTGCCCCATCATCCATTCACTCACCCCTTTCTAGGAGCCTTTCAAGGCCAAGAACCAGC  89173
                                .*   **.**..:*.** *.*:.:. . *: *:** :*..:: *.  ** :****:  ***

NC_001145.3:c720653-718575      AATCGATTTGTGGGA----CGTGTTGCTAACGGTAATAACGTTGTGCAGTTTTATAAAAA    1232
NC_000011.10:c94512412-94415570 CCTGGCATTGGGCAACCCCCACAAGGTTACCAGCTTCCTCCCTCTTCAGCTTCCAGTTTG  89233
                                ..* *.:*** * .*    *. .: * **.*.* :: .:*  * * *** ** .:.:::.

NC_001145.3:c720653-718575      AAGGTCACCTGTAACTAGATCAAAAAAATCCGGTATAAATGGAACAAGCATCA-GTGATA    1291
NC_000011.10:c94512412-94415570 TATCACCTCTCTATC-AGCTCTTGGTGCTTTTTCTCCAAAGATCTGCTCAAAATATGTTG  89292
                                :*  :*. ** **:* **.**::..:..*     : .**:*.:. .. **:.* .**:*.

NC_001145.3:c720653-718575      GAGATGTTGAGAAACTTTTCAGCGAAAGTGGCGGTGAACTAGAAGTTCAAACTTTGGTTA    1351
NC_000011.10:c94512412-94415570 GCTTACTCGATATTTTGGTCTTTATTAGTGGCAGTGG---TGCTTCTTGGCCCTGTCTAG  89349
                                *. :: * ** *:: *  **:  .::******.***.   :*.:  * ...* *   *:.

NC_001145.3:c720653-718575      ATGATCTCTTGAACAAAATGCAACTATCTTTATTACCAGAAGTTGGTTTGAATGAAGCAG    1411
NC_000011.10:c94512412-94415570 TTGGCCATCTTGTCCTTCTTCCACTAAAAATTAT--------TTTTAAAAATAGTTTCAG  89401
                                :**. *:  * .:*.::.* *.****:.::*::*         **  ::::*::*:: ***

NC_001145.3:c720653-718575      TAAAGAAGTTTGTAGATAAAGATGAGAAAACAGCTCTTAAAGAATTTATTAGCCATGAAA    1471
NC_000011.10:c94512412-94415570 GCTGGGCGCGGTAGCTCATGCCTGTAATCCCAGCACTTTGGAAGGCTGAGGCAGGCGGAT  89461
                                 .:.*..*    :. : *:. .**:.*:..****:***:...*.  *.: . . . *.*:

NC_001145.3:c720653-718575      TATCGAACGAAGTTGGAATATTATCTACGAATGAAGAATTTCTGAGAACAGATGATGCAG    1531
NC_000011.10:c94512412-94415570 CACCTGAGGTCGGGAGTTCATGACCAGCCTGACCAACATGGAGAAACCCCGTCTCTACTG  89521
                                 * * .* *:.*  .*:: ** * *:.* :.: .*..**  . .*...*.*:  .*.*:*

NC_001145.3:c720653-718575      AGGAAATGAAAGCGCTTATAAAACAGGTTAAGCGTGCTAACAGTGTTAGGCCGACTCCCC    1591
NC_000011.10:c94512412-94415570 AAAATA--------CAAAATTAGCCGGACATGGTGGCACATGCCTGTAATCCCACCTACT  89573
                                *..*:*          *::*:::*.*.**: *:*    **:.* .     **. ** **  .*

NC_001145.3:c720653-718575      CTAAAGAAAATGATGAGACAAATTTCGCATTCAATGGTAATGGGCTAGATTCCTTCCGGT    1651
NC_000011.10:c94512412-94415570 CAGGAGGCTGAGGCAGGAGAATCGCTTGAACCTGGGAAGTGGAGGTTGCAGTGAGCCAAG  89633
                                *:..**.:.:*. ..** **:       *: *:. *.:. *.* *:*.:    : **..

