Investigation of Antimutagenic Activities of *Commiphora Gileadensis* Essential Oil Against Cyclophosphamide Induced Genotoxicity in Mice

Asmaa S. Salman [a,1,*], Emad A. Alsherif [b]

[a] Genetic and Cytology Department, National Research Center, Dokki, Cairo, Egypt
[b] Botany and Microbiology Department, Beni Suef University, Egypt.
[1] drasmaasalman@gmail.com
* corresponding author

ARTICLE INFO

Article history
Received August 13, 2023
Revised November 19, 2023
Accepted December 30, 2023

Keywords
Commiphora gileadensis
cyclophosphamide
chromosomal aberrations
DNA fragmentation
sperm

ABSTRACT

Commiphora gileadensis, a wild aromatic medicinal plant, is part of the Burseraceae family, which contains 190 species under the genus Commiphora. It has both ethnobotanical and therapeutic applications. Currently, the antigenotoxic effects of Commiphora gileadensis essential oil (CEO) was investigated by assessing chromosomal anomalies in bone marrow cells, DNA fragmentation in hepatocytes, chromosomal aberrations in spermatocytes, and sperm head and tail anomalies in mice. Animals were divided into the following groups: Cyclophosphamide (CP) treated groups (20 mg /kg b.w.), the control group (received no treatment), corn oil treated group, group administered CEO for 7 days at 300 mg /kg b.w., three groups administered CEO for 7 days at (100, 200 or 300 mg /kg b.w.) plus cyclophosphamide. In vivo investigations demonstrated that preliminary treatment with the tested concentrations of CEO reduced CP-induced injury. Therefore, research has shown that the CEO is a potential factor for protection against the genetic toxicity caused by CP.

1. Introduction

Plants offer a diverse array of natural compounds which could serve as the basis of advanced conventional therapeutic systems, making them a constant source of research for the development of new drugs [1]. *Commiphora gileadensis* (L.), also known as *Commiphora opobalsamum*, Amyris gileadensis, Amyris opobalsamum, or Balsamodendron opobalsamum, is a therapeutic plant in the family Burseraceae [2-3]. These plants are found in several places, including Arabia (Yemen and Oman), Africa (Somalia, Ethiopia), and Sudan, as well as in India [4]. It's referred to as Ood-e-balsam, Bechan, Bisham, Balm of Mecca, or Balsam, Balessan. Also, the Bible was named "Balm of Gilead," whereas in Hebrew it is known as "Apharsemon" [5]. Methanolic extract of *Commiphora molmol* displayed notable antibacterial activity in Gram-positive and Gram-negative bacteria. Additionally, the significant effectiveness of its oil extract against resistant *S. aureus* was documented [6]. Al-Abdallah et al., [7] noted that the methanolic leaf extract of *Commiphora gileadensis* exhibited efficacy in reducing the growth of several bacterial species through the use of one procedure. Al-Hazmi et al. [8] documented that Pseudomonas aeruginosa was effectively inhibited by the whole *Commiphora gileadensis* methanolic extract in vivo. The results indicate that *C. leptophloeos* has promising preventive effects against *S. aureus* resistance and against various pathogenic microbes [9]. Studies of Salman et al., [10] showed that treatment with leaves extract of

C. gileadensis significantly reduced chromosomal abnormalities in bone marrow cells, DNA fragmentation in hepatocytes and sperm shape anomalies induced by CCL4.

Currently, Cyclophosphamide (CP) is used in treatment of cancer and autoimmune diseases [11-12]. Bioactivation of CP release cytotoxic alkylating phosphoramide mustard and acrolein metabolites [13]. Therefore, the goal of the current study is to provide new insights into Commiphora gileadensis essential oil (CEO) ameliorative activities against CP induced genotoxicity.

2. Materials and methods

2.1. Chemicals

Commiphora gileadensis essential oil: Aldousiah farm, Saudi Arabia. Cyclophosphamide: Sigma-Aldrich (St. Louis, MO).

2.2. Animals and treatment

The animals were male, 9-12 weeks old and weighed 25-27g. The source of this strain was the Animal House Colony at National Research Center, Egypt. CEO were examined in 3 tested doses (100, 200 and 300 mg /kg b.w diluted in corn oil for 7 days).

