Eurasian Journal of Molecular and Biochemical Sciences

https://dergi.erzurum.edu.tr/ejmbs

Determination of the potential of 5-Hydroxy-L-tryptophan and L-tryptophan as therapeutic agents for prostate cancer

Özlem Özdemir Tozlu[1*], Nursena Yüksel[1], Tuğba Gezmiş[1], Arzugül Tanas[1]

[1]*Erzurum Technical University, Faculty of Science, Department of Molecular Biology and Genetics, 25050, Erzurum, Turkey*

Cite:*Tozlu Ö.Ö, Yüksel N, Gezmiş T, Tanas A. Determination of the potential of 5-Hydroxy-L-tryptophan and L-tryptophan as therapeutic agents for prostate cancer. Eurasian Mol Biochem Sci 2022;1(2): 19-25.*

Received: 9 September 2022, Accepted: 27 October 2022 *DOI. 10.54672/ejmbs.2022.10*

Abstract

Prostate cancer is the second leading cause of death from cancer in men. Androgen deprivation therapy (ADT) is used as the standard treatment in prostate cancer, and this treatment has undesirable side effects over time. There is a need for more effective, safe compounds that occur naturally under the influence of these undesirable limitations. In this study, the anti-cancer potentials of 5-Hydroxy-L-tryptophan (5-HTP) and L-tryptophan, which are thought to have various inhibitory potential on cancer and its mechanisms, were studied. The study results showed that 5-HTP has a significant inhibition in prostate cancer cells. This study is an important preliminary screening for new and effective molecule trials. In order to develop a possible treatment strategy and to use these molecules as new therapeutic agents, further studies are needed in order to obtain more comprehensive data on the mechanisms of action of these molecules and to investigate their possible effects in other cancer types, and the obtained data should be supported by these studies.

Keywords: Prostate cancer, 5-Hydroxy-L-tryptophan, L-tryptophan , MTT assay, LDH release

Introduction

Prostate cancer, which is the most diagnosed cancer among men in Europe and America, is the second most common cause of death from cancer in men. It is observed that mortality rates have decreased due to the widespread use of prostate-specific antigen (PSA) secreted in the prostate, which is thought to contribute to the motility of spermatozoa, and developments in transrectal ultrasound-guided prostate needle biopsy (1–3).

It has long been known that prostate cancer is dependent on androgen for its growth and progression. Androgens, produced in the testicles, adrenal glands, and prostate gland, are essential for normal development and function of the prostate and prostate

Correspondence: Özlem Özdemir Tozlu
1Erzurum Technical University, Faculty of Science, Molecular Biology and Genetics Department, Erzurum, Turkey, 25050
E-mail: ozlem.ozdemir@erzurum.edu.tr.

This article licensed under Creative Commons Attribution Non-Commercial 4.0 International (CC BY-NC 4.0)

cancer proliferation. Therefore, androgen deprivation is an effective therapeutic strategy widely used in clinical practice and has become the standard treatment for this disease. Androgen deprivation therapy (ADT) is used to suppress androgenic effects and therefore prevent progression of prostate cancer (4,5). However, most patients develop resistance to metastatic castration after several years of ADT therapy and progress to prostate cancer (6). In addition, since androgens affect many other organs besides the prostate, according to the mechanism of action of the drug used, ADT may cause decreased libido, erectile dysfunction, hot flashes, loss of bone density, bone fractures, loss of muscle mass and physical strength, changes in blood lipids, insulin resistance, weight gain, It can have various side effects such as burnout and gynecomastia. Today, many new drugs are introduced to the market for use in the treatment of prostate cancer. Due to these limitations and adverse effects of current standard treatments, the search for safer and more effective molecules based on naturally occurring compounds is emerging.

L-Tryptophan is an essential aromatic α- amino acid and is required in the diet of children and adult humans. It serves as a precursor for important biomolecules such kynurenic acid, nicotinamide adenine dinucleotide, serotonin, melatonin, tryptamine, and niacin in addition to being a necessary amino acid for protein synthesis (7–9).