NC_001145.3:c720653-718575      CTAGTAATAGAGAAGTAAGAACTGGATCTC-CAGACATTACCCAATCACATGTTGATAA-    1709
NC_000011.10:c94512412-94415570 ATTGCACCATTGCACTCCAGCCTGGGCAACACGAGCGAAACTCCATCTCAAAAAAAAAAA  89693
                                .*:* *. * :*.* *.....****. .:* *...*.::** *.***:**:.:.*:**

NC_001145.3:c720653-718575      --TGAATCAAGAATAACCCATATTAGTCAAGCGGAAAGCAGTAAGCCAACGAGCAAACCC    1767
NC_000011.10:c94512414-94415570 ATAGTTTCATTGATAACCTTCCTTTGAACTATAGTTAATGTTAAGAGTTAACAAGTAACA  89753
                                  :*::***: .****** : .**:*:...  .*::*. . ****. ::.......:*.*.

NC_001145.3:c720653-718575      AAACGAGTGCGAACTGCAACGAAAAAGAAAATTCCTGCTTTTTCAGACTCAACTGTCATA    1827
NC_000011.10:c94512415-94415570 TATGTTGAGTGCCCATCAGTG-------TAATACCTCAGATATAAAAGTCCTGTAGATTT  89806
                                :*:  :*:* *..*: **. *       :***:*** . :*:*.*.* **.: *. .:*:

NC_001145.3:c720653-718575      TCCGATGCAGAAAATGAACTCGGTGATAATAACGATGCTCAAGATGATGTTGATATTGAT    1887
NC_000011.10:c94512415-94415570 TTAAAATATGATATTCACTCAGTTCTTATTTGCCCAGTTAACAGTGGGATCGGAATTTTT  89866
                                * ..*: .:**:*:* *.  .* * :**:*:.* .:* *.*...**. .* *.:*** :*

NC_001145.3:c720653-718575      GAGAATGACATAATTATGGTCAGTACTGACGAAGAG--GACGCTAGTTATGGTTTACTTA    1945
NC_000011.10:c94512415-94415570 TTTAGCTTCTACCATTTACTTATATTTGGAGAAATGATGATGATGATGATGATATCCCAA  89926
                                 : *.  :*::..:*:*. * * :: **..***.:*  ** *.*..* ***.*:*.* :*

NC_001145.3:c720653-718575      ATGGTCGAAAAACAAAAACAAAGACTCGTCCTGCTGCGAGCACCAAAACCGCTTCCAGAA    2005
NC_000011.10:c94512415-94415570 GAGCTTTTAATATTACTTTAATGAATATTTATTAAGTACTCACTATATGCTCTACATG-T  89985
                                .:* *  :**:* :*.:: **:**.*. * .* .:* .. *** *:*: * **:*.:* :

NC_001145.3:c720653-718575      GGGGAAAAGGAAGAGCATCAAGGACGCCAAAGACGGATATTCTTGGAAGTCTCCTTGCTA    2065
NC_000011.10:c94512415-94415570 GTCATGTAGAGGGTGGCGCATGGTGGTGATTTCATGACATACAGGTATTCAGGCATTGTC  90045
                                *  .:.:**...*:* . **:**: *  *:: .. ** **:*: * *:  .  *:*  *.

