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ORIGINAL ARTICLE

Molecular studies on the effect of Ivermectin and hydroxychloroquine utilized for the treatment of COVID-19 on Albino Rat

[1]Asmaa I. Bayomi, [1]Sobhy Hassab El-Nabi, [1]Islam M. El-Garawani, [1]Shimaa H. Roshdy, [2]Samah El-Ghlban*

[1]Zoology Department, Faculty of Science, Menoufia University, Menoufia, Egypt.
[2]Biochemistry Division, Department of Chemistry, Faculty of Science, Menoufia University, Shebin El-kom, Egypt

Key words:
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Corresponding Author:
Samah El-Ghlban,
Biochemistry Division,
Department of chemistry,
Faculty of science, Menoufia University, Shebin El-Kom, Egypt.
Tel. no: +201062621920
S_elghlban@yahoo.com

ABSTRACT

Background*: COVID-19 infection can cause severe disease for which currently no specific therapy is available.* ***Objective:*** *The present study was to evaluate the molecular effects of Ivermectin (IVM) and hydroxychloroquine (HCQ) as a drug for COVID-19 on different organs of albino rats.* ***Methodology:*** *Forty eight albino rats were divided into three groups as the following: distilled water rats; 50 mg/kg oral injection of Hydroxychloroquine and 0.4 mg/kg oral injection of Ivermectin.* ***Results:*** *There was a significant reduction in kidney and liver intact DNA content with both doses of Hydroxychloroquine and Ivermectin. Additionally, the flow cytometry analysis results indicated a considerable rise in the number of apoptotic cells with the treatment of IVM and HCQ. Gene expression was assessed by RT-PCR technique the results demonstrated a significant decrease in P53 and Bcl2 gene expression in rats treated with hydroxychloroquine and ivermectin.* ***Conclusion****: The findings of this investigation indicate both hydroxychloroquine and ivermectin significantly decrease the kidney DNA. IVM and HCQ therapy led to G1/S phase cell cycle arrest, which partially explains the decreased proliferation. Furthermore, the study's flow cytometry results showed that both IVM and HCQ significantly increased the number of apoptotic cells.*

INTRODUCTION

The coronavirus illness 2019 (COVID-19), which is brought on by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has spread around the globe since the December 2019 pandemic in China. While the majority of COVID-19 patients only show mild symptoms, some develop severe pneumonia[1].

Reports from the World Health Organisation[2] predict that from January 2020 to June 2021, there were 276,756 confirmed cases of COVID-19 in Egypt. Ivermectin, paracetamol, and hydroxychloroquine were included in the Ministry of Health's protocol in Egypt for the treatment of mild cases that just needed home care and isolation, as well as moderate instances that would need hospitalization (MOHP,2020). Ivermectin, or 22–23-dihydroavermectin B, is a macrolide antibiotic derived from streptomyces avermitilis, which was originally identified from a soil sample in Japan. It is a semisynthetic macrocyclic lactone. A member of the avermactine family, ivermectin is an acaricide[3]. One of the most successful medications for the treatment of parasite infections in both human and veterinary medicine is ivermectin (IVM). Ivermectin (IVM) is a crucial medication[4]. One of the avermectin groups is ivermectin. The fermentation of an actinomycete called *Streptomyces avermitilis*, which was discovered from soil samples in Japan, produces a class of chemically similar anthelmintics known as avermectins[5]. There isn't a specific treatment for COVID-19 available yet. According to Gautret et al.[6], the antimalarial medications chloroquine and hydroxychloroquine have been suggested as therapeutic agents. It was shown that these medications prevented the SARS-CoV-2 virus from replicating in monkey cells in vitro[7]. Nevertheless, there is currently no proof that these medications can impact viral replication in vivo in people[8]. Additionally, the immunomodulating qualities of both hydroxychloroquine and chloroquine may affect the progression of COVID-19 illness[9]. However, there is conflicting information regarding the effectiveness of chloroquine and hydroxychloroquine in treating COVID-19, and their route of action is not well understood [10]. The original purpose of chloroquine was as an antimalarial medication. Later on, it was found to be beneficial in treating a number of rheumatological illnesses, such as RA, SLE, and other inflammatory and dermatological disorders. The therapeutic and toxicological characteristics of these drugs are comparable[11]. According to studies by Michaelides et al.[12], hydroxychloroquine and chloroquine are related medications with varying hazardous and therapeutic

dosages. When taken at the prescribed therapeutic levels, chloroquine is a safe medication. The majority of severe toxicity follows an unintentional or deliberate overdose. An adult can become toxic after ingesting 1–1.5 g (20 mg/kg), and 5 g has the potential to be fatal[13]. Our goal was to examine the genetic impact of medications like ivermectin and hydroxychloroquine, which are used to treat Covid infection.

