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## Evaluation of SOD and MDA Levels with the Cytotoxicity of Some Plant Extracts Toward Human Rhabdomyosarcoma Cell Lines

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#### RESEARCH ARTICLE

# **Evaluation of SOD and MDA Levels with the Cytotoxicity of Some Plant Extracts Toward Human Rhabdomyosarcoma Cell Lines**

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#### **ABSTRACT**

Cytotoxicity of plant extracts has attracted more attention in the last years to assess their activities against tumor cells. Cancer chemoprevention focuses on plant extracts to be toxic toward tumor cell lines without affecting normal cells. The current study aimed to evaluate the cytotoxicity of ginger; olive leaf extracts and their mixture against Rhabdomyosarcoma cell lines (RD cell lines) and oxidative stress in terms of both Superoxide Dismutase (SOD) and Malondialdehyde (MDA). Alcoholic extracts of both ginger and olive leaves were obtained by Soxhlet method. The third extract was prepared by mixing equal quantities from both ginger and olive leaf extracts at a ratio of 1:1. Plates of RD cell lines and normal cell lines were used with different concentrations of the prepared extracts within the range (12.5–800  $\mu$ g/ml) were tested to determine their activity toward both RD and normal cell lines. Also, the levels of SOD and MDA were estimated depending on their kit procedures. The results showed significant differences in the cytotoxicity of the studied concentrations for each extract toward RD cell lines. The viability of RD cell lines was significantly decreased with increasing concentrations of the studied extracts, on the contrary, normal cells were not affected. Levels of SOD were increased with increasing extract concentration, in contrast with MDA levels. It is concluded that the current results of the studied extracts support the principles of cancer chemoprevention, besides their antioxidant properties to reduce oxidative stress and activate the normal antioxidant factors in human body.

Keywords: Antioxidant enzyme, Ginger rhizomes extract, Olive leaves extract, Peroxidation marker, Sarcoma cell line

#### Introduction

Plant extracts have received great attention to determine their extent to which they can be used in medical applications against various diseases <sup>1–3</sup> especially diabetes, <sup>4–6</sup> and tumors. <sup>7–9</sup> Numerous studies have also been conducted on the complications of these diseases to determine the factors that can be applied for their diagnosis or treated with plant extract as an antidiabetic agent. <sup>10–12</sup> The activity of the root extract of *Menyanthes trifoliata L*. was studied to estimate its effect on glioma cells. The study reported its anticancer properties against this type of cancer. It referred to the ability of root extract

to induce apoptosis in cancer cells by the action of its active components. <sup>13</sup> In another study, Bundit P. et al. <sup>14</sup> evaluated the activity of three plant extracts of medicinal herbs against cholangiocarcinoma (CCA). The study suggested that the studied extracts revealed an ability to reduce the viability of CCA cell lines via apoptosis.

The correlation between antioxidants and cytotoxicity for 57 plant extracts were investigated. It was found that the tested plant extracts have active components useful against free radicals, which lead to cancer. It was recommended to isolate these components and conduct further tests on different cancer cell lines to know their activities and the mechanisms

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of action. 15 On the other hand, the cytotoxicity of ethanolic extract of euphorbia tehranica root was examined toward colon cancer cell lines. The study suggested that the studied extract possesses a promising activity against cancer cell lines. 16 Similarly, the cytotoxicity toward human cancer cell lines for other plant extracts like Illicium verum, Glycyrrhiza glabra, Rhamnus Frangula, and Linum usitatissimum was investigated. The study suggested that these plant extracts showed a significant reduction in cancer cell line viability and recommended necessary additional studies to evaluate the cytotoxicity of more plant extracts toward different tumor cell lines. 17 Ethanoic and aqueous extracts of Rosa beggeriana Schrenk revealed anti-proliferative and cytotoxic effects on breast and human liver cell lines. Data in this work indicated that the ethanolic extract was more active than aqueous extracts and both extracts affected tumor cell lines but not normal cell lines. 18