NC_001145.3:c720653-718575      AGAAAAGAAAATAG-------------------------------------------      2079
NC_000011.10:c94512415-94415570 CGAAATGTTCTCTGGGAGGATGTCTGGAAAAAACTCCAGTTCCAGATCGCGTCTGCAACC  90105
                                .****:*::.: :*
```

Fig. 10: Gene alignment in the Clustal Omega web site between human gene MRE11 and yeast gene MRE11 ('*' indicates identical between two aligned, '-' indicates gaps missing of one) and ('.' indicates low similar, ':' indicates more similar used to denote the level of similarity that are not identical) at position.

Figure (11) depicted the findings of alignment between human sequence SLC30A10 and yeast sequence ZRC1. Vacuolar membrane zinc transporter; transports zinc from cytosol to vacuole for storage; also has role in resistance to zinc shock resulting from sudden influx of zinc into cytoplasm; human ortholog SLC30A10 functions as a Mn transporter and mutations in SLC30A10 cause neurotoxic accumulation of Mn in liver and brain; ZRC1 has a paralog, COT1, that arose from the whole genome duplication.

```
NC_001145.3:c756166-754838      ----TTAGACACGGT-----------------------------TTTTTTCCTAT-----      64
NC_060925.1:c219196857-219148333 AACCCAAAACTTTGTGTTTATATTTTATAATTTTTCTGACCAAATTTTTACCTAATTTAC  39720
                                     :*.**:  **                              *****:****:

NC_001145.3:c756166-754838      ----------------------------------TGGA------------------AATT      72
NC_060925.1:c219196857-219148333 TTACTGAATCCCAATTTAGTCTTTGGCTAACAATTGGGCACTCAAATCTTTGCTGAGGAA  39780
                                                                  ***.                   ..::

NC_001145.3:c756166-754838      ACCATAGGTTATATGTCACATTCATTGGCCTTGATTGCCGATTCATTTCACAT-------     125
NC_060925.1:c219196857-219148333 TGCATGTGTTCTATATCATAATCAATATCTTT------AGAAACAATTCCCTTAATTAAA  39834
                                 : ***. ***.***.*** *:***:*. * **      .**::**:***.*:*

NC_001145.3:c756166-754838      ---------------------------GTTGAATGAT------ATCATCTCTCTTTTAGTG     153
NC_060925.1:c219196857-219148333 GGCACCCACAATTTTTAGTATTATCTGTAGAATTTTAATTTTATTTTATTGTGATAAATG  39894
                                                  **:**** :*      ** :*.*    :*:*.**

NC_001145.3:c756166-754838      GCACTATGGGCTGTGGAT--GTGGCCAAAAACAGGGGT----------------------     189
NC_060925.1:c219196857-219148333 GCTCTTTATGCAATGGAAATGTTGCCAGAGTCCCTGTTCTTGGACACCTCTTAAACCAAA  39954
                                 **:**:*. **:.****:  ** ****.*.:*.  * *

NC_001145.3:c756166-754838      --------------CCAGACGCTAAATACACTTATGGATGGAAAAGAGCAGAAATTTTGG     235
NC_060925.1:c219196857-219148333 TGCCATTTAAATGACCACAGACTAAATACAAAGTAGGTAAA-TGCTTACAGGGAA-AGGG  40012
                                            *** * .*********.: ::**::.. :.. :.***..*: : **

NC_001145.3:c756166-754838      GTGCTTTAATCAATGCTGTTTT---TCTTATTGCCCTGTGTTTCTCTATTATGATTGAAG     292
NC_060925.1:c219196857-219148333 GAAAGTGGATCAAGGTAGAAAAAAGACAATTTGTT---TTAAACACTCTTA---------  40060
                                 *:.. * .***** * :*::::   :*:::***     * :::*:**.***

NC_001145.3:c756166-754838      CTTTACAAAGATTGATTGAACCTCAAGAAATTCAAAACCCAAG-GTTGGTTTTATACGTT     351
NC_060925.1:c219196857-219148333 -----------------ATACAACTTGTT-CTCTCTCCCCTTAAACATGTTTAAAACAAC  40102
                                                 .:**.:*::*::  **:.:.***::. . : ****:*:**.:

