Alfarama Journal of Basic & Applied Sciences
https://ajbas.journals.ekb.eg
ajbas@sci.psu.edu.eg
Faculty of Science Port Said University
http://sci.psu.edu.eg/en/
ISSN 2682-275X July 2022, Volume 3 Issue II DOI:10.21608/AJBAS.2022.118007.1085
Submitted: 24-01-2022
Accepted: 16-03-2022 Pages: 200-209

Measurement of the effect of 5-Fluorouracil as an anticancer drug on the nitric oxide levels on HepG2 cell line

Nabil M. Abdel-Hamid[1], Mohamed S. El- Gharieb [2], Fardous F. El- Senduny[3], Nadia A. Alnakib[2,*]

[1]Chemistry Department, Faculty of Pharmacy, Kafr-Elsheikh University
[2]Chemistry Department, Faculty of Sciences, Port-Said University
[3]Chemistry and Molecular Biology, Faculty of Sciences, Mansoura University
*** Corresponding author:** nadiaelnakib@yahoo.com

ABSTRACT

Background: Carcinoma of the liver is the sixth most frequent malignancy in humans and is accountable for more than 600,000 deaths annually. According to the statistics, hepatocellular carcinoma patients typically die within a year after being diagnosed. As 5-fluorouracil (5-FU), the primary chemotherapeutic agent for most different types of cancers, is now routinely monitored via therapeutic drug monitoring, it has contributed to better clinical results. Many studies indicated the role of nitric oxide (NO) in different cancers as a pro-neoplastic or anti-neoplastic effector, but its function remains unclear in hepatocellular carcinoma (HCC). **Aim of the study:** The study's overall purpose was to look into the efficacy and toxicities of 5- fluorouracil on the biological behavior of HepG2 cell line by the methyl thiazole tetrazolium (MTT) assay and evaluate nitric oxide levels in monitoring the progression of HCC. **Material and Methods:** HepG2 cell line was incubated with different concentrations of 5-Fluorouracil as a chemotherapy drug. The effects of 5-Fluorouracil on cell proliferation, morphology, and the half-maximal inhibitory concentration (IC50) values were determined by the methyl thiazole tetrazolium (MTT). **Results:** The IC50 values for 5-Fluorouracil in HepG2 cells was (12.92±0.085µg), moreover the NO levels in the HepG2 cell line treated with 5-Fluorouracil were measured. We found significant inhibition in NO levels after treating HepG 2 cell lines by 5-Fluorouracil. **Conclusion:** The change in NO levels following chemotherapy helps predict treatment response in HepG-2 cells.

Key Words:
5-Fluorouracil, Hepatocellular carcinoma, Nitric oxide, MTT assay, Proliferation assay.

1. INTRODUCTION

Because of cell lines' closeness to primary tissues, low cost, and ease of use and cultivation, they have revolutionized the scientific study. Huge efforts have been undertaken in recent decades to produce cell lines that express distinct liver functions. As a result, as an alternative to cultured hepatocytes, well-characterized human, as well as rodent liver-derived cell lines, were used in hepatology research [1]. Several human hepatoma cell lines, such as HepG2, Hep3B, HuH7, and HepaRG, are frequently employed in drug metabolism and hepatotoxicity research [2]. In pharmaco-toxicological research, HepG2 is the most commonly utilized human hepatoma cell line [3]. This cell line was created from biopsies taken from the liver of a 15-year-old Caucasian man who had a differentiated hepatocellular carcinoma. HepG2 cells are nontumorigenic, highly proliferating cells that have been grown in wide-scale

culture systems with success. When grown on a solid surface, they have an epithelial appearance, and under specific growth circumstances, polarisation of HepG2 cells can occur, with the production of bile canaliculi-like structures among adjacent cells [4].

A worldwide concern, hepatocellular carcinoma (HCC) has varying epidemiological statistics from place to place, yet the disease is a universal problem everywhere [5]. People in Egypt are the third most populous people in Africa and the 15th most globally [6]. HCC is seen as the most challenging health problem in Egypt by the government. During the last decade, the number of people with HCC rose two-fold [7]. The sixth most frequent cancer globally is hepatocellular carcinoma (HCC) [8]. It is Egypt's fourth most prevalent cancer type [6]. This form of cancer requires early discovery to be effectively treated [9]. Only a tiny percentage of individuals (between 20 and 30 percent) who are detected early enough can receive the most effective therapy [10]. Even in these situations, the prognosis and survival are unsatisfactory because the recurrence rate is greater than 70% five years after resection. Currently, available medications have failed to reduce this rate significantly [11].

HCC is linked to long-term liver problems, like cirrhosis, caused by factors like viral hepatitis, alcohol abuse, and metabolic steatohepatitis; this leads to a high rate of this neoplasia worldwide [12]. Despite the expansion of HCC, therapy remains difficult, owing to a high prevalence of late diagnosis and a lack of treatment options for advanced illness [13]. In reality, the most radical types of therapy, such as liver transplantation and major surgery, can only be used if the disease is diagnosed early [14]. Even local therapies, like transarterial chemoembolization, have a restricted number of indications, posing a significant difficulty in treating patients with advanced disease [15]. Systemic therapy is the only option in this condition, and 5- fluorouracil is one of the necessary standard treatments available [16].

