Tropical Journal of Pharmaceutical Research November 2024; 23 (11): 1887-1893
ISSN: 1596-5996 (print); 1596-9827 (electronic)

Available online at http://www.tjpr.org
http://dx.doi.org/10.4314/tjpr.v23i11.12

Original Research Article

Antitumor evaluation of hydroxyurea analogue Schiff base metal complexes

Hafize Telceken[1], Arzu Karatepe[2]*, Songül Çeribaşı[3], Zuhal Karagöz Genç[4], Ali Osman Çeribaşı[3]

[1]Department of Chemistry, Science Faculty, Firat University, Elazig, [2]Department of Chemistry, Science Faculty, Bingol University, Bingol, [3]Department of Pathology, Faculty of Veterinary Medicine, Fırat University, Elazig, [4]Metallurgy and Materials Engineering, Engineering Faculty, Adiyaman University, Adiyaman, Türkiye

For correspondence: **Email: akaratepe23a@gmail.com; **Tel:** +90 (553) 715 83 74; **Orcid ID:** 0000-0001-6649-2130*

Sent for review: 12 July 2024 *Revised accepted: 2 November 2024*

Abstract

Purpose: *The in vivo and in vitro antitumor activities of hydroxyurea derivative Schiff bases (SBs) metal complexes were investigated in immortalized human colon cancer cell lines (HT-29 cells) and rat models.*

Methods: *For the in vitro studies, three concentrations (5, 10, and 20 µM) of the 1-hydroxy-3-(E)-pyridine-3-ylmethylidene urea derivative SB (L)-metal complexes (L-Cd, L-Cu, L-Zn) were used to determine the viability of HT-29 cell line with dimethylsulphoxide (DMSO) as control. On the other hand, colorectal cancer was induced with subcutaneous administration of azoxymethane (AOM; 15 mg/kg) in 35 Wistar albino rats, which were assigned to five treatment groups consisting of DMSO (negative control), cisplatin (15 mg/kg, positive control), AOM + L-Cd (25 mg/kg), AOM + L-Cu (25 mg/kg) and AOM + L-Zn (25 mg/kg) groups, respectively. Tumor formation was observed by macroscopic and microscopic examinations.*

Results: *Tumour formation was not observed in the positive control group. The rats treated with AOM (excluding L-Cu and cisplatin group) displayed severe dysplasia and adenocarcinoma formations in the oil+DMSO, L-Cd and L-Zn groups. When compared with the cisplatin group in the in vivo studies, the Cu complex had a more favorable effect against colon cancer.*

Conclusion: *Consequently, hydroxyurea derivative SB-metal complexes exhibit antiproliferative activity in both in vitro ($p < 0.0001$) and in vivo studies.*

Keywords: *Azoxymethane, Colon cancer, Hydroxyurea, Schiff base, Antiproliferative*

Tropical Journal of Pharmaceutical Research is indexed by Scopus, Chemical Abstracts, Embase, Index Copernicus, EBSCO, African Index Medicus, JournalSeek, Directory of Open Access Journals (DOAJ), African Journal Online, Bioline International, Open-J-Gate and Pharmacy Abstracts

INTRODUCTION

Cancer is a major disease that affects a considerable proportion of people worldwide of which colon cancer is the most common type between females and males in the US [1]. In the US, approximately 136,830 people were diagnosed with colorectal cancer and around 50,310 mortalities were recorded in 2014 [2]. The World Health Organization reports approximately 1.9 million new colorectal cancer cases every year. Hydroxyurea was one of the first chemical agents developed in the 1960s to treat cancer. However, long-term treatment or high doses of hydroxyurea result in cell death due to DNA damage and oxidative stress. Hydroxyurea has

been evaluated as a single agent or in combination with other chemicals in several clinical trials [3]. On the other hand, Schiff bases (SBs) and metal complexes have various pharmacological activities and are of great interest in pharmacology, chemistry and biology [4]. They have antimicrobial, antitumor, antiviral, and antineoplastic properties. In addition, SB-Cu(II) complexes have also been reported to exhibit antioxidative activity by inhibiting lipid peroxidation [5]. Schiff base metal complexes promote antitumor activity *in vivo* and *in vitro* for colon cancer [6]. Schiff bases are obtained by condensation from a reaction of aldehyde and ketone compounds with primary amines resulting in the formation of a C=N double bond called azomethine. The biological activity of SBs, which are influenced by the redox potentials of the central atom and of the structure and conformation of the ligand in the coordination compounds, have been the subject of many *in vitro* studies [7].