Animals were divided into the following groups: CP treated groups: single intraperitoneal injection of 20 mg /kg b.w. 24h. prior sacrifice; the control group (received no treatment); corn oil treated group; group administered CEO for 7 days at 300 mg /kg b.w.; three groups administered CEO for 7 days at (100, 200 or 300 mg /kg b.w.) plus cyclophosphamide.

2.3. Chromosomal Aberration for Bone Marrow Cells

To evaluate any abnormalities at the chromosomal level, the bone marrow of mice that were administered an intraperitoneal injection of colchicine two hours prior to euthanasia was utilized [14]. Protocols of Yosida and Amano, [15] was utilized with some modifications to prepare chromosomes from bone marrow cells.

2.4. DNA Fragmentation Assay

The DNA fragmentation procedure was conducted by the methodology detailed by Perandones et al., [16].

2.5. Chromosomal abnormalities in spermatocytes

Preparation of the meiotic chromosomes was done by Evan et al., [17].

2.6. Sperm shape abnormalities assay

The method of Wyrobek and Bruce [18] was used.

3. Results and Discussion

In somatic cells, the mean percentage of metaphases with chromosomal aberrations after receiving an i.p. injection of CP was 18.2 % ($P<0.01$) (Table 1). DNA fragmentation in hepatocytes significantly increased to 10.0% ($P<0.01$) (Table 2). In germ cells the mean percentage of abnormal diakinesis metaphase I cells was 11.8% ($P<0.01$) 24 hours after receiving a single dose of CP (Table 3). Treatment with CP significantly raised the abnormal sperm's percentage compared to control untreated group (Table 4). CP were observed to induce the breakage in chromosomes, increasing the percentage of micronuclei and elevate the percentage of sister chromatid exchange [19-20]. CP were observed to affect sperm count and absence of spermatogenic cycle [21]. Animals treated with 300 mg/kg b.wt of CEO showed statistically insignificant percentages of aberrant bone marrow cells as compared to the control group. In addition, hepatocytes DNA fragmentation was observed in the control normal level. Consecutive administration of 200 or 300 mg/kg of CEO for seven days significantly ($P<0.01$) suppressed the proportion of chromosomal abnormalities triggered by CP. The inhibition percent was 40.65 and 49.45%, respectively (Fig. 1). DNA fragmentation decreased to 7.2 and 5.54% after treatment with the two doses respectively (Table 2).

Table 1. Number and mean percentage of the different types of chromosomal aberrations in mouse bone marrow cells after treatment with CEO alone or in combination with CP.

Treatment and doses (mg/kg b.wt.)	No. of metaphases with				Chromosomal aberrations	
	Gap	frag. and /or break	Deletion	Total	Excluding gaps Mean	Including gaps
CP	19	66	6	72	14.4 ± 0.5^{a}	18.2 ± 07^{a}
Control	2	6	0	6	1.2 ± 0.2	1.6 ± 0.4
Corn oil	1	6	1	7	1.4 ± 0.2	1.6 ± 0.25
CEO (300)	2	8	0	8	1.6 ± 0.22	2.0 ± 0.24
CEO (100) + CP	14	50	4	54	10.8 ± 0.54	13.6 ± 0.7
CEO (200) + CP	10	39	5	44	8.8 ± 0.56^{b}	10.8 ± 0.24^{b}
CEO (300) + CP	9	34	3	37	7.4 ± 0.27^{b}	9.2 ± 0.6^{b}

a: significant at 0.01 level (t test) compared to control (non- treated).
b: significant at 0.01 level (t test) compared to treatment (CP)

Table 2. DNA fragmentation in hepatocytes after treatment with CEO alone or in combination with CP.

Treatment and doses (mg/kg b.wt.)	DNA fragmentation Mean% ± S.E.
CP	10.0 ± 0.44^{a}
Control	3.2 ± 0.3
Corn oil	3.2 ± 0.2
CEO (300)	3.3 ± 0.25
CEO (100) + CP	8.54 ± 0.42
CEO (200) + CP	7.2 ± 0.24^{b}
CEO (300) + CP	5.54 ± 0.52^{b}

a: significant at 0.01 level (t test) compared to control (non- treated).
b: significant at 0.01 level (t test) compared to treatment (CP)