NC_001145.3:c756166-754838      GGTGTAGCAGGGTTAA-TTTCTAATGTCGTAGGTTTATTTTTGTTCCACGATCATGGCAG     410
NC_060925.1:c219196857-219148333 -GTAAAGCACAGATGATTGTCTGAAA------G-----------------AAAAA----G  40134
                                  **.:**** .*:*.* * ***.*:.      *                 *:.*:    *

NC_001145.3:c756166-754838      CTCTGCC--CTTATCACGTAGAGCTTCAAGAATTTTACTACAGGCTACTCCTTCTACAAT     905
NC_060925.1:c219196857-219148333 CC-TTCCCGCTTATCAAGGAGACCG-CTGCCATTCTGCTAC-------------------  40681
                                 *  * **  *******.* *** *  *:. .*** *.****

NC_001145.3:c756166-754838      TTCTGCTGATCAGATTCAAAGAGAGATTTTGGCAGTACCTGGCGTG--------ATAGCG     957
NC_060925.1:c219196857-219148333 ---AGATGGTCCCAA--AAGGAGTCAACATGGAAGAGCTGAGTAAGTAGACTGAATTTTG  40736
                                    :*.**.**. *:  **.***: *: :***.**:.*  .* .:*        **:  *

NC_001145.3:c756166-754838      GTCCATGACTTCCACGTCTGGAACTTAACTGAATCAATATATATTGCATCTATCCAC-GT     1016
NC_060925.1:c219196857-219148333 ATCCAGAACCAC---------------TC----TCAATTTAATGTTATTCAGGCCAAAGG  40777
                                 .**** .** :*                :*    *****:**:: * .:**:. ***. *

NC_001145.3:c756166-754838      T----CAAATA-----GACTGTGCACCTGA--TAAATTCATGAGCTCC-------GCCAA     1058
NC_060925.1:c219196857-219148333 ACAAGCATTATTTAAGAGCAGTGTTTGAGTTATGTATTCTGAAACACCTTAAATCACCAG  40837
                                 :    **::::     ..*:*** :  :*:  *.:****: .*.*:**       .***.

NC_001145.3:c756166-754838      GCTGATAAGAA-------------AAA--------------------------------     1072
NC_060925.1:c219196857-219148333 GTGGTAGAGATGTCATCATATGTTTTATAGCCTATTAAGATGACTCAGCTCCAAGTACCA  40897
                                 *  *::.***:             ::*

NC_001145.3:c756166-754838      -TATTCCAT--CAACACGGTATTCA-------TTCTGCAACTGTTCAACCAGAATTTGTT     1122
NC_060925.1:c219196857-219148333 AAGGTCCTTTGCAATCCGTGATTCTCTGACTCAACTGCAAATTTT---TAAGAATGCAAT  40954
                                  :. ***:*  *** .**  ****:       ::******.* **    .*****  .:*

NC_001145.3:c756166-754838      TCTGGAGATGTTAATGAGGATATTCGCAGAAGATTTTCTATCAT-------------AGC     1169
NC_060925.1:c219196857-219148333 ACTTGGTCTGGGCATGGTGGCTCACGCC--TGTAATCCTGGCACTCTGGGAGGCAGAGGT  41012
                                 :** *. .**  .***. *. : :***.  :*:::* **. **             .*

NC_001145.3:c756166-754838      AGGTGGTTCACCA-------------------------------TCT--------------     1185
NC_060925.1:c219196857-219148333 GGGTGAGTCACCTGAGGTCAGAAGTTCAAGACCAGACTGACCAACATGGTGAAACCCTGT  41072
                                 .****. *****:                                 :*:

NC_001145.3:c756166-754838      -TCGTCTCAAGA------AGCCTTTGACAGCCATGGAAACACTGAGCATGGTAGAAAAAA     1238
NC_060925.1:c219196857-219148333 CTCTACTAAAAATACAAAAAAATTAGCCAGGCGTGGTAGCACGC-GCCTGT---------  41122
                                  ** :**.**.*      *...**:*.*** *.***:*.***   **.**
```