METHODOLOGY

Experimental animals

At the onset of the tests, forty-eight male *Rattus norvegicus* albino rats, weighing 160±10 grams were originally acquired from the Biological Products and Vaccines Authority (BOVA-VACSERA), Egypt. Rats were kept in normal laboratory cages at Menoufia University's animal house in the Zoology Department of the Faculty of Science. They were fed commercial pelleted diet. Rats were housed in the lab for one week prior to the start of the tests to allow them to acclimatize to the settings. There were three groups of rats. The current study closely followed the National Institutes of Health's standards for the use and care of laboratory animals (NIH Publications No.8023, received 1978) and Menoufia University's Faculty of Science in Egypt (Approval No.MN S GE 4 24). We also followed these guidelines when conducting our research.

Drugs

a- Hydroxychloroquine:

The comversional pills containing hydroxychloroquine sulphate (HCQ) (Molecular Formula: C18H26ClN3O.H2O4S) were procured from Menoufia pharmacy, Shebin El-Kom, Menoufia Government, Egypt. The tablets are manufactured by MINAPHARM for Pharmaceutical and Chemical Industries Company, 10 Ramadan City, Egypt.

Freshly made HCQ tables (200 mg each tablet) were ground and given to rats in five doses, each equal to 50 mg/kg body weight, over the course of five days [14].

b-Ivermectin:

The manufacturer of iverzine pills is Universal Pharmaceutical Co. (UNIPHARMA), located in AL Obour City-1 Industrial region, Egypt. The ivermectin tablets were made right away. According to Arise and Melomo, [15], tablets (6 mg each) were ground and given to rats in 15 doses, each of which was 0.4 mg/kg. Other Chemical were purchased from Sigma company

Experimental Groups:

- Group I: Twenty-four rats served as the normal control (NC).
- Group П: Hydroxychloroquine (50 mg/kg) was administered orally to twelve rats for five days.
- Group Ш: consisted of Twelve rats that received an oral injection of Ivermectin (0.4 mg/kg) for fifteen days.

Electrophortic pattern of nucleic acids (DNA)

- Extraction of Whole Genomic DNA and Identification of Apoptosis in Liver and Kidney Tissue**:**

For DNA extraction and apoptosis detection (DNA fragmentation assay), the salting out extraction method of Aljanabi and Martinez[16] was used, with modifications made by El-Nabi and Elhassaneen[17].

Flow cytometric analysis of the distribution of the cell cycle.

Both treated and control cells' cell cycle distribution and DNA content were evaluated using flow cytometry after propidium iodide (PI) labeling. Cells were trypsinised after varying treatment times, twice cleaned with PBS, and then fixed for two hours in cold ethanol (70%). After removing all traces of alcohol, cells were gathered and given a PBS wash for five minutes at 1,200 rpm. For two hours at 37°C, 50 µg/mL of RNase A was added to cells in PBS. Then, they were combined with 25 µg/mL of PI stain as directed by the manufacturer [18,19] and examined on a Becton Dickinson FACS flow cytometer (United States). The cell cycle phase studies were carried out with the use of BD FACS Diva software. All reagents utilized were from Sigma-Aldrich in Germany.

Reverse transcription polymerase chain reaction (RT-PCR):

RNA extraction

Cytokines P53 and BCL2 exhibit elevated expressions during the inflammatory process. their expressions were investigated using RNA-real-time PCR in hepatic tissue. All groups' homogenized tissues had their total RNA extracted using the Direct-zol RNA Miniprep Plus (Cat# R2072, ZYMO RESEARCH CORP. USA). Additionally, the amount and quality of the isolated RNA were measured using the Beckman dual spectrophotometer (USA).

Real time PCR:

PCR was carried out after the extracted RNA was reverse-transcribed using the SuperScript IV One-Step RT-PCR kit (Cat# 12594100, Thermo Fisher Scientific, Waltham, MA, USA). In the following thermal profile, a 48-well plate StepOne instrument (Applied Biosystem, USA) was utilised: Reverse transcription takes place for 10 minutes at 45 ºC, RT inactivation takes place for 2 minutes at 98 ºC, and initial denaturation is carried out using 40 cycles of 10 seconds at 98 ºC, 10 seconds at 55 ºC, and 30 seconds at 72 ºC for the amplification phase. Following the RT-PCR, the target gene and housekeeping gene data were expressed as Cycle threshold (Ct). Using the ΔΔCt method, normalisation for variance in target gene expression was carried out for P53 and BCL2, with reference to the mean critical threshold (Ct) expression values of the housekeeping gene GAPDH. Every target gene's relative quantitation (RQ) is measured using the -ΔΔct method of calculation.