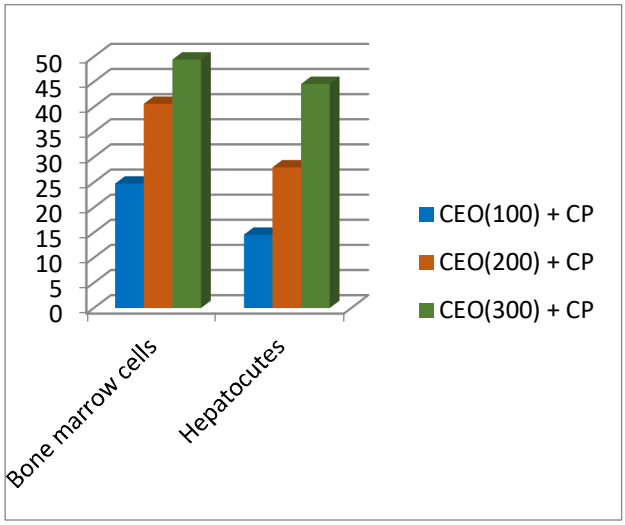

Fig. 1. Inhibition percentage of the tested doses of CEO in bone marrow chromosomal aberration and hepatocytes DNA fragmentation.

In germ cells the mean percentage of abnormal diakinesis metaphase I cells was 11.8 % (P<0.01) 24 hours after receiving a single dose of CP. Treatment with CP significantly (P<0.01) raised the abnormal sperm's percentage compared to control untreated group (Table 4).

The antigenotoxic role of CEO was examined in germ cells. Treatment with 200 or 300 mg/kg b.wt. of CEO significantly (P<0.01) reduced the CP-induced abnormalities in spermatocytes reached 7.2 and 5.4% (P<0.01) respectively (Table 3). The inhibition percentage observed in sperm abnormalities reached 35.36 and 46.34 % after cotreatment with these doses respectively (Fig.4).

Table 3. Number and mean percentage of the different types of chromosomal aberrations in mouse spermatocytes after treatment with CEO alone or in combination with CP.

Treatment and doses (mg/kg b.wt.)	No. of metaphases with			Total aberrations	
	XY Univalent	Autosomal univalent	XY+ Autosomal Univalent	NO.	Mean% ± S.E.
CP	37	14	8	59	11.8 ± 0.21[a]
Control	7	3	0	10	2.0 ± 0.14
Corn oil	8	3	0	11	2.2 ± 0.2
CEO (300)	9	3	1	13	2.6 ± 0.12
CEO (100) + CP	32	12	5	49	9.8 ± 0.22
CEO (200) + CP	20	9	7	36	7.2 ± 0.2 [b]
CEO (300) + CP	15	5	7	27	5.4 ±0.2 [b]

a: significant at 0.01 level (t test) compared to control (non- treated).
b: significant at 0.01 level (t test) compared to treatment (CP)

Table 4. Number and percentage of different types of sperm shape abnormalities in male mice after treatment with CEO alone or in combination with CP.

Treatment and doses (mg/kg b.wt.)	No. of sperms with					Abnormal sperm No.	Abnormal sperms Mean % ± S.E.
	Head abnormalities				Tail abnormalities		
	Triangle	Amorphous	Without hook	Banana	Coiled		
CP	144	102	81	18	65	410	8.2 ± 033^{a}
Control	50	31	17	0	12	110	2.2 ± 0.3
Corn oil	42	36	9	1	12	100	2.0 ± 0.21
CEO (300)	45	28	11	2	19	105	2.1 ± 0.18
CEO (100) + CP	114	94	66	12	79	365	7.3 ± 0.15
CEO (200) + CP	85	69	36	9	66	265	5.3 ± 0.31^{b}
CEO (300) + CP	72	58	29	9	52	220	4.4 ± 0.23^{b}

a: significant at 0.01 level (t test) compared to control (non- treated).
b: significant at 0.01 level (t test) compared to treatment (CP)

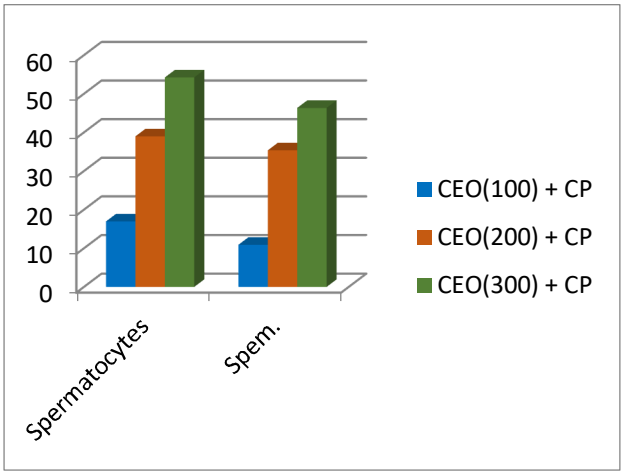

Fig. 2. Inhibition percentage of the tested doses of CEO in spermatocytes and sperm shape abnormalities.