5-Hydroxy-L-tryptophan (5-HTP), a serotonin pathway metabolite of L-tryptophan in the brain that regulates serotonin levels. As a result, 5-HTP is a key player in the serotonin pathway. Additionally, 5-HTP is a naturally occurring aromatic amino acid that has a variety of antioxidant properties (10–13). Additionally, 5-Hydroxy-l-tryptophan (5-HTP) is a well-known dietary supplement that has taken the place of l-tryptophan (l-Trp) as a treatment for depression (14,15), fibromyalgia's incapacitating symptoms (16,17), weight loss assistance (18), headache prevention (19), and assistance for insomniacs (20,21). Taking together all these data, the aim of this study was to elucidate the anticancer potentials L-tryptophan and 5-HTP on prostate cancer cells. Therefore, L-tryptophan and 5-HTP were screened for their cytotoxicity using 3-(4,5-dimethyl-thiazol-2-yl) 2,5-diphenyltetrazolium bromide (MTT) assay and LDH release assay. Also, they were examined for antioxidative and oxidative status using total antioxidant capacity (TAC) and total oxidant status (TOS) assay.

Materials and Methods

Cell culture: PC3 and DU-145 cells cell line were kindly provided from Dr. Ömer Faruk Karataş (Erzurum Technical University). The cells were grown in Dulbecco's Modified Eagle Medium/Nutrient Mixture F-12 (DMEM/F-12) (Gibco, Life Science, USA) (1:1) medium supplemented with 10% fetal bovine serum (PAN Biotech®), 1% streptomycin/penicillin and maintained at 37°C in a 5% CO2 incubator.

MTT Assay: For MTT assay, $1x10^4$-$1x10^5$ cells were seeded in 96-well plates and kept under appropriate culture conditions (37 °C, 5% CO2) for 24 h for cell attachment. Then, cells were incubated with different concentrations of 5-HTP and L-tryptophan for 48h. After incubation period, MTT solution (5 mg/ml MTT in PBS; Sigma-Aldrich®, Germany) was added and incubated for 3 h. Then, dimethyl sulfoxide (DMSO) (Merck®, Germany) is used to dissolve formazan crystals. In a microplate reader, the optical density was measured at 570 nm of wavelength (Synergy-HT; BioTek Winooski, VT, USA). Cells were given a treatment with 0.1% (w/v) Triton X-100 as a positive control. The untreated cells were used as a negative control. With the use of Probit-log concentration graphs, the IC_{50} value was calculated.

LDH Release Assay: Following the manufacturer's instructions, the LDH test was carried out using the CytoSelectTM LDH Cytotoxicity Assay Kit (Cell BioLabs, San Diego, CA). Briefly, after the above-mentioned treatments, 90 µl of the supernatant from the cells were transferred to a fresh plate, and 10 L of the reaction mixture were applied to each well. The reaction was incubated for 30 minutes at room temperature in the dark. Ultimately, a microplate reader was used to detect the optical density at a wavelength of 450 nm. (Synergy-HT; BioTek Winooski, VT, USA). As a positive control, cells were treated with 0.1% (w/v) Triton X-100. The cells without treatment served as negative control.

Oxidative analysis: TAC assay and TOS assay were carried out according to provider's manual. Briefly, the cells were cultured in 96-well plate and treated with 5-HTP in a concentration of 95.23 mg/ml for 48 h. At the end of incubation, commercially available TAC and TOS assay kits (Rel Assay Diagnostics®, Turkey) were used according the manufacturer's instructions to measure antioxidative/oxidative capacity of 5-HTP. Ascorbic acid (10 µM) and hydrogen peroxide (25 µM) from Sigma-Aldrich Company were preferred as positive control treatments in determining TAC and TOS levels, respectively.

Statistical Analysis: Statistical analysis was conducted using SPSS® 21.0 program. The results are given as mean ± standard deviation. Duncan's test was used as a post-hoc followed by one-way analysis of variance (ANOVA). $p < 0.05$ was set as the minimal level of significance.