Table 1: Primers sequence of all studied genes:

Gene	Forward sequence	Reverse sequence	Gene accession number
BCL2	TGATAACCGGGAGATCGTGA	AAAGCACATCCAATAAAAAGC	NM_016993.1
P53	TGGGTCACCTCCACACCTCC	GGATGTTGCAGAGTTGTTAG	XM_032912337.1
GAPDH	CACCCTGTTGCTGTAGCCATATTC	GACATCAAGAAGGTGGTGAAGCAG	XM_017592435.1

Statistical analysis

The Mean ± Standard error (M ± SE) is used to display the data. The groups that received and did not receive treatment were compared.

In each experiment, the significance of the differences between the mean values of the experimental and control groups was determined at a level of significance of $P \leq 0.05$ using the Student's t-test for normally-distributed data[20].

RESULTS

Electrophortic pattern of nucleic acids (DNA)

The hepatic and renal intact DNA of normal rats treated with hydroxychloroquine for five days demonstrated a significant decrease in the mean value of maximal optical density (128±0.49 and 144±1.49, respectively) when compared to the normal control group (148.8±0.96 and 158±1.56, respectively), as shown in table (2) and figure (1). The mean maximal optical density of fragmented DNA was significantly higher than that of the normal control group (70±0.54 and 60±0.64, respectively), with values of 95±0.78 and 88±0.83, respectively. The maximal optical density of intact DNA in normal rat liver and kidney treated with Ivermectin for 15 days showed a significant decrease in mean values (135±1.41 and 144±1.6, respectively) when compared to the normal control group (156±1.64 and 158±1.1, respectively) as shown in table (3) and figure (2). As opposed to the normal control group, which had mean maximal optical densities of 80±0.54 and 58±0.94, respectively, fragmented DNA had a markedly higher mean maximal optical density of 109±0.39 and 17±0.38, respectively.

Table 2: Mean of maximal optical density of DNA apoptotic fragments of normal rats treated with Hydroxychloroquine for 5days. liver; kidney.

Mol. Wt. (bp) / Groups	Intact DNA (Mean ± SE)	DNA fragmentation (Mean ± SE)
Liver		
Normal control	148.8±0. 96	70±0.54
Normal+ treated with hydroxychloroquine liver	128±0.49*	95±0.78*
Kidney		
Normal control	158±1.56	60±0.64
Normal+ treated with hydroxychloroquine kidney	144±1.49*	88±0.83*

The number of rats/group=12. The normal Rats treated with Hydroxychloroquine for 5days.

Mean ± Standard error (SE) is used to express the data, and * indicates a significant difference from the normal control group at $P \leq 0.05$.

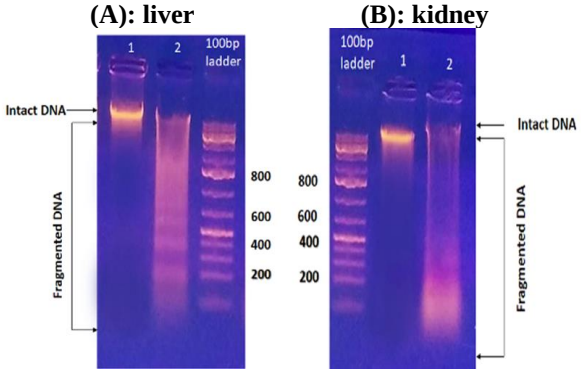

Fig. 1: Mean of maximal optical density (MOD)of apoptotic fragments DNA of normal control rats treated with Hydroxychloroquine for five days for**(A)** liver; **(B)** kidney. Lane 1: normal rats & lane 2: rats treated with Hydroxychloroquine.

Table 3: Mean of maximal optical density of DNA apoptotic fragments of normal control rats treated with Ivermectin for 15 days for liver; kidney.

Mol. Wt. (bp) / Groups	Intact DNA (Mean ± SE)	DNA fragmentation (Mean ± SE)
Liver		
Normal control	156±1.64	80±0.54
Normal+ treated with Ivermicten liver	135±1. 41*	109 ±0.39*
Kidney		
Normal control	158±1.1	58±0.94
Normal+ treated with Ivermicten kidney	144±1.6*	17±0.38*

The number of rats /group=12. The normal Rats treated with Ivermectin for15 days.

Mean ± Standard error (SE) is used to express the data, and * indicates a significant difference from the normal control group at P≤ 0.05.

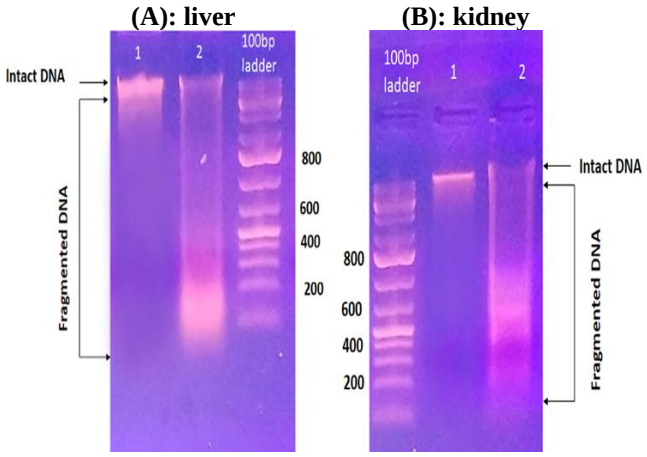

Fig. 2: Mean of maximal optical density(MOD)of apoptotic fragments DNA of normal control rats treated with Ivermectin for 15 days for **(A)** liver ;**(B)** kidney. **Lane 1**: normal rats & **lane 2**: rats treated with Ivermectin.