It is vital to mention that the Commiphora genus emerged as a good source of traditional medicine and was found to contain multiple phytochemicals that exhibit antioxidant properties as evidenced by several studies. Research conducted by Gowri Shankar et al. [22] implies that bark extract of Commiphora berryi could be considered a hepatoprotective agent against CCl4-induced oxidative liver damage in rats. They concluded that this effect may be attributed to its capacity to neutralize free radicals and antioxidant properties. Ahamed et al. [23] observed that ethanolic extract of Commiphora myrrh possesses the capacity to mitigate oxidative stress and lipid peroxidation triggered by D-galactosamine/lipopolysaccharide (D-GaIN/LPS) in liver. Compaoré et al. [24] reported that Commiphora. africana had a significant in vitro antioxidant and anti-inflammatory properties due to the presence of bioactive compounds including p-Coumaric acid, ferulic acid, isoquercitrin, quercitrin, and quercetin. The ethyl acetate extracts of Commifora mukul were observed to display promising antioxidant properties [25]. Ezenyiet et al. [26] revealed that the ethanol extract from stem bark of Commiphora kerstingii demonstrated protective effects against liver damage triggered by carbon tetrachloride, providing some basis for its traditional use in treating liver diseases. Studies conducted by Farida et al. [27] showed that stem bark of Commiphora opobalsamum is a potentially effective natural remedy for liver injury, hyperglycemia, oxidative stress, inflammation, hyperlipidemia, and DNA damage. Moreover, Research conducted by Alahmari et al. [28] suggested that treatment of rats with 500 mg Commiphora myrrha /kg for one month significantly reduced ethanol consumption-induced liver toxicity. The results of this study indicate that Commiphora myrrha extract contains abundant sesquiterpenoids, which possess antioxidant properties and can neutralize reactive oxygen species.

In the present study the ameliorative activity of CEO against CP induced injury in somatic and germ cells could be attributed to its antioxidant activity. Antioxidants could act through various mechanisms: (1) preventing or reducing the formation of free radicals; (2) neutralizing free radicals; (3) transforming free radicals into less harmful substances; (4) minimizing the production of secondary toxic active species; (5) preventing the chain propagation reaction; (6) improving the body's natural antioxidant response by acting in association with other antioxidants; and (7) capturing metal ions (Losada-Barreiro et al., [29]. ROS are tiny, very reactive molecules that contain oxygen and have short 74 half-life. Superoxide anions, hydrogen peroxide (H2O2), and hydroxyl radicals are the three most significant subtypes of ROS that cause cell damage and even death out of the more than 20 different forms of ROS [30].

4. Conclusion

The current study demonstrated the ameliorative effects of CEO, which included a considerable reduction in the defective chromosomes in bone marrow cells, fragmentation of DNA in hepatic

cells, and sperm shape abnormalities caused by CP. Furthermore, this study showed clear evidence of the capability of CEO to minimize the strong oxidative damage caused by chemical substances and cytogenetic abnormalities. Additional studies on CEO are needed to identify the mechanisms engaged in these antigenotoxic activities.

Acknowledgement

Authors are grateful for the National Research Centre for all the facilities and tools provided.

References

[1] Nasim, N., Sandeep, I. S., Mohanty, S. (2022). Plant-derived natural products for drug discovery: current approaches and prospects. The Nucleus: an international journal of cytology and allied. *Topics, 65(3),* 399–411.

[2] Amiel, E., Ofir, R., Dudai, N., Soloway, E., Rabinsky, T., Rachmilevitch, S. (2012). β-Caryophyllene, a compound isolated from the biblical balm of gilead (Commiphora gileadensis), is a selective apoptosis inducer for tumor cell lines. *Evidence-based complementary and alternative medicine,* 2012.