Previous studies have shown the *in vitro* antioxidant and antitumor activities of hydroxyurea derivative SB-metal complexes. The complexes were found to exhibit effective *in vitro* antiproliferative activity against MCF-7 breast cancer cell lines. In addition, treatment of *Saccharomyces cerevisiae* cells with the complexes resulted in moderate antioxidative effects on antioxidant vitamins and malondialdehyde (MDA) levels in cell media and *in vitro* 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity [8]. This study aimed to investigate the *in vitro* and *in vivo* antitumor activity potential of 1-hydroxy-3-(E)-pyridine-3-ylmethylidene urea derivative SB (L)-metal complexes (L-Cd, L-Zn and L-Cu) against colon cancer.

EXPERIMENTAL

Chemical compounds

Hydroxyurea derivative SB metal complexes used in this study were synthesized and characterized by Şekerci *et al*. in the Chemistry Department, Faculty of Science, Firat University [9,10].

Cytotoxic activity of hydroxyurea derivative-SB metal complexes in HT-29 cell line

Cell culture studies were conducted at Inonu University Medical Faculty, Department of Physiology, Türkiye. Cells were cultured in RPMI-1640 medium consisting of 10 % FCS, 100 U/mL penicillin, and 0.1 mg/mL streptomycin. The culture media was changed twice a week and the cells were kept in a 5 % CO_2 incubator (Nuaire Co., Plymouth, MN, USA) at 37 ^oC in 25 cm^2 culture flasks. The hydroxyurea derivative-SB metal complexes were used at three concentrations (5, 10, and 20 µM) and solvent (DMSO) was used as control. After incubation, cell viability was assayed with 0.4 % trypan blue and a haematocytometer. If the viability was below 90 %, experiments were not started.

Also, the MTT assay was used to determine the cytotoxic effects of the test compounds. With this assay, living cells are able to generate water-insoluble formazan by catalysis via mitochondrial succinate dehydrogenase, which is quantified with a spectrophotometer. The stock MTT solution was prepared in sterile PBS at a final concentration of 0.5 mg/mL MTT. The SB-metal complexes, in addition to the cell lines, were incubated with the MTT solution at 37 ^oC for 3 hours, after which the absorbances of cells were read with a spectrophotometer. The experiment was repeated ten times and on different days.

In vivo studies for anticancer and antitumor activities

The animal studies were conducted with the approval of the Fırat University Ethical Committee for Animal Experiments (approval no. 2014/22:204) and followed international guidelines for animal studies. The rats (Wistar albino) were obtained from the Fırat University Experimental Research Center (FUDAM) and housed in cages cleaned daily. They were kept at a temperature of 22 ± 2 °C and a relative humidity of 55 ± 5 % with a ventilation system. Thirty-five (35) male Wistar albino rats (2 months old and 200 - 250 g) were used and were equally assigned to five groups as follows: an azoxymethane (AOM, 25 mg/kg) + (Oil + DMSO) group acting as the negative control, an azoxymethane + cisplatin (AOM + cis-15) group, which served as positive control, received 15 mg/kg dose of cisplatin after the tumor has developed, an azoxymethane + L-Cd (AOM + L-Cd) group administered 25 mg/kg L-Cd after the tumor had developed, an azoxymethane + L-Cu (AOM + L-Cu) group given 25 mg/kg L-Cu after the tumor had developed and an azoxymethane + L-Zn (AOM + L-Zn) group which was treated with 25 mg/kg L-Zn after the tumor had developed. All solutions were prepared in DMSO and corn oil before injection due to DMSO toxicity (final concentration of 10 % DMSO or less). The final concentration of 25 mg/kg in 1 mL was injected subcutaneously. For the control group, 1 mL of subcutaneous injection of DMSO-containing corn oil was administered [11].