Flow cytometry analysis

Figures 3&4 show the impact of hydroxychloroquine and ivermectin treatment on liver cells utilising flow cytometric examination of the cell cycle distribution in both treated and normal rats. The proportion of apoptosis in rats treated with hydroxychloroquine for 5 days and ivermectin for 15 days was significantly higher (P≤0.05) than in rats treated with standard medication. The mean percentage value G0/1 in normal rats treated with hydroxychloroquine showed a significant decrease to 46.86± 1.71 from the normal value of 56.48± 1.14. Rats treated with ivermectin had a higher mean percentage number of G0/1 (61.9±1.47) in contrast to normal rats (56.48±1.14). However, compared to the normal rat value of 1.03±0.088, the mean percentage of S phase in the treated Ivermectin-treated rats shows a substantial increase of 1.41±.158. However, as indicated by figures 3, normal rats treated with hydroxychloroquine and ivermectin respectively demonstrated a significant drop in the mean percentage value of G2/M with values of 17.7±1.43 and 3.3±0.551, compared to normal rats (38.1±1.05).

Results of RT-PCR:

The RT-PCR method was used to measure gene expression; the results are displayed in Figure 5. The hydroxychloroquine-treated rats' P53 and Bcl2 gene expression levels were significantly lower than those of the control group, according to the findings. Additionally, rats given ivermectin demonstrated decreased Bcl2 and P53 expression.

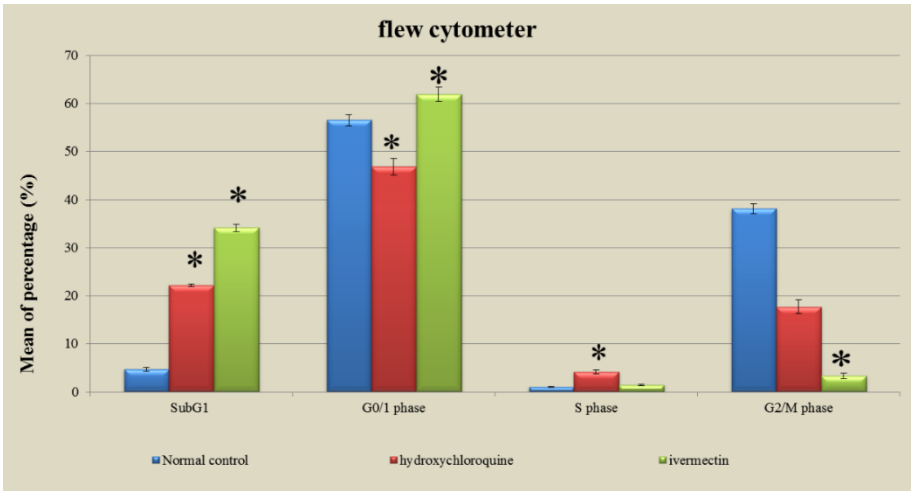

Fig. 3: Mean of percentage (%)value of flow cytometer for normal control rats liver treated with Hydroxychloroquine for 5days and another normal Rats treated with Ivermectin for 15 days.
Mean ± Standard error (SE) is used to express the data, and * indicates a significant difference from the normal control group at P≤0.05.

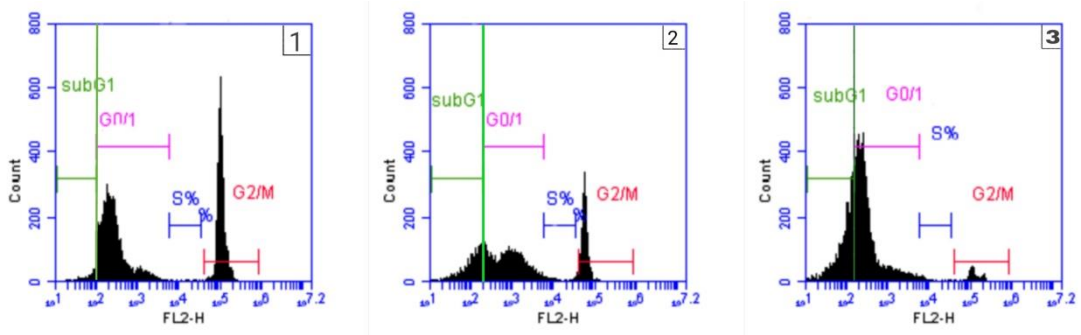

Fig. 4: Mean of percentage (%) value of flow cytometer for **(1)** normal control rats liver **(2)** treated rats liver with Hydroxychloroquine and **(3)** normal Rats treated with Ivermectin.