Fluorouracil has an anticancer activity that can help fight breast, liver, colon cancers, and other solid tumors [17]. An analog of uracil called 5-fluorouracil has the same structure as uracil, but the hydrogen atom at position five has been replaced by fluorine [18]. After it turns into an active deoxynucleotide, it acts as an antimetabolite; this stops DNA synthesis by blocking turns of deoxyuridylic acid into thymidylic acid by thymidylate synthetase enzyme, which slows down tumors' growth [19]. Antimetabolites are analogous to the nucleotides found in DNA and RNA [20]. Because those medications are so close to natural substances found in the cells, they effectively fool the body into thinking they are biological nucleotide bases. Nevertheless, the cell is rendered incapable of dividing and replicating when they are metabolized, thereby halting growth. It is critical to understand that 5-FU is a pyrimidine antagonist [21]. This distinction indicates any specific natural material with which it interferes: in this case, pyrimidines consider the building blocks of DNA [22]. Moreover, 5-fluorouracil acts as a radiosensitizer, an immunosuppressive agent, and a xenobiotic [23].

5-Fluorouracil metabolism and mechanism of action, 5-FU penetrates cells through enhanced transportation, the same method used by uracil to enter cells. After that, 5-Fluorouracil (FU) is metabolized intracellularly to various active metabolites, including fluorodeoxyuridine monophosphate (FdUMP), fluorodeoxyuridine triphosphate (FdUTP), and fluouridine triphosphate (FUTP) [24]. 5-FU is converted to several metabolites that are active following intravenous delivery, disrupting the activity of thymidylate synthase (TS) and the synthesis of DNA/RNA, resulting in damage of DNA/RNA and cell death as shown in (Figure 1) [24]. 5-FU enters the cells and is transformed to 5-fluorouridine monophosphate (FUMP) either directly via orotate phosphorylase (OPRT) and phosphoribosyl transferase (PRPP) or indirectly via uridine phosphorylase (UP) and uridine kinase (UK) through Fluorouridine (FUR). FUMP is then phosphorylated to become fluorouridine diphosphate (FUDP), which is then transformed by ribonucleotide reductase (RNR) to either fluorodeoxyuridine triphosphate (FUTP) or fluorodeoxyuridine diphosphate (FdUDP). Finally, instead of uridine -5'-triphosphate/ 2′-deoxythymidine -5'-triphosphate (UTP/ dTTP), those active metabolites are integrated into RNA and DNA [24]. On the other hand, when thymidine phosphorylase (TP) and thymidine kinase (TK) work sequentially, 5-FU can

be transformed to fluorodeoxyuridine monophosphate (FdUMP) via an indirect mechanism (Figure 1) [24].