```
NC_001145.3:c756166-754838      CGATAGTCTGCACTCACAC---------TCTCATGGCTCT-------GTGGAAAGCGGGA     454
NC_060925.1:c219196857-219148333 AAATCATTTGCACAGCCCCTGTTTTCCAGCTTGGTGCCCTTTGATGGGTGGTGAGTG---  40191
                                 ..**..* *****: .*.*          ** .  ** **       ****:.** *

NC_001145.3:c756166-754838      ATAACGATTTGGACATAGAATCTAATGCGACTCATTCCCACTCTC----------------     499
NC_060925.1:c219196857-219148333 GTAGCGATGTGGCCCAG-----TGAGACGGCACAGTCGCTCTTCCTGCAGGTCAGAATCT  40246
                                 .**.**** ***.*.:.     *.* .**.*:** ** *:**  *

NC_001145.3:c756166-754838      -------ATGCATCTCTTCCAAACGATAATTTGGCCATCGATGAAGATGCTATTTCGAGT     552
NC_060925.1:c219196857-219148333 TCCTAGGCTGTTGCTCTTCCAAGGTACTCTGAGTCCTGCGATGGT-------------GG  40293
                                        .** : *********.  * :.* :* **: *****.:             *

NC_001145.3:c756166-754838      CCTGGGCCCTCAGGGCAAATTGGTGAAGTGTTGCCACA--------------ATCAGTAG     598
NC_060925.1:c219196857-219148333 CCTGGGGTTTTGTTTCC-TAAGA--AGTAGTTGCCATTATTTTGATTCATCCTACTGTTC  40350
                                 ******   * .   *. :::*.  *. :******* :               ::*:**:

NC_001145.3:c756166-754838      TAAACAGATTATCAAACGAAAGCCA----AC-CCTTATTGAACCACGATGATCATGACCA     653
NC_060925.1:c219196857-219148333 TAACCAAA-------ACAAAAACCAAAAAACTCCAAATGGTAA-CAGATATTCATATACC  40402
                                 ***.**.*       **.***.***    ** **::** *:*. ..***.:****.:.*.

NC_001145.3:c756166-754838      CAG----CCA----------TGAATCGAAGAAACCAGGTCATCG------C--------T     685
NC_060925.1:c219196857-219148333 CGGACACCCAAGTCAGTGAGTGTCACGTGGCTTCCAGATTTGCATCTGTGCTGCAGTAGT  40462
                                 *.*    ***           **:.:**:.*.::****.* : *.       *        *

NC_001145.3:c756166-754838      CTTTGAATATGCATGGTGTCTTCTTACATGTACTAGGTGATGCTCTGGGTAATATTGGTG     745
NC_060925.1:c219196857-219148333 CTTTCTTCCTGGCAGGTGTACTTTTGCATGTGATGGGAGATGCCCTGGGGTCCGTGGTTG  40522
                                 **** :: .** .:*****. * **.*****..*.**:***** ***** :. .* * **

NC_001145.3:c756166-754838      TTATTGCAGCTGCTTTGT----TTATTTGGAA-AACTGAA-------TA------TTCTT     787
NC_060925.1:c219196857-219148333 TGGTCATCACGGCCATCATATTCTATGTGCTTCCCCTGAAGAGTGAGGACCCGTGTAACT  40582
                                 * .* . ..* ** :* :     *** ** :: ..*****        *      *:. *

NC_001145.3:c756166-754838      GGAGATATTACTCGGATCCAATCGTTTCTTTAATCATCACCATTATTATTTTCTCTTCCG     847
NC_060925.1:c219196857-219148333 GGCAGTGTTACATTGACCCCAGCCTGACTGTCCTCATGGTCATCATCATTTTGTCATCTG  40642
                                 **...*.****:  ** **.* * * :** *..**** . *** ** ***** **:** *