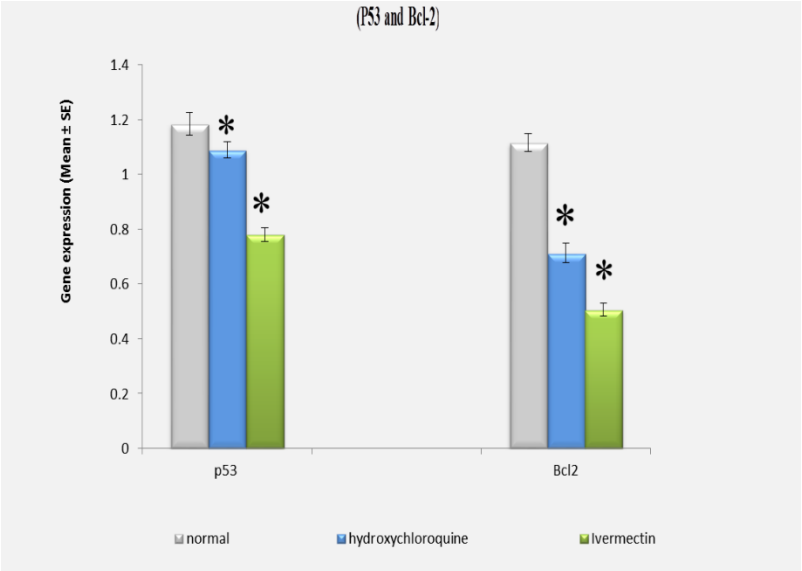

Fig. 5: Mean of (P53 and Bcl-2) genes expression in normal control rats liver treated with Hydroxychloroquine for 5days and another normal Rats treated with Ivermectin for 15 days.

DISCUSSION

Global human health was significantly impacted by COVID-19, which was caused by SARS-CoV-2. WHO statistics suggest that from January 2020 to June 2021, there were 276,756 confirmed cases of COVID-12 in Egypt[21]. Ivermectin, paracetamol, and hydroxychloroquine were included in the Egyptian Ministry of Health's protocol for treating mild cases that just needed home care and isolation, as well as moderate cases that would need hospitalization. One of the most useful medications in both human and veterinary medicine for the treatment of parasite infections is ivermectin (IVM). Among the most significant is ivermectin (IVM) [22].

According to Liu et al.[23], CQ and HCQ have comparable chemical structures and cellular modes of action. Whereas HCQ is provided as a sulphate, CQ is administered as a phosphate salt. The upper gastrointestinal system absorbs both medications[24]. Acidic intracellular organelles like lysosomes and endosomes, which need a low pH for maturation and function, can have their pH raised by both CQ and HCQ, which are weak bases [25].

In terms of molecular research, the current study showed that rats given hydroxychloroquine for five days had far higher liver DNA fragmentation than the control group. Conversely, there was a notable reduction in DNA fragmentation in the kidney as compared to normal rats. This finding is supported by Farombi's[26] study, which showed that CQ might cause purine and pyrimidine base oxidation as well as DNA strand breakage in rat liver cells. Additionally, they suggest that some free radical scavengers may be able to lessen the oxidative DNA-damaging effects of CQ in rat liver cells, which would explain the drug's genotoxic impact. Chemicals, especially medications that release oxidants, have the ability to cause possibly mutagenic DNA damage by the direct action of reactive oxygen species (ROS) on DNA or indirectly through the breakdown products of aldehydic lipid per oxidation[27]. Previous studies have demonstrated that CQ interferes with the intracellular GSH balance to cause oxidative stress and hepatotoxicity[28]. In vitro investigations on mammalian systems, reports on patients with rheumatoid or aplastic anemia treated with CQ, and multiple test systems investigations (i.e, Wistars rats, mouse bone marrow cells, and African common toad) in terms of chromosomal aberrations, sister-chromatid exchange, sex-linked recessive lethal, DNA damage, inhibition of DNA repair, micronuclei formation, and genesis of tumors (lymphosarcomas, myeloblastic leukemia) were all used to demonstrate the mutagenic, genotoxic, carcinogenic, and co-carcinogenic effects of CQ [29]. Conversely, when compared to the normal control group, the Ivermectin-treated group had a substantial drop in intact DNA of the liver and kidney tissues, while fragment DNA (200–400 bp) demonstrated a significant rise in the amount of rna tissue lysate. This finding is supported by Qureshi[30], who discovered that ivermectin medication drastically decreased the amounts of DNA in the liver. Ivermectin was found to inhibit the hepatic contents of DNA and RNA based on data on nucleic acids. Ivermectin and ivomec-induced genotoxicity and cytotoxicity in Chinese hamster ovary cells have been confirmed in vitro by[31]. Ivermectin's action on nucleic acids is thought to be due to the catecholamine and GABA receptor stimulation that produces cAMP[32]. Lauterburg and Mitchell[33] proposed that the decrease of the intrahepatic free glutathione/mixed disulphide ratio is mediated by cAMP. The detrimental effects of cAMP on DNA synthesis, repair, and integrity have also been demonstrated in earlier studies[34] The suppression of nucleic acids reported by Bemba-Meka et al.[35] could perhaps due to the generation of free radicals resulting from disruptions in chloride and Ca2+ homeostasis produced by ivermectin.