Results

Two different cell viability assays were carried out to measure the anti-proliferative effect of 5-HTP and L-tryptophan on the human prostate cancer cells in order to obtain more reliable data. Markers of energy metabolism of cell is measured by MTT reduction assay and loss of cell membrane integrity was determined by LDH release assay. The cytotoxicity assays demonstrated that 5-HTP led to decreased cell growth depending on dose ($p<0.05$) (Figure 1).

As a result of the study, IC_{50} values of 5-HTP was determined as 95.23 mg/ml for PC3 and 108.58 mg/ml for Du-145 cells. On the other hand, IC_{50} values of L-tryptophan was calculated as 543.67 mg/ml for PC3 and 793.12 mg/ml for Du-145 cells. Also, LDH release assay exhibited similar results with MTT assay (Figure 2).

Since 5-HTP showed highest cytotoxic activity on PC-3 cells, antioxidative capacity was determined using TAC and TOC assays. Treatments of cells with 5-HTP (at IC20 concentration) and positive control agents (as ascorbic acid and hydrogen peroxide) resulted in changes of TAC and TOS levels as compared to untreated cells. 5-HTP considerably slightly expanded TAC level (1.9x fold) on PC3 cells while this concentration did not cause a change in TOS level. (Table 1).

Discussion

One of the most used criteria for cytotoxicity is cell membrane integrity, and many approaches have been established for its evaluation (22). Numerous investigations have employed the release of stable intracellular enzymes like lactate dehydrogenase (LDH)1 as well as the release of a label like 51Cr or calcein (23–26). Other options for assessing general cytotoxicity include tests that evaluate cell metabolic activity. For instance, many tetrazolium compounds have been utilized for this (27,28). In the present study, we assessed MTT and LDH release assay and both results showed a dose-dependent reduction in viability of both prostate cancer cells after 48 h incubation with 5-HTP.