Animal treatment

Azoxymethane was administered subcutaneously once a week (repeated 3 times) at a dose of 15 mg/kg body weight (BW) dose to induce colon cancer formation [11]. The rats were observed for 5 months while the tumors formed. At the end of this period, two animals from each group were sacrificed and the colorectal tissues were harvested and examined. Tumor formation was observed by macroscopic and microscopic examinations. The rats then received cisplatin (15 mg/kg body weight) and SB- metal complexes (L-Cd, L-Zn, L-Cu; 25 mg/kg body weight) via subcutaneous injection which was repeated after three days, for up to 64 days. Pathological examinations of the animals that died during the study were also recorded. At the end of the study, the rats were anesthetized with ether and were sacrificed. Colorectal tissues were removed and processed for histological analyses. Paraformaldehyde (4 %) was used for fixing the tissues for macroscopic examination of tumors after which sections were photographed with a light microscope.

Histological procedure

After the abdominal cavity was opened, the colorectal tissues were immediately removed for histological examinations. The 2 cm tissue samples, which were taken from the 10 cm column section starting from the rectum border, were fixed in 10 % formaldehyde, dehydrated in ethanol and embedded in paraffin. Conventional tissue monitoring procedures were performed for the samples. The samples (5 µm thick, 300 µm-spaced serial block sections) were stained with hematoxylin and eosin (H&E) and then examined under light microscope.

Data analysis

The Statistical Package for Social Sciences (SPSS) was used for all statistical analyses and the data were expressed as mean ± standard error of the mean (SEM). Normal distribution of the groups was assessed by using the Kolmogorov-Smirnov test and the comparison of groups was by one-way ANOVA. Levene test was used for the homogeneity of the variances while the Tamhane T2 test was used for multiple comparisons. A p-value of < 0.05 was considered statistically significant.

RESULTS

In vitro antitumor effect on HT-29 cells

The *in vitro* antitumor effects of metal complexes of hydroxyurea derivative-SB metal compounds on HT-29 cell line are shown in Figure 1. Schiff base complexes had significant cytotoxic effects on the cells ($p < 0.05$). The results reveal statistically significant differences when comparing between the groups at 5, 10 and 20 µM concentrations of all compounds (Figure 1). It was also observed that the SB complexes used in the study were more effective on the HT-29 cells than cisplatin which was used as a positive control.

Macroscopic and histopathology examinations

The macroscopic investigations revealed lesions located in the proximal or distal colonic area and were poly-shaped (Figure 2). The macroscopic and microscopic lesions of the experimental groups are given in Table 1.

Two masses were observed in the Oil + DMSO negative control group and the L-Zn group, respectively, with one mass seen in the L-Cd group. Invasive adenocarcinoma structures were seen in three Oil + DMSO-treated rats and a signet ring formation was evident in one of them. (Figure 2, c-i). Dysplasia lesions in the nuclei of glandular epithelium cells were detected including hyperchromasia and nuclear growth.

Table 1: Macroscopic and microscopic lesions

Group	Macroscopic mass	Location			Weak dysplasia	Strong dysplasia	Malignant tumoral lesion	Total lesions
		P	*M*	*D**				
Oil+DMSO	2	1	-	1	9	2	4	15
Cis-15	-	-	-	-	4	1	-	5
L-Cd	1	-	-	1	12	2	2	16
L-Cu	-	-	-	-	8	-	-	8
L-Zn	2	1	-	1	9	1	3	13