[3] Al-Abdallat, A. M., Adayileh, B. K., Sawwan, J. S., Shibli, R., Al-Qudah, T. S., Abu Irmaileh, B., Bastani, Y. (2023). Secondary metabolites profiling, antimicrobial and cytotoxic properties of Commiphora gileadensis L. leaves, seeds, callus, and cell suspension extracts. *Metabolites, 13(4),* 537.

[4] Shadid, K. A., Shakya, A. K., Naik, R. R., Al-Qaisi, T. S., Oriquat, G. A., Atoom, A. M., Farah, H. S. (2023). Exploring the chemical constituents, antioxidant, xanthine oxidase and COX inhibitory activity of Commiphora gileadensis commonly grown wild in Saudi Arabia. *Molecules, 28(5),* 2321.

[5] Ahmed, H., Rashed, M. M., Almoiliqy, M., Abdalla, M., Bashari, M., Zaky, M. Y., ... & Jiang, L. P. (2023). Antioxidant activity and total phenolic compounds of Commiphora gileadensis extracts obtained by ultrasonic-assisted extraction, with monitoring antiaging and cytotoxicity activities. *Food Science & Nutrition. 11(6),* 3506–3515.

[6] Mohammed, E. A., & Samy, A. S. (2013). Antimicrobial activity of essential oil and methanol extract from Commiphora molmol (Engl.) resin.International Journal of *Current Microbiology and Applied Sciences, 2* (12).

[7] Abdallah, H. M., Mohamed, G. A., Ibrahim, S. R., Koshak, A. E., Alnashri, I., Alghamdi, A., ... & Khairy, A. (2022). Commigileadin A: A new triterpenoid from: Commiphora Gileadensis: Aerial parts. *Pharmacognosy Magazine, 18(78),* 256-260.

[8] Al-Hazmi, A.S., Alherthi, M. N., Aljuaid, M. M., Alfifi, O. A., Alshaer, R. S., Alsaadi, R. S., Almehmadi, M. M., ... & Hawash, Y. A. (2020). In vitro and in vivo antibacterial effect of Commiphora gileadensis methanolic extract against methicillin-resistant staphylococcus aureus (MRSA) and pseudomonas aeruginosa. Pakistan Journal of Biological Sciences ,23(12), 1676-1680.

[9] de Souza Pereira, J. J., Pereira, A. D. P., Jandú, J. J., Da Paz, J. A., Crovella, S., dos Santos Correia, M. T., & de Azevêdo Silva, J. (2017). Commiphora leptophloeos phytochemical and antimicrobial characterization. *Frontiers in microbiology*, 8, 52.

[10] Salman A.S., Alsherif E.A., Althobiti S.M. (2023). Evaluation of the potential protective effect of Commiphora gileadensis on CCL4 induced genotoxicity in mice *Egypt. J. Chem. Vol. 66, No. SI 13* 1935 - 1944

[11] Bergmann M., Wendtner C.M. [Chronic lymphocytic leukemia]. *Dtsch Med Wochenschr* 2015; 140(7): 479-82. German.

[12] Dan D, Fischer R, Adler S, Förger F, Villiger PM. Cyclophosphamide: as bad as its reputation? Long-term single centre experience of cyclophosphamide side effects in the treatment of systemic autoimmune diseases. Swiss Med Wkly 2014; 144: w14030.

[13] Vredenburg G, den Braver-Sewradj S, van Vugt-Lussenburg BM, Vermeulen NP, Commandeur J, Vos JC. (2014). Activation of the anticancer drugs cyclophosphamide and ifosfamide by cytochrome P450 BM3 mutants. *Toxicol Lett*,*232(1),*182-92.

[14] Moore, P. D., Patlolla, A. K., & Tchounwou, P. B. (2011). Cytogenetic evaluation of malathion-induced toxicity in Sprague-Dawley rats. Mutation Research/Genetic Toxicology and *Environmental Mutagenesis, 725(1–2),* 78–82.

[15] Yosida, T. H., Amano, K. (1965). Autosomal polymorphism in laboratory-bred and Wild Norway rats, rattus norvegicus, found in Misima. *Chromosoma, 16(6),* 658 - 667.

[16] Perandones, C. E., Illera, V. A., Peckham, D., Stunz, L. L., & Ashman, R. F. (1993). Regulation of apoptosis in vitro in mature murine spleen T cells. *The Journal of Immunology, 151(7),* 3521–3529.