RTPCR results showed that rats treated with hydroxychloroquine expressed significantly less of the P53 and Bcl2 genes than the normal control group. These findings are consistent with those of Lagneaux et al.[36], who state that HCQ activates caspase-3 and downregulates Bcl-2 to cause apoptosis in B-CLL cells. They also suggest that HCQ may provide a novel therapeutic option for the management of B-CLL patients.

Additionally, compared to normal rats, rats treated with ivermectin displayed decreased expression of Bcl2 and P53. Additionally, Ping Zhang et al. [37] concur with our findings that IVM decreased the expression of the apoptosis protein Bcl-2. First, Xu et al.[38] simultaneously assessed the expression levels of two proteins linked to apoptosis, Bax and Bcl-2, and calculated the ratio of Bax/Bcl-2. After receiving ivermectin treatment, the pro-apoptotic factor Bax's expression was markedly increased, whilst Bcl-2, an anti-apoptotic component expression was markedly reduced in comparison to the control group. By downregulating Bcl-2 and upregulating p53 and Bax expression, IVM induced apoptosis, which resulted in the release of cytochrome c. Dandan Song and others [39]. It is commonly recognised that the p53 gene, a tumour suppressor gene closely linked to the development and spread of numerous human tumours, primarily triggers the apoptosis of tumour cells. Wawryk et al.[40]. Two essential proteins, proapoptotic protein (Bax) and antiapoptotic protein (Bcl-2), both of which are members of the Bcl-2 family, control apoptosis.

As everyone is aware, the apoptosis process may be separated into two main categories using flow cytometry analysis: the intrinsic pathway, which is mediated by mitochondria, and the extrinsic pathway,

which is mediated by death receptors [41]. The proportion of apoptosis in rats treated with ivermectin for 15 days and hydrochloroquine for 5 days was much higher than in normal rats, according to the results. This conclusion is consistent with that of Zhang et al.[37], who discovered that IVM treated with flow cytometry analysis showed a significant increase in apoptotic cells. According to Xu et al.[38], ivermectin therapy dramatically reduces the number of cells in the S and G2/M phase and increases the population of G1. These research results corroborated those of Izunya et al.[42], who discovered that prolonged oral chloroquine therapy may result in nuclear enlargement, cytoplasmic vacuolation, and cell death. By using a lysosomal mechanism, hydroxychloroquine (HCQ) caused apoptosis in cells. The death of the HCQ-induced cells was preceded by signs of "type II cell death," such as increased autophagy, organellar sequestration in autophagosomes, and cytoplasmic vacuolization, and was later followed by signs of "type I cell death," such as chromatin condensation and caspase activation. Boya et al.[43]. When CQ is applied to a colony of human lymphocytes at stage G1, it suppresses cell mitotic function at concentrations of 60 and 100 μg/ml, according to Shalumashvili and Sigidin's [44] research.

CONCLUSION

In conclusion, Both Ivermectin and hydroxychloroquine-treated rats' P53 and Bcl2 gene expression levels were significantly lower. Finally, both Hydroxychloroquine and Ivermectin significantly increase the number of apoptotic cells.

Declarations:

Consent for publication: Not applicable

Availability of data and material: Data are available upon request.

Competing interests: The author(s) declare no potential conflicts of interest with respect to the research, authorship and/or publication of this article.