*Proximal (P), middle (M), and distal (D) colon

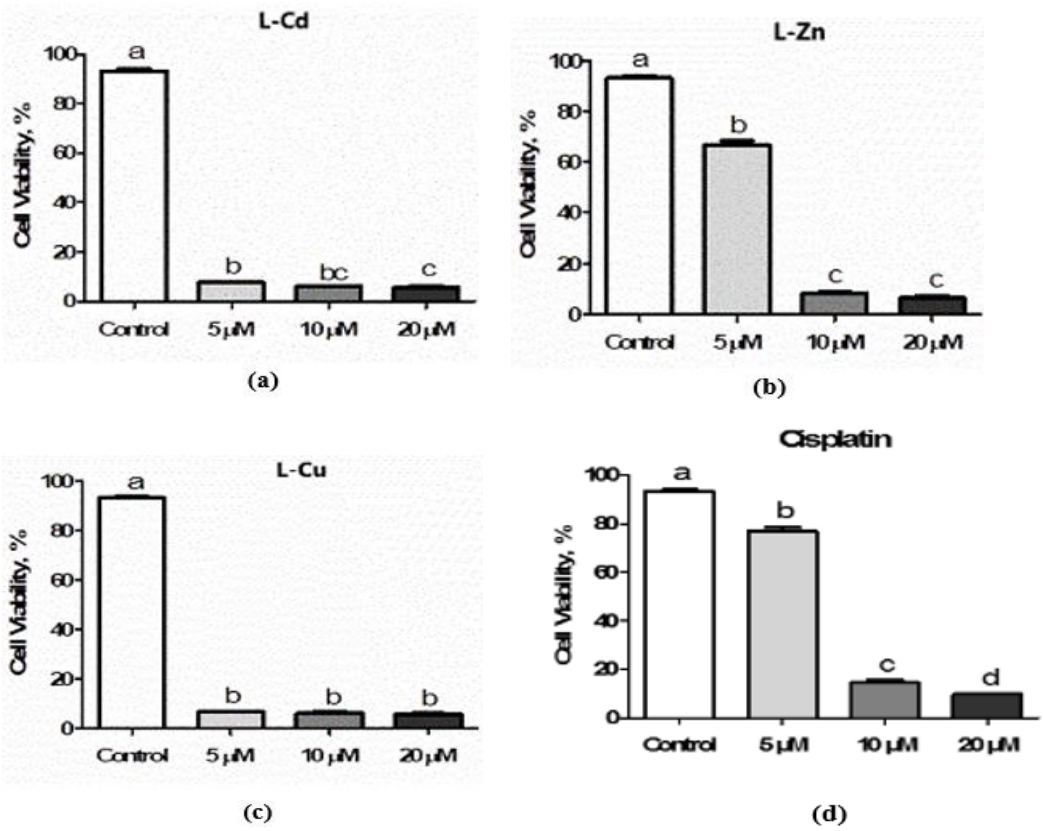