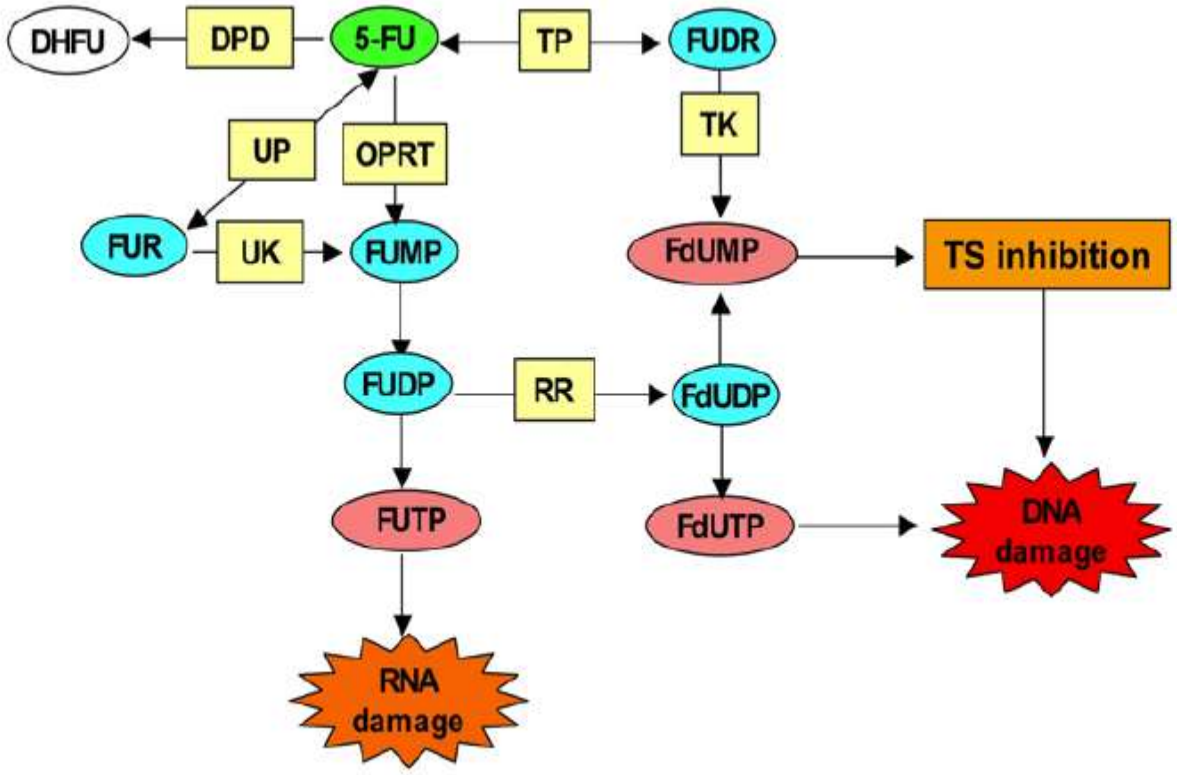

Figure 1. Illustration of the mechanism of action of 5-Fluorouracil [25].

The viral persistence and liver damage are the two critical characteristics of the natural history of Hepatitis C virus infection [26]. Nitric oxide (NO) is a highly versatile mediator in controlling viral infections, as it is the first antiviral response of the host [27]. NO works as an inducer of apoptosis in some cell types and as an inhibitor of apoptosis in others, including hepatocytes [28]. Additionally, it was discovered that patients with HCV have increased inducible nitric oxide synthase (iNOS) expression, signaling excessive NO production that correlates favorably with viral load with hepatic inflammation [29]. The most conspicuous characteristic of HCV is its strong proclivity towards chronicity [30]. NO may impede antiviral response by inhibiting the activity of type 1 helper T cell response [31]. Additionally, NO promotes viral escape mutations, allowing for viral persistence [32]. This study aimed to study the efficacy and toxicities of 5- fluorouracil as an anticancer drug on the biological behavior of HepG2 cell line and evaluate nitric oxide levels in monitoring the progression of HCC.

2. MATERIALS AND METHODS

2.1 Cell Lines and cell culture Samples collection and analysis:

HepG2 cells came from the Medical Research Institute (MRI) in Smouha, ALEX, Egypt. fetal bovine serum (FBS), streptomycin and penicillin, and Dulbecco's modified Eagle's medium (DMEM) were used to keep HepG2 cell lines alive. Every cell was grown at 37°C in 5% CO_2. After the HepG2 cancer cells became confluent, they were taken out and put into the same complete medium with $1x10^5$ cells using 0.25 % trypsin EDTA. DMEM was changed every three days [33].

2.2 Reagents and drug treatments:

The following products were purchased from GIBCO® (Invitrogen): Fetal bovine serum (FBS), streptomycin, penicillin, and Dulbecco's modified Eagle's medium (DMEM). Dimethylsulfoxide (DMSO) and standard anticancer medications (5- Fluorouracil) were bought from Sigma-Aldrich.

2.3 Estimation of Nitric oxide (NO):

Cells were plated in a growth medium at a density of 3×10^5 cells per well until they reached 80% confluence in 6-well plates and then treated with the chemotherapeutic medication dosage for 24 hours. Following treatment, we put 100ul Griess reagent to each 100ul sample volume. The sample and the reagent were mixed right inside each well of a 96 well plate. Then, we add 100 ul of deionized water and 100 ul of Griess reagent to another well to make a blank. We blanked the sample reagent combination and left it in the dark for 30 minutes to develop. The absorbance at 548 nm of the sample and the blank solutions was measured using a Biochrom EZ Read 800 Microplate Reader. The plate reader can concurrently monitor the absorbance of the blank and sample absorbance, and nitrite was measured using a standard curve generated from $NaNO_2$ (0–100 M). It was done three times for every experiment [34].

.

2.4 Cell proliferation:

The viability of cells (MTT Assay) was used to determine 5- Fluorouracil's inhibitory effect on HepG-2 cell proliferation [28]. To check that the number and density of cells were suitable, they were diluted and counted. The cells were then seeded at a density of 2×10^5 cells/ml in 96-well flat-bottom plates, and the cells had adhered for 24 hours at 37 °C. In complete media, cells were treated with drug dilutions of(50, 25, 12.5, 6.25, 3.125, and 1.56 µg). Each well was treated with ten microliter/liter of stock MTT solution (5 mg/mL) after three days of exposure to the test medication. Incubation of the cells at 37°C continued for another four hours. To determine the absorbance, we used a Biotek plate reader (Gen 5th) and a set of wavelengths spanning from 570 to 630 nm.

2.5 Statistical analysis:

Calculations for statistical analysis were performed using GraphPad Prism 8. (GraphPad, San Diego, CA, USA). A one-way analysis of variance was used to determine the statistical significance of the inhibition data (ANOVA). We defined statistical significance for our findings as less than or equal to 0.05 for the P-value. Using the median and standard deviation to summarize all available information (SD) [35].