NC_001145.3:c756166-754838      GCGTTCACCTACTGCCTATGGTGCTACTACAGCATCATCTAA-----TTGTATTGTAGAT    1293
NC_060925.1:c219196857-219148333 --A-ATCCCAGCTACTCGGGAGGCTGAGGCAGGAGAATCGCTTGAACCCGGGAGGCGGAG  41179
                                   . : .**:.**.*  . *. ***.. .*** * .*** .:       * .: * .**

NC_001145.3:c756166-754838      GACGCTGTAAACTGCAATACTTCCAA---------------TTGCCTGTAA---------    1329
NC_060925.1:c219196857-219148333 GTTGCAGGGAGCTGAGATTGCACCACTGCACTCCAGGCTGGGTGACAGAATGAGACTCTG  41239
                                 *: **:* .*.***..**:  :***.                **.*:*:*:

NC_001145.3:c756166-754838      ------------------------------------------------------------    1329
NC_060925.1:c219196857-219148333 ACTCAAAAAAAAAAAAAAAAAAAAGGAAAGAAAAAAAAGAAAAGAATGCAATGCTTGGATA  41299
```

Fig. 11: Gene alignment in the Clustal Omega web site between human gene SLC30A10 and yeast gene ZRC1 ('*' indicates identical between two aligned, '-' indicates gaps missing of one) and ('.' indicates low similar, ':' indicates more similar used to denote the level of similarity that are not identical) at position.

3.5. Yeast protein-protein interaction prediction (Networking).

Predicting protein-protein interactions in yeast and humans could lead to a determination of the degree of deliberate resemblance between two animals' genes linked to cancer (Figures 12). Gene MANIA displays the prognostic value of each data set selected for the inquiry. Currently, two organisms (Homo sapiens and Saccharomyces cerevisiae) aresupported in addition to or above distinct sequencing execute prediction methodologies on yeast and humans. The GeneMANIA prediction algorithmic program's excellent accuracy, Associate in Nursing intuitive computer programmers, and vast knowledge make sequence MANIA a helpful tool for any scientist (**Rashad *et al.*, 2021**).

Four yeast inquiries are being displayed by GeneMANIA (Fig. 12). The networks that result are completely distinct, with various totally differentabsolutely different relationships and four separate relevant genes in yeast that are linked by a pathway to the query list. Physical interaction (48.13%), co-expression (6.89%), Predicted (4.47%), co-localization (2.10%), other (1.02%), genetic interaction (36.83%), Shared protein domains (0.34%), Pathway (0.22%) and common macromolecule domains are some of the other levels of question customization (0.59 percent). GeneMANIA displays the results of gene queries in effects of Knowledge Set Choice on Topology. Mistreatment of all default parameters in the yeast default question. Mistreatment default network weight approach, which is a yeast default question. YKOs lacking genes that are similar to cancer genes in human were chosen. The ability to predict protein-protein interactions in yeast and human could lead to an assessment of the degree of deliberate similarity of some cancer-related genes between the two organisms.