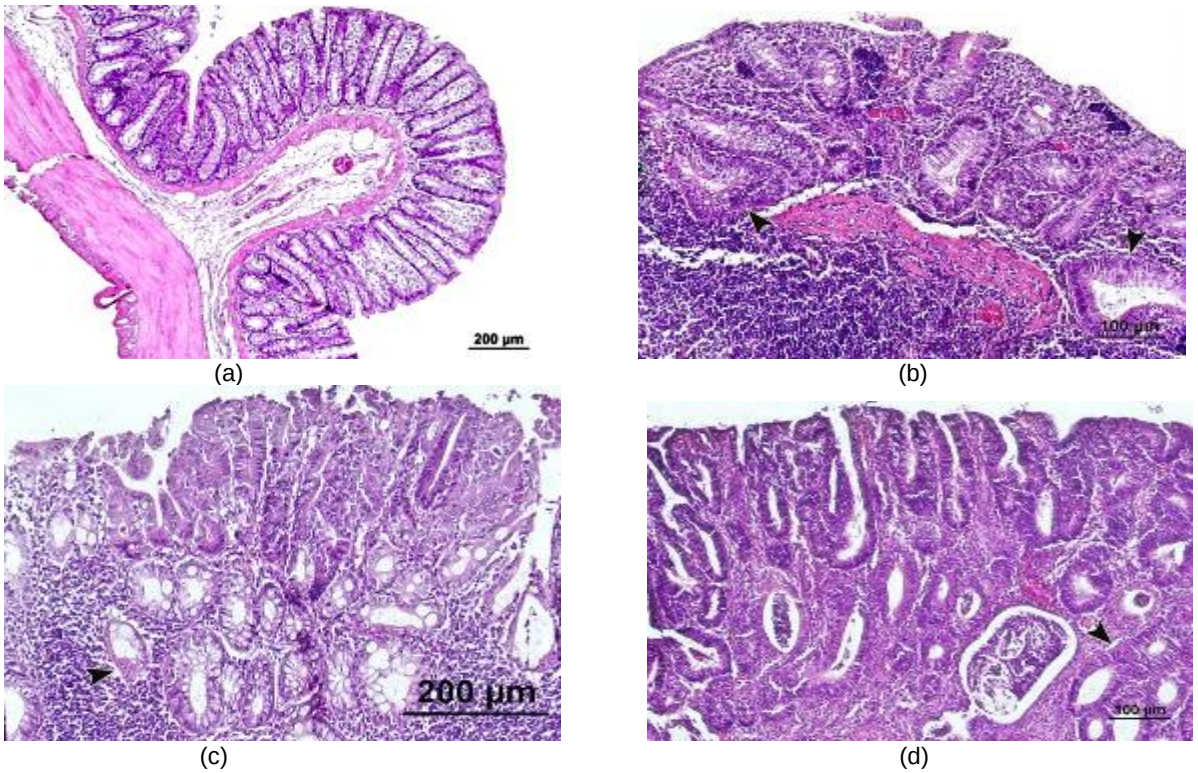
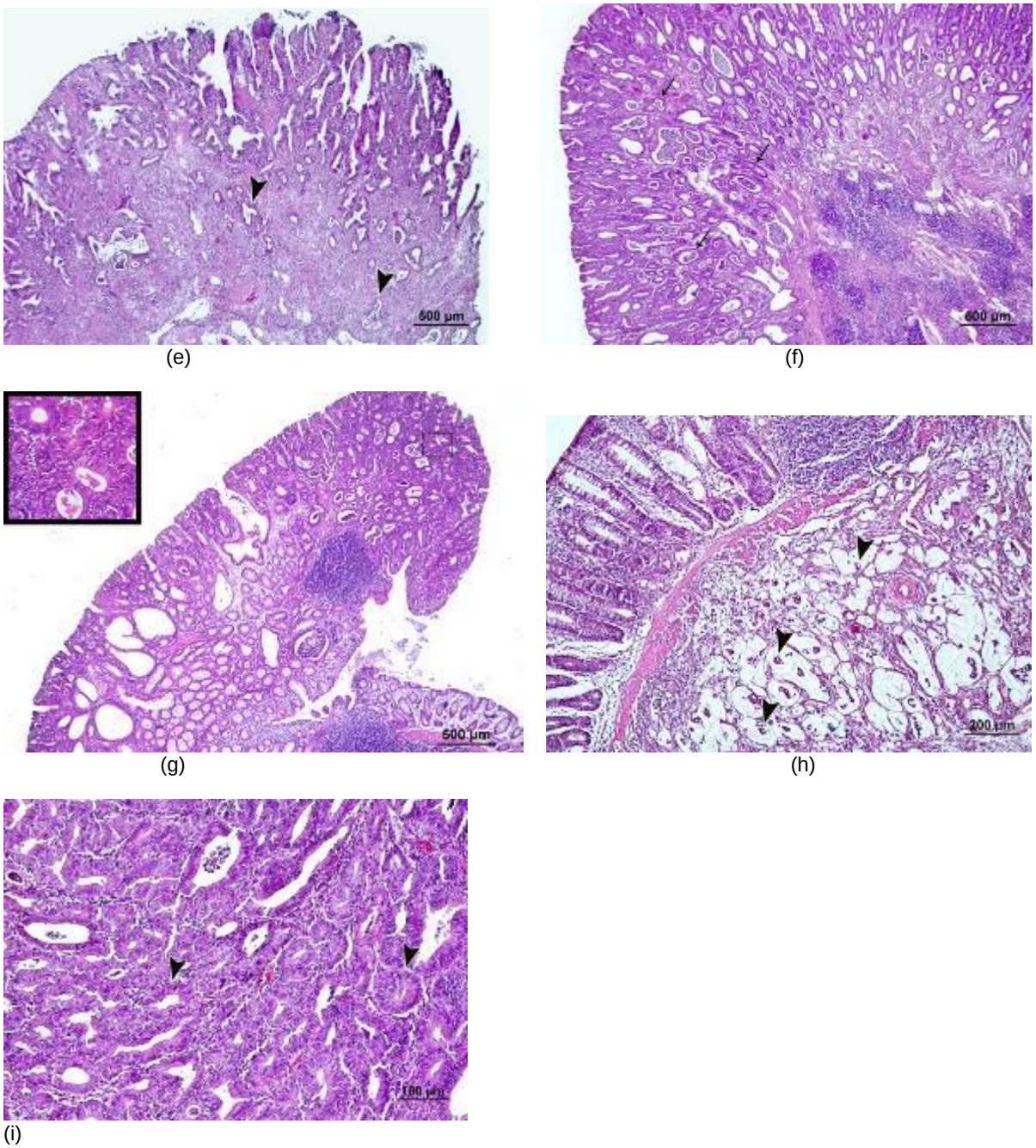