3. RESULTS

3.1 Effect of the treatment on (NO) levels:

As shown in Table (1), the value of absorbance means were reduced significantly ($P<0.001$) after the treatment of hepG2 cells with 5- Fluorouracil as a chemotherapy drug in comparison to the control group (DMSO). A more potent reduction was noticed after treating with 5- FU. Likely, the levels of NO were reduced significantly (5.041±0.121) after treatment with the previous chemotherapy treatment as shown in figure (2). The resulting absorbance and NO levels were significantly decreased and this decrease was found to be dependent on the mechanism of action of 5- Fluorouracil chemotherapy drug.

Table (1): NO levels after treatment of HepG-2 cells with 5-Fluorouracil. Data are expressed as means ± SD, Significant difference between the treatments is analyzed by one-way ANOVA test, then there's the t-test., where: ***P≤0.001.

Drug	NO (µg/ml)
Control (DMSO)	9.135±0.103
5-Fluorouracil (5-FU)	5.041***±0.121

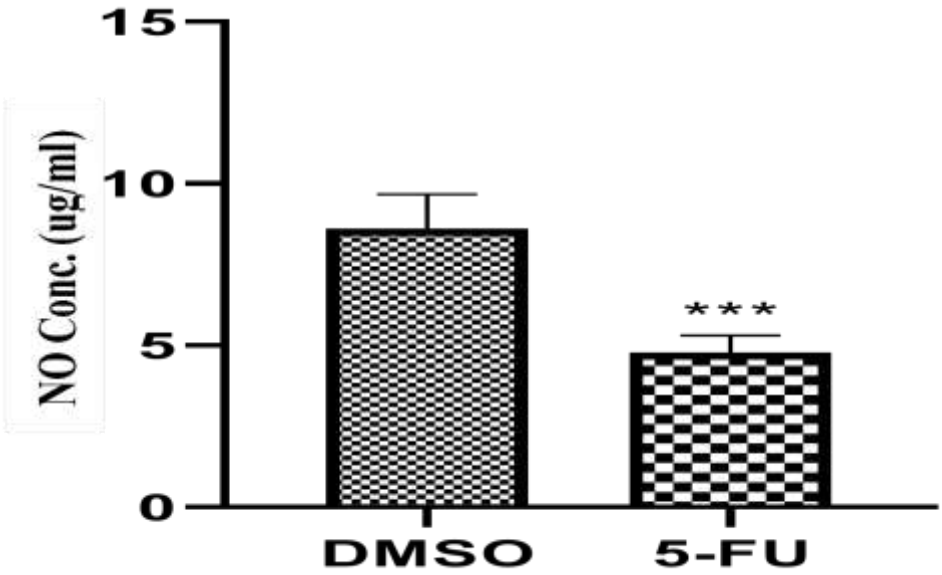

Figure 2. NO levels of treated hepG2 cells with 5-Fluorouracil. Data are expressed as means ± SD, *** significant at P< 0.001.

3.2 Morphological changes and cell proliferation inhibition of Hepg-2 cells by 5-Fluorouracil.

As demonstrated in tables (2), cells treated with 5-Fluorouracil at various concentrations showed a significant reduction in proliferative activity of HepG-2 cells. An MTT assay was used to determine the viability or proliferation percent of HepG-2 cells treated with 5-Fluorouracil. As shown in table (3) and figure (4), 5-Fluorouracil used at a concentration of (12.92±0.085µg/ml) for an indicated time demonstrated a significant inhibition effect against HepG-2 cells when compared to DMSO. These findings showed that 5-Fluorouracil has a considerable cytotoxic effect on the proliferation of HepG-2 cells. Furthermore, morphological variations were seen in HepG-2 cells treated with 5-Fluorouracil, as shown in Figure (3), when compared to DMSO, after treatment for 48 hours with 1.56 to 12.5 µg of 5-Fluorouracil did not cause notable changes in morphology or cell number. Furthermore, treatment with 25-50 µg of 5-Fluorouracil resulted in significant morphological alterations and notably suppression.

Table 2. Effect of the 5-Fluorouracil on cell proliferation percentage on HepG-2 cells.

5-Fluorouracil Conc. (µg)	Proliferation for HepG-2 (%)
50	38±1
25	41±2
12.5	50±2
6.25	57±2
3.125	67±1
1.56	72±2

Table 3. The IC50 value of HepG-2 after treatment with 5-Fluorouracil.