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REFERENCES

1. Wang M, Cao R, Zhang L, Yang X, Liu J, Xu M, Xiao G. Remdesivir and chloroquine effectively inhibit the recently emerged novel coronavirus (2019-nCoV) in vitro. Cell research. 2020;30(3), 269-271.
2. World health organization: Egypt: WHO corona virus disease dashboard. 2021.
3. Chavasse DC, Post RJ, Lemoh PA, Whitworth J. A. The effect of repeated doses of ivermectin on adult female Onchocerca volvulus in Sierra Leone. Tropical medicine and parasitology: official organ of deutsche tropenmedizinische gesellschaft and of deutsche gesellschaft fur technische zusammenarbeit (gtz) .1992; 43(4), 256-262.
4. Laing R, Gillan V, Devaney E. Ivermectin–old drug, new tricks?. Trends in parasitology. 2017; 33(6), 463-472.
5. Burg RW, Miller BM, Baker EE, Birnbaum J, Currie SA, Hartman R, Ōmura S. Avermectins, new family of potent anthelmintic agents: producing organism and fermentation. Antimicrobial agents and Chemotherapy.1979;15(3), 361-367.
6. Gautret P, Lagier JC, Parola P, Meddeb L, Mailhe M, Doudier B, Raoult D. Hydroxychloroquine and azithromycin as a treatment of COVID-19: results of an open-label non-randomized clinical trial. International journal of antimicrobial agents.2020;56(1), 105949.
7. Wang M, Cao R, Zhang L, Yang X, Liu J, Xu M, Xiao G. Remdesivir and chloroquine effectively inhibit the recently emerged novel coronavirus (2019-nCoV) in vitro. Cell research.2020;30(3), 269-271.
8. Savarino A, Boelaert JR, Cassone A, Majori G, Cauda R. Effects of chloroquine on viral infections: an old drug against today's diseases. The Lancet infectious diseases. 2003; 3(11), 722-727.
9. Schrezenmeier, E, Dörner, T. Mechanisms of action of hydroxychloroquine and chloroquine: implications for rheumatology. Nature Reviews Rheumatology.2020;16(3), 155-166.
10. Boulware DR, Pullen MF, Bangdiwala AS, Pastick KA, Lofgren SM, Okafor EC, Hullsiek KH. A randomized trial of hydroxychloroquine as postexposure prophylaxis for Covid-19. New England journal of medicine.2020;383(6), 517-525.
11. Pasadhika S, Fishman GA. Effects of chronic exposure to hydroxychloroquine or chloroquine on inner retinal structures. Eye.2010; 24(2), 340-346.
12. Michaelides M, Stover NB, Francis PJ, Weleber RG. Retinal toxicity associated with hydroxychloroquine and chloroquine: risk factors, screening, and progression despite cessation of therapy. Archives of ophthalmology.2011; 129(1), 30-39.
13. Riou B, Barriot P, Rimailho A, Baud FJ. Treatment of severe chloroquine poisoning. New England Journal of Medicine.1988; 318(1), 1-6.
14. Kaptein SJF, Jacobs S, Langendries L, et al. Favipiravir at high doses has potent antiviral activity in SARS-CoV-2-infected hamsters,

whereas hydroxychloroquine lacks activity. Proc Natl Acad Sci U S A. 2020;117(43):26955-26965. doi:10.1073/pnas.2014441117