Figure 1: Cell viability levels of a) L-Cd group, b) L-Zn group, c) L-Cu group and d) cisplatin group ($p < 0.0001$)

Furthermore, the glands had increased nucleus-to-cytoplasm ratios, deformities and structural irregularities. In addition, karyomegaly in nuclei was present and instances of adenocarcinoma in neoplastic cells revealed pleomorphism in the nuclei. However, the L-Cu and cisplatin treatment groups had no mass formation in the column mucosa (Figure 2, b).





(e) (f) (g) (h) (i)

Figure 2: Histopathological examination of the colorectal tissues **(a)** colon histology of the control group, HE x50; **(b)** mild severe dysplasia (arrowheads) in the L-Cu group appearance in the colon epithelium, HE x100; **(c)** severe dysplasia (arrowhead) in the oil + DMSO group, HE x200; **(d)** severe dysplasia (arrowhead) of the column mucosa in the cis-15 group, HE x100; **(e)** Adenocarcinoma with submucosal invasion (arrowheads) in the oil + DMSO group, HE x20; **(f)** Good differential tubular adenocarcinoma (arrows) in the L-Cd group, HE x20; **(g)** Adenocarcinoma in L-Zn group, inset: tubular adenocarcinoma cells, HE x20; **(h)** Mucinous adenocarcinoma with submucosal localization in the form of stony rings (arrowheads) in the oil + DMSO group, HE x50; **(i)** severe dysplasia (arrowheads) of the column mucosa in the cis-15 group, HE x100

DISCUSSION

Schiff base (SB) ligands play an important role in biological applications and have antiproliferative, antiviral, anti-malarial, antipyretic, anti-inflammatory, antibacterial, and antifungal properties [12]. The *in vitro* biological activities of SBs have been investigated by several groups and the research has become even more important because of the wide range of applications of SBs. Hydroxyurea (HU) is known to have biological effects on living organisms, including well-reported genotoxic abilities and anti-tumor activity in myeloproliferative disorders [13], and sickle cell anemia [14]. Additionally, hydroxyurea was shown to improve spatial

memory in a mouse model of Alzheimer's disease [15]. Cadmium (Cd) is a toxic metal that displays osteotoxicity, immunotoxicity, and nephrotoxicity and promotes tumor formation after long-term exposure to the kidney, testes, lungs, and liver after acute poisoning [16].