Compound	Chemical name	IC50(µg/ml)
5- FU	5- Fluorouracil	12.92±0.085

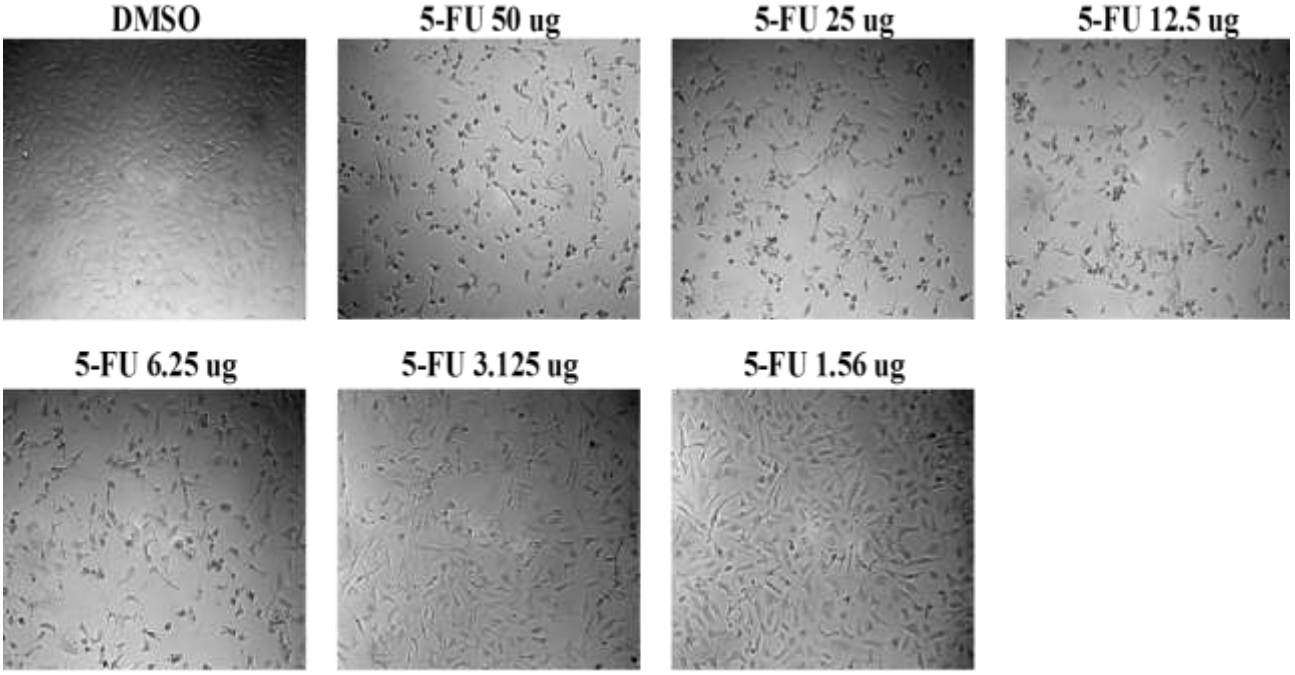

Figure 3. Effect of treatment with 5-Fluorouracil on the morphology of HepG-2 cells (an inverted microscope).

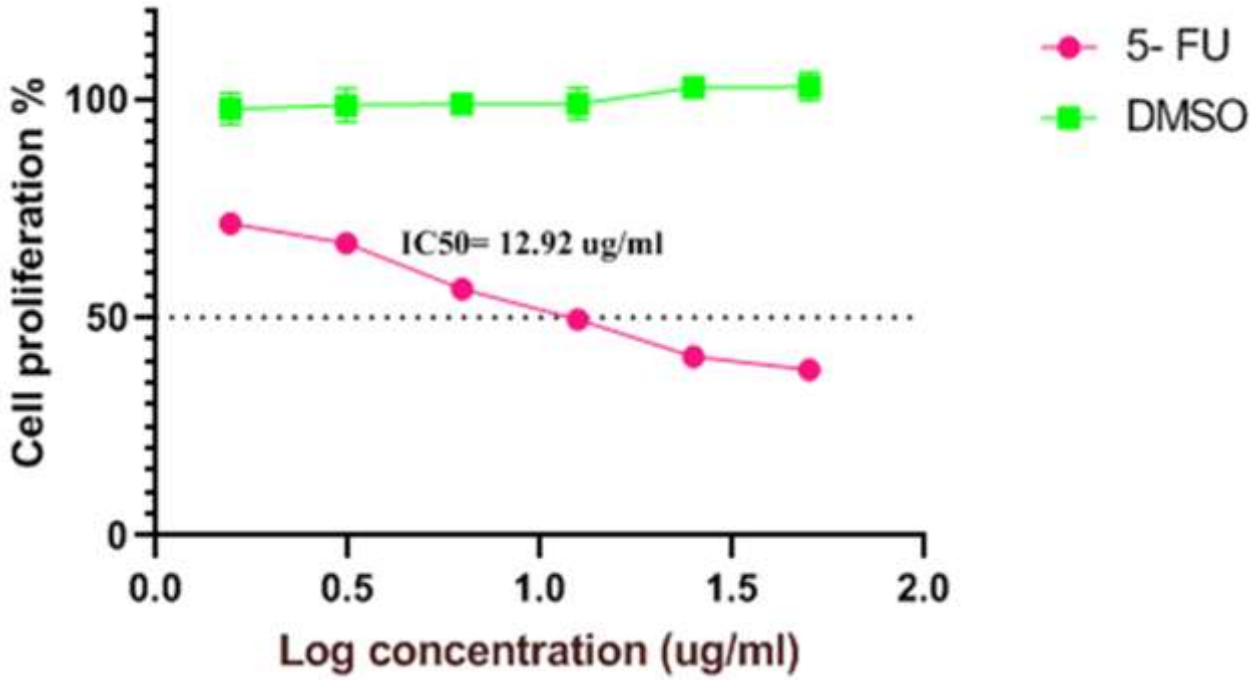

Figure 4. Effect of treatment with 5-Fluorouracil on the proliferation activity on HepG-2 cells.