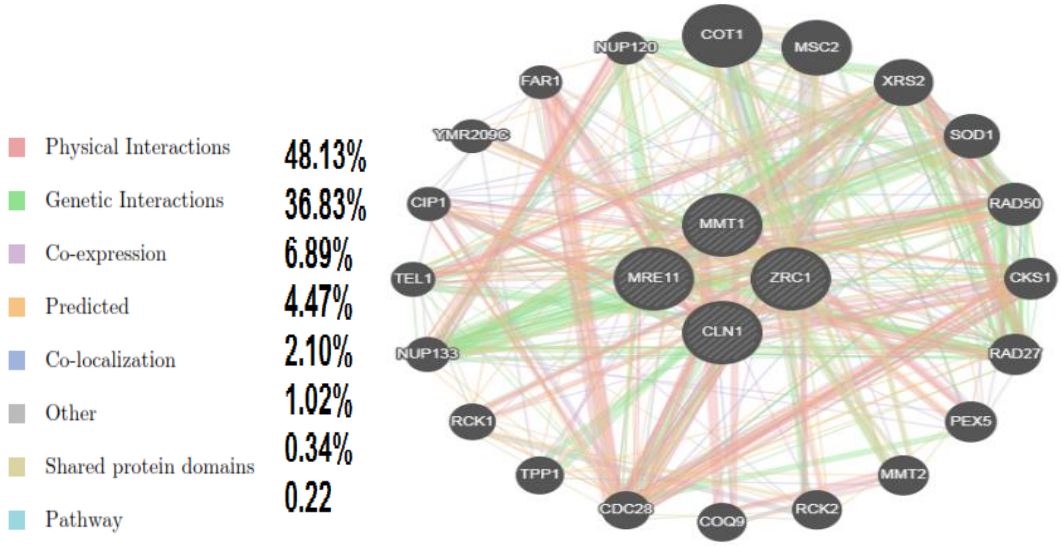


Fig. 12: The yeast cell-cycle default query with all default parameters. The yeast cell-cycle default query with all default parameters. Using the default network weighting approach, the yeast cell-cycle default query.

Four yeast inquiries are being displayed by Gene MANIA (Fig. 13). The networks that result are completely distinct, with various totally different absolutely different relationships and four separate relevant genes in yeast that are linked by a pathway to the query list. Physical interaction (48.13%), co-expression (6.89%), Predicted (4.47%), co-localization (2.10%), other (1.02%), genetic interaction (36.83%), Shared protein domains (0.34%), Pathway (0.22%) and common macromolecule domains are some of the other levels of question customization (0.59 percent). Gene MANIA displays the results of gene queries in Effects of Knowledge Set Choice on Topology. Mistreatment of all default parameters in the human default question. Mistreatment default network weight approach, which is a human default question. YKOs lacking genes that are similar to cancer genes in humans were chosen. The ability to predict protein-protein interactions in yeast and human could lead to an assessment of the degree of deliberate similarity of some cancer-related genes between the two organisms.

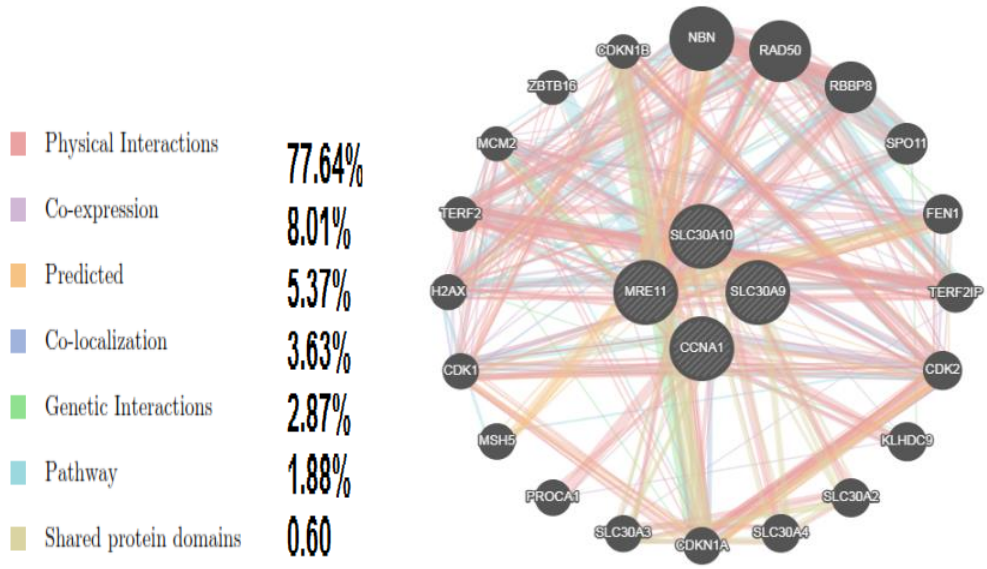


Fig. 13: The human default query, using all default parameters. The human default query, using default network weighting method

4. Conclusions

In similar to **Yuan *et al* (2012)** the cell viability of A549 first increased and then decreased with increasing zinc concentration, the turning point occurring at 50 mM $ZnSo_4$. A higher zinc concentration (≥75 mM) finally decreased theA549 cell viability. Also **Wang *et al* (2013)** indicted the cell viability of HepG2 decreased with increasing $ZnSo_4$ concentration, when treated for 48 h. **Wang *et al* (2016)** Viability of MDAMB231 cells decreased to ~80 % after being treated with 50 µM $ZnSo_4$ for24h,The significant increase on intracellular zinc content in $ZnSo_4$ -treated cells promoted cell death**.** **Zhao *et al* (2015)** showed an elevated $ZnSo_4$ concentrations reduced A549 cell viability, although the viability of A549 cells remained about 50% and 20% after treatment with 500 µM $ZnSo_4$ for 9 and 24 h, respectively. Results in this investigation are in parallel with those obtained by **Cui *et al.* 2002**. They found that the cell cycle progression of HepG2 cells was readily altered by depressed intracellular zinc status. An elevated percentage of the ZD cells were found to be in G1 phase, and the proportion ofS phase cells was markedly reduced by zinc depletion. It was suggested that zinc is critical for the progression of HepG2 cells from G1 to S phase. However, the mechanism of how zinc depletion impairs the G1-to-S phase transition remains unclear.