Copper (II) is essential for normal development and organ functions in early childbirth as its deficiency (hypocopperia) is a factor that causes low birth weight. Furthermore, Cu is necessary for the function of many enzymes, such as ascorbate oxidase, copper lysyl oxidase, uricase, cytochrome oxidase, trizonase, and Zn-Cu superoxide dismutase enzymes [17]. The trace element, Zn (II), is important for the function and regular development of different organs in the body. Negative nitrogen balance in Zn deficiency, weakness in the immune system, psychiatric symptoms, delay in wound healing, and impaired development have been reported [18].

In this study, the viability of HT-29 cell line was determined by the trypan blue method. An increase in the concentration of the test compound (5, 10 and 20 µM) was used to challenge each cell line for 24 h. Consequently, dose-dependent cell viability levels were observed in the results and the treated samples were significantly different from the control. Furthermore, HU derivative-SB metal complexes produced dose-dependent anti-proliferative activity in the HT 29 cell culture. Cytotoxicity of the xenobiotic could be explained by the impairment of either the cellular regulation system or intracellular synthesis of macromolecular or cellular transduction signaling [19]. In this study, 1-hydroxy-3-(E)-pyridine-3-ylmethylidene urea derivative-SB metal complexes were used. It is well established that the amine group in the molecule increases the inhibitory effect [20]. The complexes used in this study were intended to quickly kill the proliferating cells and both hydroxyurea as well as SBs show anticancer properties. All tested compounds decreased cell viability in HT-29 cancer cell line in a dose-dependent manner.

For the *in vivo* experiments, there was no tumor formation in the control group colon tissues and rats treated with AOM (except for the L-Cu group) displayed severe dysplasia. Adenocarcinoma formations were observed in the oil + DMSO, L-Cd, and L-Zn groups, and no tumor formations were detected in the colon mucosa of the L-Cu and cisplatin groups. The redox potentials of the metal ions and the conformation of the organic ligands significantly influence the biological behavior of the coordination compounds [7]. The scientific data in this study supports these published studies.

CONCLUSION

Based on the results obtained, L-Cu and cisplatin compounds exhibit strong antitumor properties. Hydroxyurea derivative SB-Cu complex is effective in preventing colon cancer in rats. Therefore, further studies of the L-Cu compound would be beneficial for the validation of its antitumor properties.

DECLARATIONS

Acknowledgements

We thank The Management Unit of Scientific Research Projects of Firat University (FUBAP, Project no. F.F.15.05) for their financial support. We also thank Dr Suleyman SANDAL for his help with the antitumor activity aspects of the study. our appreciation goes to Dr Mustafa Karatepe for his contribution and help.

Funding

None provided.

Ethical approval

The studies were conducted with the approval of the Fırat University Ethical Committee for Animal Experiments (approval no. 2014/22:204).

Availability of data and materials

The datasets used and analyzed during the current study are available from the corresponding author upon reasonable request.

Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

The authors declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. Hafize Telceken and Ali Osman Ceribasi designed the experiments, and Ali Osman Ceribasi conducted the experiments. Zuhal Karagöz Genç synthesized the compounds. Hafize Telceken and Songül Ceribasi analyzed and interpreted the data, while Ali Osman Ceribasi and Arzu

Karatepe prepared the manuscript with contributions from all the co-authors.