4. DISCUSSION

Hepatocellular carcinoma was the fifth most frequently occurring type of cancer globally, accounting for 55% of occurrences worldwide [36]. HCC metastasized easily and was harder to diagnose in its early stages [37]. Moreover, less than 20% of people with liver cancer can be surgically treated [38]. As a result, systemic chemotherapy has become the primary method of treating liver cancer [39].

Chemotherapy is the primary therapeutic option for liver cancer that has progressed or recurred [40]. 5-Fluorouracil (5-FU) as a first-line chemotherapy agent is used to treat malignant tumors such as the liver, breast, and other digestive systems [41]. 5-Fluorouracil is a cytotoxic medication that interferes with nucleic acid synthesis, suppresses DNA synthesis, and finally stops cells growth [42]. It is widely utilized in the treatment of solid malignancies. However, because 5-FU is rapidly metabolized, therapeutic blood levels must be maintained via intravenous injection or infusion [43]. Because 5-FU has a lot of side effects like most chemotherapy drugs, there are still a lot of restrictions on how it can be used clinically in the field of medicine. 5-FU has a highly narrow therapeutic index and a very short half-life in blood and bodily tissues in the range of minutes [44]. Localized administration of 5-FU would minimize systemic toxicity and provide an effective and safe treatment.

There is a strong epidemiologic link between Hepatitis C or Hepatitis B infection and HCC [45]. However, the molecular pathways underlying the development of Hepatocellular carcinoma remain unknown [46]. While the integration of virus Gene sequences into the liver cell genome can activate cellular protooncogenes, this incorporation is extremely rare [47]. It does not explain the bulk of virus-induced HCCs. Increased NO synthesis by liver cells has been demonstrated in various hepatic disorders, including those caused by the parasite Opisthorchis viverrini, hepatitis viruses, and cirrhosis. Because of the potential for long-term exposure to high NO concentrations, the higher risk of hepatocellular carcinoma linked to various chronic liver illnesses could be explained by an increase in the body's natural ability to produce NO [48].

The significance of NO in the development of cancer is a controversial topic [49]. It is reported to have anticancer and mutagenic properties. High levels of NO have been shown to produce nitrosative deamination or DNA base oxidation, leading to DNA damage and mutation in human cells [50]. Aside from these effects, NO may also mediate capillary leakiness and support angiogenesis as tumors grow [51]. But because of its long-known cytotoxic and cytostatic effects on tumor cells and its antiapoptotic activity, NO also has anti-cancer characteristics [52].

The present study demonstrated that the control group's NO level was much higher in HepG-2 cells before chemotherapy. Our results are agreement with BURNEY et al, who announced that increased NO production occurs as a result of viral hepatitis in two ways. First, NO may act as an antiviral agent, assisting the host's immune system in combating viral infection. Second, given NO's recognized genotoxicity, chronic hepatitis's enhanced generation of NO free radicals may directly promote mutagenesis and the development of compounds of hepatocarcinogenic N-nitroso [53]. Moreover, PAN et al., proved that The relation between infection caused by viral hepatitis and a rising risk of liver cancer could be due to high levels of NO production and the antiapoptotic characteristics of some hepatitis viral proteins (apoptosis is the primary way for virus-infected hepatocytes to be eliminated) [54]. In this study, we used 5-Fluorouracil as one of the most effective treatments for liver cancer. After noticing the effect of this drug on HepG-2 cell lines, we found highly decrease in the levels of NO in the treated group with 5-Fluorouracil. Our results are in accordance with Jung et al, who approved that Inhibiting the generation of NO and reducing the production of iNOS was achieved by pretreatment with 5-FU [55].

5. CONCLUSION

This study may provide essential evidence for the selection of potential therapeutic agents for hepatocellular carcinoma management. Our study indicated that nitric oxide levels were greatly raised in

HCC, and 5-fluorouracil was found to be particularly efficient in the treatment of HCC as chemotherapy. Furthermore, we have established that nitric oxide is a useful diagnostic marker for hepatocellular carcinoma.

List of abbreviations:
5- FU: 5- Fluorouracil
HCC: Hepatocellular carcinoma
NO: Nitric oxide
MTT: Methyl thiazole tetrazolium
DMSO: Dimethylsulfoxide

REFERENCES

[1] Kaur, G., & Dufour, J. M., Spermatogenesis, 2(1), 1-5, (2012), doi.org/10.4161/spmg.19885

[2] Ren, Z., Chen, S., Ning, B., & Guo, L., In Drug-Induced Liver Toxicity (pp. 151-177), (2018), doi.org/10.1007/978-1-4939-7677-5_8

[3] Bulutoglu, B., Rey-Bedón, C., Mert, S., Tian, L., Jang, Y. Y., Yarmush, M. L., & Usta, O. B., PloS one, 15(2), e0229106, (2020), doi.org/10.1371/journal.pone.0229106

[4] Müsch, A., & Arias, I. M., The Liver: Biology and Pathobiology, 36-49, (2020), doi.org/10.1002/9781119436812.ch4