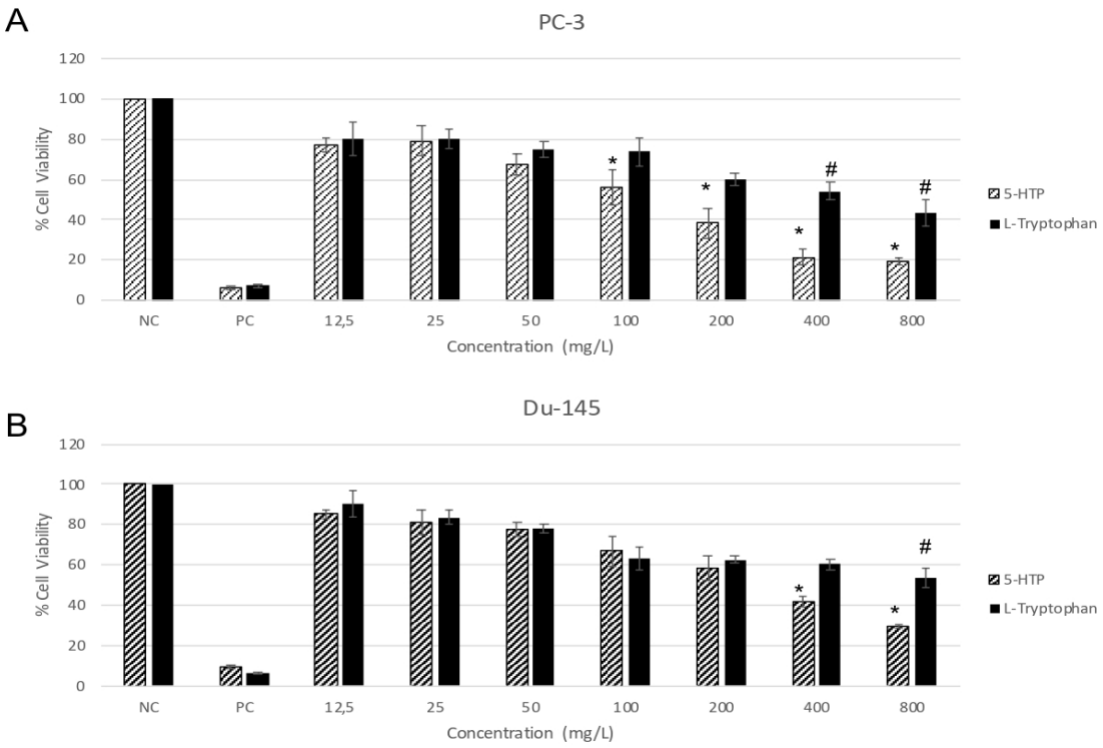

Figure 1. Effects of 5-HTP and L-tryptophan (0-800 mg/ml) on viability of PC-3 (A) and Du-145 (B) cells. Data represented as mean ± SEM (*p <0.05).

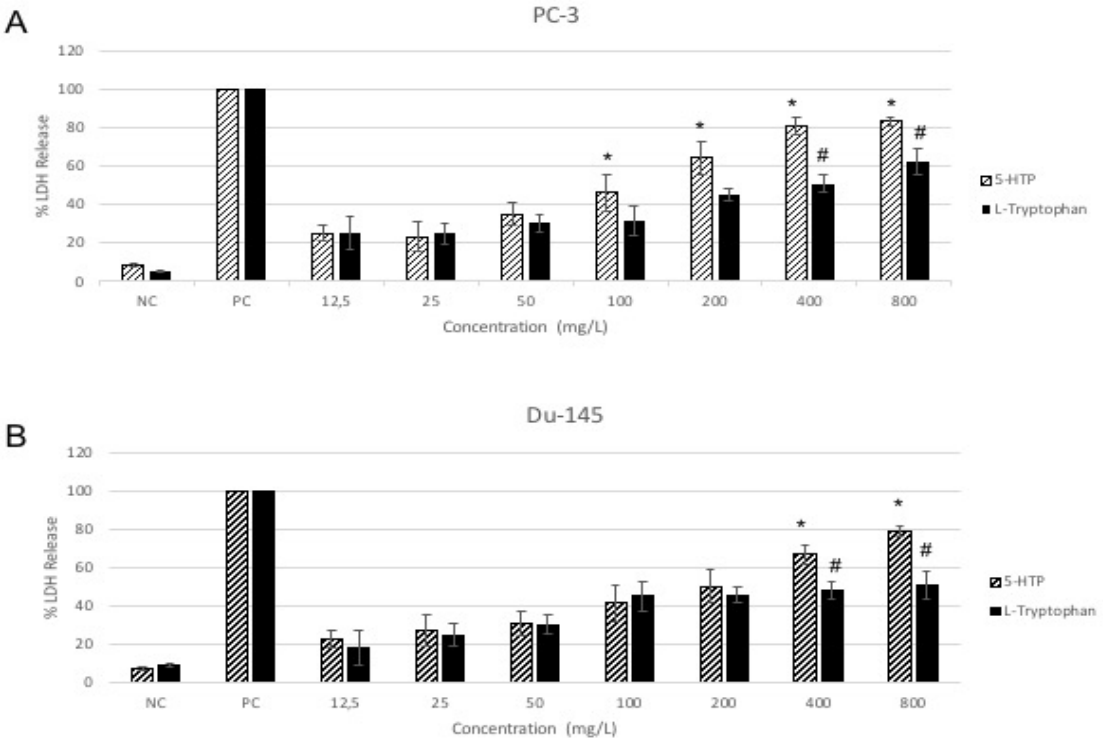

Figure 2. Effects of 5-HTP and L-tryptophan (0-800 mg/ml) on LDH activity of PC-3 (A) and Du-145 (B) cells. Data represented as mean ± SEM (*p <0.05).

Oxidative stress is a factor in the development of a number of diseases and is brought on by the interaction of chemically reactive oxygen species with biomolecules. Angiogenesis, uncontrolled proliferation, apoptosis escape, uncontrolled proliferation, tissue invasion, and metastasis are all associated with oxidative stress at different phases of cancer development (29–31). As a result, substances having antioxidant properties may play a crucial role in chemoprevention by lowering oxidative stress.