Open Access

REFERENCES

1. *Siegel RL, Wagle NS, Cercek A, Smith RA, Jemal A. Colorectal cancer statistics, 2023. CA Cancer J Clin 2023; 73: 233.*
2. *American Cancer Society. Colorectal cancer facts and figures. Available at www.cancer.org/research/cancer-facts-statistics/colorectal-cancer-facts-figures.html. Accessed 2014.*
3. *Musiałek MW, Rybaczek D. Hydroxyurea - the good, the bad and the ugly. Genes 2021; 12: 1096*
4. *Liberta AE, West DX. Antifungal and antitumor activity of heterocyclic thiosemicarbazones and their metal complexes: current status. Biometals 1992; 5: 121.*
5. *Santos MLP, Faljoni-Alário A, Mangrich AS, da Costa Ferreira AM. Antioxidant and pro-oxidant properties of some di-Schiff base copper (II) complexes. J Inorg Biochem 1998; 71: 71.*
6. *Dogan A, Basak N, Demirci S, Telci D, Dede B, Tuzcu M, Ozercan IH, Sahin K, Sahin F. A novel Schiff base derivative for effective treatment of azoxymethane-induced colon cancer. Int J Pharm Sci Res 2014; 5: 3544.*
7. *Ďuračková Z, Mendiola MA, Sevilla MT, Valent A. Thiohydrazone copper (II) complexes. The relationship between redox properties and superoxide dismutase mimetic activity. Bioelectrochem Bioenerg 1999; 48: 109.*
8. *Kelestemur U. Investigation of antioxidant and antitumor properties of hydroxyurea derivatives Schiff bases and some metal complexes. (Dissertation). Firat University; 2010.*
9. *Adiguzel R, Esener H, Ergin Z, Aktan E, Sekerci M. Synthesis and characterization of novel Ni (II), Cu (II) and Cd (II) complexes of 4-(2-Chlorphenylazo)-1H-pyrazole-3, 5-diamine. Asian J Chem 2011; 23: 1846.*
10. *Esener H, Adiguzel R, Ergin Z, Aktan E, Turan N, Sekerci M. Synthesis and characterization of novel Mn (II), Co (III), Ni (II) and Cd (II) complexes from 4-(2-nitrophenylazo)-1H-pyrazole-3, 5-diamine. Adv Sci Lett 2011; 4: 3669.*
11. *Parlak AE, Tekin S, Karatepe A, Koparir P, Telceken H, Ceribası AO, Karatepe M. In vitro and histological investigation of antitumor effect of some triazole compounds in colon cancer cell line. J Cellular Biochem 2019; 1-11.*
12. *Sinha D, Tiwari AK, Singh S, Shukla G, Mishra P, Chandra H, Mishra AK. Synthesis, characterization and biological activity of Schiff base analogues of indole-3-carboxaldehyde. Euro J Med Chem 2008; 43: 160.*
13. *Spivak JL, Hasselbalch H. Hydroxycarbamide: A user's guide for chronic myeloproliferative disorders. Expert Rev Anticancer Ther 2011; 11: 403.*
14. *Yogev O, Anzi S, Inoue K, Shaulian E. Induction of transcriptionally active Jun proteins regulates drug-induced senescence. J Biol Chem 2006; 281: 34475.*
15. *Brose RD, Lehrmann E, Zhang Y, Reeves RH, Smith KD, Mattson MP. Hydroxyurea attenuates oxidative, metabolic and excitotoxic stress in rat hippocampal neurons and improves spatial memory in a mouse model of Alzheimer's disease. Neurobiol Aging 2018; 72: 121.*
16. *Liu J, Qu W, Kadiiska MB. Role of oxidative stress in cadmium toxicity and carcinogenesis. Toxicol Applied Pharmacol 2009; 238: 209.*
17. *Al-Bader A, Mathew T, Khoursheed M, Asfar S, Al-Sayer H, Dashti H. Thioacetamide toxicity and the spleen: histological and biochemical analysis. Anat Histol Embryol 2000; 29: 3.*
18. *Jomova K, Makova M, Alomar SY, Alwasel SH, Nepovimova E, Kuca K, Rhodes CJ, Valko M. Essential metals in health and disease. Chemico-Biological Interact 2022; 367: 1.*
19. *Wen L, Han Z. Identification and validation of xenobiotic metabolism-associated prognostic signature based on five genes to evaluate immune microenvironment in colon cancer. J Gastrointestinal Oncol 2021; 12: 2788.*
20. *Hosomi H, Akatsuka A, Dan S, Iwasaki H, Nambu H, Kojima N. Synthesis of acetogenin analogs comprising pyrimidine moieties linked by amine bonds and their inhibitory activity against human cancer cell lines. Chem Pharm Bull 2022; 70: 823.*