[17] Evans, E. P., Breckon, G., & Ford, C. E. (1964). An air-drying method for meiotic preparations from mammalian testes. *Cytogenetic and Genome Research, 3(5),* 289–294.

[18] Wyrobek, A.J.; Bruce, W.R. (1978). The induction of sperm-shape abnormalities in mice and humans. In Hallaender, A., & De Serres, F.J. (Eds.), Chemical Mutagens: Principles and methods for their detection. (pp. 257-285). New York and London: Plenum Press.

[19] Jain R., Jain S.K. (2012). Effect of Buchanania lanzan Spreng. bark extract on cyclophosphamide induced genotoxicity and oxidative stress in mice. *Asian Pac J Trop Med, 5(3),* 187-91.

[20] El-Souda S.S.E., Mohammed R.S., Marzouk M.M., Fahmy M.A., Hassan Z.M., Farghaly A.A. (2014). Antimutagenicity and phytoconstituents of Egypitan Plantago albicans L. *Asian Pac J Trop Dis; 4(2),* S946-51.

[21] Green D.M., Liu W., Kutteh W.H., Ke R.W., Shelton K.C.(2014). Sklar C.A., Cumulative alkylating agent exposure and semen parameters in adult survivors of childhood cancer: a report from the St Jude Lifetime Cohort Study. Lancet Oncol, 15(11): 1215-23.

[22] Gowri Shankar, N. L., Manavalan, R., Venkappayya, D., David Raj, C. (2008). Hepatoprotective and antioxidant effects of Commiphora berryi (Arn) Engl bark extract against CCl(4)-induced oxidative damage in rats. *Food and Chemical Toxicology, 46(9),* 3182–3185.

[23] Ahmad, A., Raish, M., Ganaie, M. A., Ahmad, S. R., Mohsin, K., Al-Jenoobi, F. I., Al Mohizea, A. M., Alkharfy, K. M. (2015). Hepatoprotective effect of Commiphora myrrha against d-GalN/LPS-induced hepatic injury in a rat model through attenuation genes. *Pharmaceutical of pro-inflammatory cytokines Biology, 53(12),* 1759–1767.

[24] Compaoré, M., Meda, R. N. T., Bakasso, S., Vlase, L., & Kiendrebeogo, M. (2016). Antioxidative, anti-inflammatory potentials and phytochemical profile of Commiphora africana (A. Rich.) Engl. (Burseraceae) and Loeseneriella africana (Willd.) (Celastraceae) stem leaves extracts. Asian Pacific *Journal of Tropical Biomedicine, 6(8),* 665–670.

[25] Mathur, S., Mulani, R., George, L.B., Barot, C., Thakkar, A., Bhingradiya, V., & Patel, R. (2016). Antioxidant and cytotoxic activity of Commiphora Mukul (GuGGul) extracts against HeLa cell- line. *International Journal of Approximate Reasoning, 4,* 1258-1267.

[26] Ezenyi, I.C., I. Okoro and C.A. Ufondu. (2021). Hepatoprotective potential of Commiphora kerstingii Engl. stem bark against carbon tetrachloride-induced acute liver injury. *Trends Med. Res, 16*, 7-13.

[27] Farid, M. M., Aboul Naser, A. F., Salem, M. M., Ahmed, Y. R., Emam, M., & Hamed, M. A. (2022). Chemical compositions of Commiphora opobalsamum stem bark to alleviate liver complications in streptozotocin-induced diabetes in rats: Role of oxidative stress and DNA damage. Biomarkers: biochemical indicators of exposure, response, and susceptibility to chemicals, 27(7), 671–683.

[28] Alahmari, A. S., El-Mekkawy, H. I., Al-Doaiss, A. A., & Alduwish, M. A. (2022). Effect of natural Commiphora myrrha extract against hepatotoxicity induced by alcohol intake in Rat Model. *Toxics, 10(12),* 729.

[29] Losada-Barreiro, S., Sezgin-Bayindir, Z., Paiva-Martins, F., Bravo-Díaz, C. (2022). Biochemistry of Antioxidants: Mechanisms and Pharmaceutical Applications. Biomedicines, 10(12), 3051.

[30] Yang, Y., Karakhanova, S., Werner, J., Bazhin, A. V. (2013). Reactive oxygen species in cancer biology and anticancer therapy. *Current medicinal chemistry, 20(30),* 3677–3692