15. Arise RO, Malomo SO. Effects of ivermectin and albendazole on some liver and kidney function indices in rats. Afr J Biochem Res.2009;3(5), 190-197.
16. Aljanabi SM, Martinez I. Universal and rapid salt-extraction of high quality genomic DNA for PCR-based techniques. Nucleic acids research. 1997;25(22), 4692–4693.
17. El-Nabi SE, Elhassaneen Yousif. Detection of DNA Damage, Molecular Apoptosis and Production of Home-Made Ladder by Using Simple Techniques. Biotechnology.2008; 7. 10.3923/biotech.2008.514.522.
18. Dassonneville L, Lansiaux A, Wattelet A, Wattez N, Mahieu C, Van Miert S, Pieters L, Bailly C. Cytotoxicity and cell cycle effects of the plant alkaloids cryptolepine and neocryptolepine: relation to drug-induced apoptosis. European journal of pharmacology.2000;409(1), 9–18.
19. Dengler R, Münstermann U, al-Batran S, Hausner I, Faderl S, Nerl C, Emmerich B. Immunocytochemical and flow cytometric detection of proteinase 3 (myeloblastin) in normal and leukaemic myeloid cells. British journal of haematology. 1995; 89(2), 250–257.
20. Mc-Clave, J. T. and Dietrich, F. H.: Statistics. 4th Edition, Dellien Publishing Company, a division of Macillan publishing company. New York. 1991.
21. World health organization: Covid19- Global .Available from 2020.Https://www.who.int/emergencies/disease-Outbreak-news/item/2020-DON305
22. Laing, R, Gillan, V, & Devaney, E. (2017). Ivermectin–old drug, new tricks?. Trends in parasitology, 33(6), 463-472.
23. Liu J, Cao R, Xu M, Wang X, Zhang H, Hu H, Wang M. Hydroxychloroquine, a less toxic derivative of chloroquine, is effective in inhibiting SARS-CoV-2 infection in vitro. Cell discovery.2020;6(1), 16.
24. Schrezenmeier E, Dörner T. Mechanisms of action of hydroxychloroquine and chloroquine: implications for rheumatology. Nature Reviews Rheumatology.2020; 16(3), 155-166.
25. Kumar R, Srivastava JK, Singh R, Siddiqui MH, Mansouri RA, Abdulhakim JA, Uddin MS. Available compounds with therapeutic potential against COVID-19: antimicrobial therapies, supportive care, and probable vaccines. Frontiers in pharmacology. 2020;11, 582025.
26. Farombi EO. Genotoxicity of chloroquine in rat liver cells: protective role of free radical scavengers. Cell biology and toxicology.2006;22, 159-167.
27. Collins AR. Oxidative DNA damage, antioxidants, and cancer. Bioessays.1999; 21(3), 238-246.
28. Olatunde Farombi E, Shyntum YY, Emerole GO. Influence of chloroquine treatment and Plasmodium falciparum malaria infection on some enzymatic and non-enzymatic antioxidant defense indices in humans. Drug and chemical toxicology.2003;26(1), 59-71.
29. Giri, A, Das, A, Sarkar, AK, Giri AK. Mutagenic, genotoxic and immunomodulatory effects of hydroxychloroquine and chloroquine: a review to evaluate its potential to use as a prophylactic drug against COVID-19. Genes and Environment.2020;42(1), 25.
30. Qureshi S. Biochemical toxicity of ivermectin in Wistar albino rats. Am-Eur J Toxicol Sci.2013;5(1), 15-9.
31. Molinari GM, Kujawski A. Scuto S. Soloneski and m.L. Larramendy. DNA damage kinetics andapoptosis in ivermectin-treated chinese hamsterovary cells. Journal of Applied Toxicology(wileyonlinelibrary.com) .2012; doi: 10.1002/jat.2782.
32. Vlahovlć P, Stefanović V. Effect of dopamine on ecto-5′-nucleotidase expression in human glomerular mesangial cells. Archives internationales de physiologie, de biochimie et de biophysique.1994; 102(3), 171-173.
33. Lauterburg, B.H. and J.R. Mitchell. In vivo regulation of hepatic glutathione synthesis effects of food deprivation or glutathione depletion by electrophillic compounds. Advances in experimentalmedicine and Biology. 1981;136(pt A): 453-461.
34. Qureshi, S, Tariq, M, El-Feraly, F. S, Al-Meshal, I. A. Genetic effects of chronic treatment with cathinone in mice. Mutagenesis.1988; 3(6), 481-483.
35. Payne GT, Soderlund DM. Activation of γ-aminobutyric acid insensitive chloride channels in mouse brain synaptic vesicles by avermectin B1a. Journal of biochemical toxicology.1991; 6(4), 283-292.
36. Kellner U, Rudolph P, Parwaresch R. Human DNA-topoisomerases–diagnostic and therapeutic implications for cancer. Oncology Research and Treatment.2000; 23(5), 424-430.
37. Dassonneville, L, Lansiaux, A, Wattelet, A, Wattez, N, Mahieu, C, Van Miert, S, Bailly, C. Cytotoxicity and cell cycle effects of the plant alkaloids

cryptolepine and neocryptolepine: relation to drug-induced apoptosis. European journal of pharmacology. 2000;409(1), 9-18.

38. Zhang, P, Zhang, Y, Liu, K, Liu, B, Xu, W, Gao, J, Tao, L. Ivermectin induces cell cycle arrest and apoptosis of HeLa cells via mitochondrial pathway. Cell Proliferation. 2019; 52(2), e12543.
39. 39. Xu, N, Lu, M, Wang, J, Li, Y, Yang, X, Wei, X, Bao, D. Ivermectin induces apoptosis of esophageal squamous cell carcinoma via mitochondrial pathway. BMC cancer. 2021;21, 1-11.
40. Song, D, Liang, H, Qu, B, Li, Y, Liu, J, Zhang, Y, Gao, A. Ivermectin inhibits the growth of glioma cells by inducing cell cycle arrest and apoptosis in vitro and in vivo. Journal of Cellular Biochemistry. 2018;doi:10.1002/jcb.27420
41. Wawryk-Gawda E, Chylińska-Wrzos P, Lis-Sochocka M, et al P53 protein in proliferation, repair and apoptosis of cells. Protoplasma. 2013; 251(3):525-533
42. Letai, A. Apoptosis and cancer. Annual Review of Cancer Biology.2017; 1(1), 275-294.
43. Izunya, A. M, Nwaopara, A. O, Anyanwu, L. C, Odike, M. A. C, Oaikhena, G. A, Bankole, J. K, & Okhiai, O. Effect of Chronic Oral Administration of Chloroquine on the Histology of the Liver in Wistar Rats. British Journal of Pharmacology and Toxicology.2011; 2(3), 97-103.
44. Boya P, Gonzalez-Polo RA, Poncet D, Andreau K, Vieira HL, Roumier T, Kroemer G. Mitochondrial membrane permeabilization is a critical step of lysosome-initiated apoptosis induced by hydroxychloroquine. Oncogene.2003; 22(25), 3927-3936.