[5] Caines, A., Selim, R., & Salgia, R., Clinics in Liver Disease, 24(4), 535-547, (2020), doi.org/10.1016/j.cld.2020.06.001

[6] Rashed, W. M., Kandeil, M. A. M., Mahmoud, M. O., & Ezzat, S., Journal of the Egyptian National Cancer Institute, 32(1), 1-11, (2020), doi.org/10.1186/s43046-020-0016-x

[7] Konyn, P., Ahmed, A., & Kim, D., Expert review of gastroenterology & hepatology, 15(11), 1295-1307, (2021), doi.org/10.1080/17474124.2021.1991792

[8] Samant, H., Amiri, H. S., & Zibari, G. B., Journal of Gastrointestinal Oncology, 12(Suppl 2), S361–S373, (2021), doi.org/10.21037/jgo.2020.02.08

[9] Yang, J. D., Hainaut, P., Gores, G. J., Amadou, A., Plymoth, A., & Roberts, L. R., Nature reviews Gastroenterology & hepatology, 16(10), 589-604, (2019), doi.org/10.1038/s41575-019-0186-y

[10] Singal, A. G., Lok, A. S., Feng, Z., Kanwal, F., & Parikh, N. D., Clinical Gastroenterology and Hepatology, 20(1), 9-18, (2022), doi.org/10.1016/j.cgh.2020.09.036

[11] Yang, X., Sun, H., Hu, B., Wu, S. Y., Shi, Y. H., Wang, X. Y., & Fan, J., Annals of Oncology, 32(5), S824, (2021), doi.org/10.1016/j.annonc.2021.08.164

[12] Suresh, D., Srinivas, A. N., & Kumar, D. P., Frontiers in Oncology, 10, 601710, (2020), doi.org/10.3389/fonc.2020.601710

[13] Rimassa, L., Pressiani, T., & Merle, P., Liver cancer, 8(6), 427-446, (2019), doi.org/10.1159/000499765

[14] Junco, P. T., Cano, E. M., Dore, M., Gomez, J. J., Galán, A. S., Vilanova-Sánchez, A., & Santamaria, M. L., European Journal of Pediatric Surgery, 29(01), 028-032, (2019), doi.org/10.1055/s-0038-1668148

[15] Kloeckner, R., Galle, P. R., & Bruix, J., Hepatology, 73, 137-149, (2021), doi.org/10.1002/hep.31424

[16] Fondevila, F., Méndez-Blanco, C., Fernández-Palanca, P., González-Gallego, J., & Mauriz, J. L., Experimental & molecular medicine, 51(9), 1-15, (2019), doi.org/10.1038/s12276-019-0308-1

[17] Vodenkova, S., Buchler, T., Cervena, K., Veskrnova, V., Vodicka, P., & Vymetalkova, V., Pharmacology & therapeutics, 206, 107447, (2020), doi.org/10.1016/j.pharmthera.2019.107447

[18] Derissen, E. J., & Beijnen, J. H., Clinical Pharmacokinetics, 59, 1521–1550, (2020), doi.org/10.1007/s40262-020-00934-7

[19] Cho, C. H., & Hu, T., Academic Press, (2020), Hardcover ISBN: 9780128199374 eBook ISBN: 9780128199381

[20] Huang, X., Chen, L., Li, Z., Zheng, B., Liu, N., Fang, Q., & Ouyang, D., Toxicology, 460, 152858, (2021), doi.org/10.1016/j.tox.2021.152858