Because of their low molecular weight, simple absorption, and great action, researchers have recently focused a lot of attention on antioxidative peptides. It has been observed that several amino acids and their derivatives, including cysteine, histidine, tryptophan, lysine, arginine, leucine, valine, and 5-hydroxytryptophan, have antioxidant properties. (32–35). A naturally occurring amino acid containing amino and hydroxy groups, 5-hydroxytryptophan serves as a metabolic key component for the production of the neurotransmitters melatonin and serotonin. The powerful ability of melatonin to scavenge free radicals is influenced by 5-hydroxytryptophan. (36,37). Various tissues have been shown to be protected against oxidative stress by indoles, both natural and synthetic, mostly by scavenging harmful reactive oxygen species (37–39). Several researches have discovered that substituted indoles have antioxidant properties (40–42).

Notably, we found a significant reduction in TOS level and an increased TAC level in 5-HTP treated cells. These results support the antiproliferative potential of 5-HTP as a chemotherapeutic agent in cancer treatment. Collectively, it can be suggested that 5-HTP may have a good potential for anticancer activity in prostate cancer via decreasing oxidative stress and promoting cell death. However, further investigations are still needed to understand the precise mechanism of the anticarcinogenic effect of 5-HTP in prostate cancer.

Funding: This study is supported with project by Erzurum Technical University, Scientific Research Project Support Coordinator (Project number: 2019/06).

Declaration of Interest: No potential conflict of interest relevant to this article was reported.

Authors' Contributions: ÖÖT, NY, TG and AT contributed to the study conception, design and laboratory work. Writing the article (OOT, NY). All authors read and approved the final manuscript. ÖÖT; Özlem Özdemir Tozlu, NY; Nursena Yüksel, TG: Tuğba Gezmiş, AT; Arzugül Tanas.

ORCID:

Özlem Özdemir Tozlu 0000-0002-7776-1188

References

1. Carroll PR, Coakley F V, Kurhanewicz J. Magnetic Resonance Imaging and Spectroscopy of Prostate Cancer. Rev Urol. 2006;8(Suppl 1):S4.
2. Oguz U, Şimşek G, Demirelli E, Şenocak Ç, Reşorlu B, Faruk Bozkurt Ö, et al. Prostat Dokusundaki Kanserin Tespitinde Kanser Lokalizasyonunun Önemi ve Dağılımı The İmportance of Cancer Location on The Detection of Cancer in Prostate Tissue and Cancer Distribution in Prostate. Kocaeli Med J. 2021;10(1):160–5.
3. Serin Akbayır NM. Prostate Specific Antigen and Derivatives in Prostate Cancer Diagnosis. Türk Klin Biyokim Derg. 2016;14(3):189–204.
4. Fujita K, Nonomura N. Ⅱ.Treatment of Advanced Prostate Cancer. Gan To Kagaku Ryoho. 2020;47(1):27–9.
5. Nguyen-Nielsen M, Borre M. Diagnostic and Therapeutic Strategies for Prostate Cancer. Semin Nucl Med. 2016;46(6):484–90.
6. Zhua J, Liao R, Su C, Liang D, Wu J, Qiu K, et al. Toxicity profile characteristics of novel androgen-deprivation therapy agents in patients with prostate cancer: A meta-analysis. Expert Rev Anticancer Ther. 2018;18(2):193–8.
7. Nayak BN, Buttar HS. Evaluation of the antioxidant properties of tryptophan and its metabolites in in vitro assay. J Complement Integr Med [Internet]. 2016 Jun 1 [cited 2022 Sep 20];13(2):129–36. Available from: http://www.ncbi.nlm.nih.gov/pubmed/26641976
8. Christen S, Peterhans E, Stocker R. Antioxidant activities of some tryptophan metabolites: possible implication for inflammatory diseases. Proc Natl Acad Sci U S A [Internet]. 1990 Apr [cited 2022 Sep 20];87(7):2506–10. Available from: http://www.ncbi.nlm.nih.gov/pubmed/2320571
9. Elisia I, Tsopmo A, Friel JK, Diehl-Jones W, Kitts DD. Tryptophan from human milk induces oxidative stress and upregulates the Nrf-2-mediated stress response in human intestinal cell lines. J Nutr [Internet]. 2011 Aug [cited 2022 Sep 20];141(8):1417–23. Available from: http://www.ncbi.nlm.nih.gov/pubmed/21677072
10. Ghosh G, De K, Maity S, Bandyopadhyay D, Bhattacharya S, Reiter RJ, et al. Melatonin protects against oxidative damage and restores expression of GLUT4 gene in the hyperthyroid rat heart. J Pineal Res [Internet]. 2007 Jan [cited 2022 Sep 20];42(1):71–82. Available from: https://onlinelibrary.wiley.com/doi/10.1111/j.1600-079X.2006.00386.x
11. Mollaoglu H, Topal T, Ozler M, Uysal B, Reiter RJ, Korkmaz A, et al. Antioxidant effects of melatonin in rats during chronic exposure to hyperbaric oxygen. J Pineal Res [Internet]. 2007 Jan