Consistent with previous studies in HepG2 cells, zinc depletion led to a reduction in DNA content per plate. **Libin *et al.* 2002** stated that addition of only 0.4 M zinc significantly restored the DNA content per plate, indicating that minimal changes of cellular zinc status have profound influence on cell proliferation and DNA synthesis. On the other hand, the zinc depletion-reduced DNA content in zinc-depleted HepG2 cells may also reflect the possibility that some of these cells were undergoing apoptosis (**Nakatani *et al* 2000**).

Flow cytometry analysis caused inhibition of the rate of liver cancer (HepG2) cell viability, it was necessary to assess cytotoxic effect of food additives on cell cycle arrest based cell cycle distribution. The results showed significant accumulation of HepG2 cells in G2/M phase, and confirmed that additives has cytotoxic effect via induction of G2/M phase arrest of the cell cycle (**Rashad *et al*., 2022**).

Kocdor *et al.*, (2015) zinc showed cytotoxicity in p53-wild lung cancer cells but not in null cells at different supra physiological concentrations. Suggested that many cytotoxic molecules induce mitotic cell death (apoptosis) which occurs in parallel with G2/M arrest.

Rashad *et al.*, 2018 The quantitative real time-PCR was used to measure the mRNA levels of *p53, Bax, and Bcl-2* genes. The data showed that food additives changed transcriptional levels of these related genes. The mRNA expression of *p53* and *Bax* were up-regulated, but, the transcription of *Bcl-2* wassignificantly down-regulated compared to the control.

According to the present data, $ZnSo_4$ led to *p53* activation and *Bcl-2* reduction which then activated mitochondria-mediated downstream molecular events including activation of caspase3. In conclusion, $ZnSo_4$ can effectively induce apoptosis of HepG2 cells. The induction of apoptosis by $ZnSo_4$ involved the activation of a mitochondria-mediated caspase cascade and the inhibition of the anti-apoptotic protein *Bcl-2*. Also $ZnSo_4$ at (50, 75, 100 µg/ml) considered as cytotoxic for HepG2 cells but not for normal Wi-38, Higher than this concentration decreased cell viability in malignant and non-malignant cells as well as confirmed the occurrence of their cytotoxic effects.

5. Conflicts of interest

"There are no conflicts to declare".

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