[21] Thalambedu, N., & Khan, Y., Cureus, 11(7), e5162, (2019), doi.org/10.7759/cureus.5162
[22] Chatterjee, K., & Dopfer, O., The European Physical Journal D, 75(3), 1-16, (2021), doi.org/10.1140/epjd/s10053-021-00065-z
[23] Shakerzadeh, E., Journal of Molecular Liquids, 343, 116970, (2021), doi.org/10.1016/j.molliq.2021.116970
[24] Trivedi, S., Murthy, S., Sharma, H., Hartlage, A. S., Kumar, A., Gadi, S. V., & Kapoor, A., Hepatology, 68(2), 435-448, (2018), doi.org/10.1002/hep.29494
[25] ChemicalBook.,(2017),https://www.chemicalbook.com/ChemicalProductProperty_EN_CB8162744.htm
[26] Pieretti, J. C., Rubilar, O., Weller, R. B., Tortella, G. R., & Seabra, A. B., Virus Research, 291, 198202, (2021), doi.org/10.1016/j.virusres.2020.198202
[27] DePace, N. L., & Colombo, J., Springer, Cham, (pp. 71-138), (2019), doi.org/10.1007/978-3-030-17016-5_4
[28] Munkhjargal, B., Enkhtuvshin, B., Ulziisaikhan, U., Tuvdenjamts, B., Unurbuyan, K., Sandagdorj, D., & Lkhagvasuren, E., bioRxiv, (2021), doi.org/10.1101/2021.02.15.431273
[29] Suarez, A. A. R., Baumert, T. F., & Lupberger, J., Journal of hepatology, 69(3), 564-566, (2018), doi.org/10.1016/j.jhep.2018.05.033
[30] Koushki, K., Salemi, M., Miri, S. M., Arjeini, Y., Keshavarz, M., & Ghaemi, A., Biomedicine & Pharmacotherapy, 144, 112346, (2021), doi.org/10.1016/j.biopha.2021.112346
[31] Chigbu, D. I., Loonawat, R., Sehgal, M., Patel, D., & Jain, P., Cells, 8(4), 376, (2019), doi.org/10.3390/cells8040376
[32] Tsikas, D., Free radical research, 39(8), 797-815, (2005), doi.org/10.1080/10715760500053651
[33] Bokhari, M., Carnachan, R. J., Cameron, N. R., & Przyborski, S. A., Journal of anatomy, 211(4), 567-576, (2007), doi.org/10.1111/j.1469-7580.2007.00778.x
[34] Morgan, D. M., Humana Press, (pp. 179-184), (1998), doi.org/10.1385/0-89603-448-8:
[35] Miceli, M. H., Bernardo, S. M., Ku, T. N., Walraven, C., & Lee, S. A., Antimicrobial agents and chemotherapy, 56(1), 148-153, (2012), doi.org/10.1128/AAC.05061-11
[36] Petruzziello, A., The open virology journal, 12, 26–32, (2018), doi.org/10.2174/1874357901812010026
[37] Guo, S., Hu, C., Zhai, X., & Sun, D., American Journal of Translational Research, 13(6), 6001, (2021), PMID: 34306340 PMCID: PMC8290788
[38] Orcutt, S. T., & Anaya, D. A., Cancer Control, 25(1), 1073274817744621, (2018), doi.org/10.1177/1073274817744621
[39] Kow, A. W. C., Journal of gastrointestinal oncology, 10(6), 1274, (2019), doi.org/10.21037/jgo.2019.08.06
[40] Longo, V., Brunetti, O., Gnoni, A., Licchetta, A., Delcuratolo, S., Memeo, R., & Argentiero, A., Medicina, 55(10), 698, (2019), doi.org/10.3390/medicina55100698
[41] Entezar-Almahdi, E., Mohammadi-Samani, S., Tayebi, L., & Farjadian, F., International Journal of Nanomedicine, 15, 5445, (2020), doi.org/10.2147/IJN.S257700
[42] Ahmed, N., Journal of Nuclear Technology in Applied Science, 8(1), 125-134, (2020), doi.org/10.21608/jntas.2020.36757.1025
[43] Goirand, F., Lemaitre, F., Launay, M., Tron, C., Chatelut, E., Boyer, J. C., & Schmitt, A., Expert opinion on drug metabolism & toxicology, 14(12), 1303-1313, (2018), doi.org/10.1080/17425255.2018.1550484
[44] Sethy, C., & Kundu, C. N., Biomedicine & Pharmacotherapy, 137, 111285, (2021), doi.org/10.1016/j.biopha.2021.111285
[45] Vodenkova, S., Buchler, T., Cervena, K., Veskrnova, V., Vodicka, P., & Vymetalkova, V., Pharmacology & therapeutics, 206, 107447, (2020), doi.org/10.1016/j.pharmthera.2019.107447
[46] Longley, D. B., Harkin, D. P., & Johnston, P. G., Nature reviews cancer, 3(5), 330-338, (2003), doi.org/10.1038/nrc1074
[47] Petruzziello, A., The open virology journal, 12, 26–32, (2018), doi.org/10.2174/1874357901812010026

[48] Xie, C., Li, S. Y., Fang, J. H., Zhu, Y., & Yang, J. E., Cancer Letters, 500, 281-291, (2021), doi.org/10.1016/j.canlet.2020.10.042

[49] Poletti, V., & Mavilio, F., Viruses, 13(8), 1526, (2021), doi.org/10.3390/v13081526

[50] Sun, M. H., Han, X. C., Jia, M. K., Jiang, W. D., Wang, M., Zhang, H., & Jiang, Y., World journal of gastroenterology: WJG, 11(38), 5931, (2005), doi.org/10.3748/wjg.v11.i38.5931

[51] Mandal, P., Biochemical and biophysical research communications, 495(2), 1766-1768, (2018), doi.org/10.1016/j.bbrc.2017.12.051

[52] Khan, F. H., Dervan, E., Bhattacharyya, D. D., McAuliffe, J. D., Miranda, K. M., & Glynn, S. A., International journal of molecular sciences, 21(24), 9393, (2020), doi.org/10.3390/ijms21249393

[53] Chakraborty, S., Njah, K., & Hong, W., Trends in cancer, 6(2), 81-85, (2020), doi.org/10.1016/j.trecan.2019.12.002

[54] Vahora, H., Khan, M. A., Alalami, U., & Hussain, A., Journal of cancer prevention, 21(1), 1., (2016), doi.org/10.15430/JCP.2016.21.1.1

[55] Burney, S., Caulfield, J. L., Niles, J. C., Wishnok, J. S., & Tannenbaum, S. R., Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis, 424(1-2), 37-49, (1999), doi.org/10.1016/S0027-5107(99)00006-8