[cited 2022 Sep 20];42(1):50–4. Available from: https://onlinelibrary.wiley.com/doi/10.1111/j.1600-079X.2006.00382.x
12. Ortega-Gutiérrez S, Fuentes-Broto L, García JJ, López-Vicente M, Martínez-Ballarín E, Miana-Mena FJ, et al. Melatonin reduces protein and lipid oxidative damage induced by homocysteine in rat brain homogenates. J Cell Biochem [Internet]. 2007 Oct 15 [cited 2022 Sep 20];102(3):729–35. Available from: https://onlinelibrary.wiley.com/doi/10.1002/jcb.21327
13. Saravanan KS, Sindhu KM, Mohanakumar KP. Melatonin protects against rotenone-induced oxidative stress in a hemiparkinsonian rat model. J Pineal Res [Internet]. 2007 Apr [cited 2022 Sep 20];42(3):247–53. Available from: https://onlinelibrary.wiley.com/doi/10.1111/j.1600-079X.2006.00412.x
14. Angst J, Woggon B, Schoepf J. The treatment of depression with L-5-Hydroxytryptophan versus imipramine. Arch Psychiatr Nervenkr [Internet]. 1977 Jun [cited 2022 Sep 20];224(2):175–86. Available from: https://link.springer.com/10.1007/BF00346485
15. Lancet TP-T, 1967 undefined. 5-Hydroxytryptophan for depression. thelancet.com [Internet]. [cited 2022 Sep 20]; Available from: https://www.thelancet.com/journals/lancet/article/PIIS0140-6736(67)90824-0/fulltext
16. Caruso I, Puttini PS, Cazzola M, Azzolini V. Double-Blind Study of 5-Hydroxytryptophan versus Placebo in the Treatment of Primary Fibromyalgia Syndrome. J Int Med Res [Internet]. 1990 May 25 [cited 2022 Sep 20];18(3):201–9. Available from: http://journals.sagepub.com/doi/10.1177/030006059001800304
17. Nicolodi M, Sicuteri F. Fibromyalgia and Migraine, Two Faces of the Same Mechanism. In 1996 [cited 2022 Sep 20]. p. 373–9. Available from: http://link.springer.com/10.1007/978-1-4613-0381-7_58
18. Cangiano C, Ceci F, ... AC-... A journal of, 1992 undefined. Eating behavior and adherence to dietary prescriptions in obese adult subjects treated with 5-hydroxytryptophan. academic.oup.com [Internet]. [cited 2022 Sep 20]; Available from: https://academic.oup.com/ajcn/article-abstract/56/5/863/4715513
19. Bono G, Criscuoli M, ... EM-A in, 1982 undefined. Serotonin precursors in migraine prophylaxis. moh-it.pure.elsevier.com [Internet]. [cited 2022 Sep 20]; Available from: https://moh-it.pure.elsevier.com/en/publications/serotonin-precursors-in-migraine-prophylaxis
20. Wyatt R, Zarcone V, Engelman K, ... WD-E, 1971 undefined. Effects of 5-hydroxytryptophan on the sleep of normal human subjects. Elsevier [Internet]. [cited 2022 Sep 20]; Available from: https://www.sciencedirect.com/science/article/pii/0013469471901477
21. Guilleminault C, Cathala J, neurophysiology PC clinical, 1973 undefined. Effects of 5-hydroxytryptophan on sleep of a patient with a brain-stem lesion. Elsevier [Internet]. [cited 2022 Sep 20]; Available from: https://www.sciencedirect.com/science/article/pii/001346947390045X
22. Eisenbrand G, Pool-Zobel B, Baker V, ... MB-F and chemical, 2002 undefined. Methods of in vitro toxicology. Elsevier [Internet]. [cited 2022 Sep 20]; Available from: https://www.sciencedirect.com/science/article/pii/S0278691501001181
23. Decker T, methods ML-M-J of immunological, 1988 undefined. A quick and simple method for the quantitation of lactate dehydrogenase release in measurements of cellular cytotoxicity and tumor necrosis factor (TNF) activity. Elsevier [Internet]. [cited 2022 Sep 20]; Available from: https://www.sciencedirect.com/science/article/pii/0022175988903109
24. Korzeniewski C, methods DC-J of immunological, 1983 undefined. An enzyme-release assay for natural cytotoxicity. Elsevier [Internet]. [cited 2022 Sep 20]; Available from: https://www.sciencedirect.com/science/article/pii/0022175983904386
25. Wang X, Terasaki P, Jr GR, immunology DC-H, 1993 undefined. A new microcellular cytotoxicity test based on calcein AM release. Elsevier [Internet]. [cited 2022 Sep 20]; Available from: https://www.sciencedirect.com/science/article/pii/0198859993905108
26. Lichtenfels R, Biddison W, Schulz H, ... AV-J of, 1994 undefined. CARE-LASS (calcein-release-assay), an improved fluorescence-based test system to measure cytotoxic T lymphocyte activity. Elsevier [Internet]. [cited 2022 Sep 20]; Available from: https://www.sciencedirect.com/science/article/pii/0022175994901104
27. Goodwin C, Holt S, Downes S, immunological NM-J of, 1995 undefined. Microculture tetrazolium assays: a comparison between two new tetrazolium salts, XTT and MTS. Elsevier [Internet]. [cited 2022 Sep 20]; Available from: https://www.sciencedirect.com/science/article/pii/0022175994002774
28. Gutleb A, Morrison E, and AM-ET, 2002 undefined. Cytotoxicity assays for mycotoxins produced by Fusarium strains: a review. Elsevier [Internet]. [cited 2022 Sep 20]; Available from: https://www.sciencedirect.com/science/article/pii/S1382668902000200
29. Reuter S, Gupta SC, Chaturvedi MM, Aggarwal BB. Oxidative stress, inflammation, and cancer: how are they linked? Free Radic Biol Med [Internet]. 2010 Dec 1 [cited 2022 Sep 20];49(11):1603–16. Available from: http://www.ncbi.nlm.nih.gov/pubmed/20840865
30. Pani G, Galeotti T, Chiarugi P. Metastasis: cancer cell's escape from oxidative stress. Cancer Metastasis Rev [Internet]. 2010 Jun [cited 2022 Sep 20];29(2):351–78. Available from: http://www.ncbi.nlm.nih.gov/pubmed/20386957
31. McCubrey JA, Steelman LS, Chappell WH, Abrams SL, Wong EWT, Chang F, et al. Roles of the Raf/MEK/ERK pathway in cell growth, malignant transformation and drug resistance. Biochim Biophys Acta [Internet]. 2007 Aug [cited 2022 Sep 20];1773(8):1263–84. Available from: http://www.ncbi.nlm.nih.gov/pubmed/17126425
32. Uchida K, Kawakishi S. Sequence-dependent reactivity of histidine-containing peptides with copper(II)/ascorbate. J Agric Food Chem [Internet]. 1992 Jan 1 [cited 2022 Sep 20];40(1):13–6. Available from: https://pubs.acs.org/doi/abs/10.1021/jf00013a003
33. Chen H-M, Muramoto K, Yamauchi F, Nokihara K. Antioxidant Activity of Designed Peptides Based on the Antioxidative Peptide Isolated from Digests of a Soybean Protein. J Agric Food Chem [Internet]. 1996 Jan 1 [cited 2022 Sep 20];44(9):2619–23. Available from: https://pubs.acs.org/doi/10.1021/jf950833m
34. Lysek N, Kinscherf R, Claus R, Lindel T. L-5-Hydroxytryptophan: Antioxidant and Anti-Apoptotic Principle of the Intertidal Sponge Hymeniacidon heliophila. Zeitschrift für Naturforsch C [Internet]. 2003 Aug 1 [cited 2022 Sep 20];58(7–8):568–72. Available from: https://www.degruyter.com/document/doi/10.1515/znc-2003-7-821/html
35. Suzen S, Cihaner SS, Coban T. Synthesis and Comparison of Antioxidant Properties of Indole-Based Melatonin Analogue Indole Amino Acid Derivatives. Chem Biol Drug Des [Internet]. 2012 Jan [cited 2022 Sep 20];79(1):76–83. Available from: https://onlinelibrary.wiley.com/doi/10.1111/j.1747-0285.2011.01216.x
36. Poeggeler B, Reiter RJ, Tan D-X, Chen L-D, Manchester LC. Melatonin, hydroxyl radical-mediated oxidative damage, and aging: A hypothesis. J Pineal Res [Internet]. 1993 May [cited 2022 Sep 20];14(4):151–68. Available from: https://onlinelibrary.wiley.com/doi/10.1111/j.1600-079X.1993.tb00498.x
37. Reiter RJ, Tan D, Cabrera J, D'Arpa D. Melatonin and Tryptophan Derivatives as Free Radical Scavengers and Antioxidants. In 1999 [cited 2022 Sep 20]. p. 379–87. Available from: http://link.springer.com/10.1007/978-1-4615-4709-9_48
38. Štolc S. Indole derivatives as neuroprotectants. In: Life Sciences [Internet]. 1999 [cited 2022 Sep 20]. p. 1943–50. Available from: https://www.sciencedirect.com/science/article/pii/S0024320599004531
39. Padillo FJ, Cruz A, Navarrete C, Bujalance I, Briceño J, Gallardo

JI, et al. Melatonin Prevents Oxidative Stress and Hepatocyte Cell Death Induced by Experimental Cholestasis. Free Radic Res [Internet]. 2004 Jul 7 [cited 2022 Sep 20];38(7):697–704. Available from: http://www.tandfonline.com/doi/full/10.1080/10715760410001705131

40. Keithahn C, Lerchl A. 5-Hydroxytryptophan is a more potent in vitro hydroxyl radical scavenger than melatonin or vitamin C. J Pineal Res [Internet]. 2005 Jan [cited 2022 Sep 20];38(1):62–6. Available from: https://onlinelibrary.wiley.com/doi/10.1111/j.1600-079X.2004.00177.x
41. Shertzer HG, Tabor MW, Hogan ITD, Brown SJ, Sainsbury M. Molecular modeling parameters predict antioxidant efficacy of 3-indolyl compounds. Arch Toxicol [Internet]. 1996 Oct 14 [cited 2022 Sep 20];70(12):830–4. Available from: http://link.springer.com/10.1007/s002040050346
42. Benabadji S, Wen R, ... JZ-AP, 2004 undefined. Anticarcinogenic and antioxidant activity of diindolylmethane derivatives. researchgate.net [Internet]. [cited 2022 Sep 20]; Available from: https://www.researchgate.net/profile/Jianbin-Zheng-2/publication/8572652_Anticarcinogenic_and_antioxidant_activity_of_diindolylmethane_derivatives/links/55c371a308aea2d9bdc17302/Anticarcinogenic-and-antioxidant-activity-of-diindolylmethane-derivative.