Also, it was reported that the methanolic extract of Euphorbia lathyris mature seed revealed a high content of polyphenols, potent antioxidant activity together with antitumor activity toward colon cancer cell lines. It was suggested that the extract could form the basis for developing colon cancer treatment while emphasizing the need for more in vivo studies to control its side effects. 19 In a more recent study, it was found that superoxide dismutase (SOD) possesses high activity against canine mammary gland tumor cell lines. The study referred to the suggested mechanism of SOD effect in terms of inhibition of the migration and proliferation of tumor cells, so, the study confirms the potential activity of SOD to be a chemotherapeutic model.<sup>20</sup> In another recent study, levels of SOD with other two antioxidant enzymes including catalase and glutathione peroxidase were evaluated in patients with breast cancers treated with doxorubicin. The study showed that the levels of these enzymes were significantly decreased in patients without treated with doxorubicin. In contrast, the enzymatic levels increased to their normal level range during the treatment with doxorubicin. <sup>21</sup>

Most cancer drugs have severe side effects and poor selectivity, thus, many attempts were conducted to develop new anti-cancer agents as safer and more effective ones. Ogbole1 et al., <sup>22</sup> studied the activity of some medicinal plant extracts toward Rhabdomyosarcoma cell lines and they were referred to that both Macaranga *barteri* and *Calliandra portoricensis* extracts possess cytotoxic effects on this type of cancer cell lines. In a recent study, the cytotoxic effect of African plant extract (Nauclea latifolia) on breast cancer and RD cell lines was estimated. The study showed a cytotoxic activity of this type of extract which is

considered as an excellent chemotherapeutic agent. It was reported that the exogenous SOD causes effects on prostate cancer cell lines in terms of apoptosis index and the expression of manganese superoxide dismutase. <sup>23</sup> Furthermore, the level of SOD decreased in the case of cancer disease, especially in the late stage with disease progression and cancer cell migration and invasion, subsequently, the study suggested that SOD could represent a therapeutic agent at the end stage of prostate cancer. <sup>24</sup>

It was found that treatment of breast cancer monitored by the level of SOD and some other relevant biochemical factors. Therefore, the level of SOD is decreased during disease period due to the increasing of ROS that consider primers or carcinogens cause the cancer development, so the level of SOD is suggested to be a marker for treatment of breast cancer when cancer cells are prevented by the induced apoptosis process. <sup>25</sup> In the case of oral squamous cell carcinoma (OSCC), the level of SOD was determined. The literature referred to that the decrease of SOD level in tissue samples of OSCC is attributed to increase of oxidative stress. <sup>26</sup>

The effect of some plant extracts rich in phenolic compounds and flavonoids on breast cancer cell lines was studied. The extracts of chloroform, ethanol, methanol, ethyl acetate, and aqueous, revealed promising results as antioxidants and antiproliferation agents against cancer cells. Moreover, ethanol extract also showed high effectiveness as an anti-proliferation of breast cancer cells in mice, which showed a significant reduction in tumor size. <sup>27</sup> The activity of incensole acetate nanoemulsion which is obtained from Catharanthus roseus essential oil was estimated toward breast cancer. Potential effects of this extracted substance on oxidative stress, inflammatory factors, and tumor growth were recorded. <sup>28</sup>

Malondialdehyde compound can occur as a result of the polyunsaturated fatty acids peroxidation. Therefore, this compound has been employed as a good marker for oxidative stress in patients with a wide range of diseases. Furthermore, the determination of MDA level applied for different types of cancer samples. <sup>29</sup> Oxidative stress (OS) occurs as a result of the development of degenerative diseases like cancer and diabetes. The production of ROS increases under the increase of OS, which leads to the destruction of beta cells or damage to DNA to develop diabetes or cancer, respectively. The effects of Clinacanthus nutans leaf extracts on inflammatory and oxidative stress markers were assessed in diabetic rats. Moreover, the extract of Clinacanthus nutans leads to decreased MDA with each of TNF $\alpha$  and IL-6 levels and improves  $\beta$ -cells area. <sup>30</sup> The objectives of the current study are

Table 1. Effect of ginger extract on RD and normal cell lines.

Cell line type	Extract concentrations in $\mu$ g/Ml						
	12.5	25	50	100	200	400	800
	Inhibition rate (IR%)						
RD Cell Lines	39.81	52.25	63.70	76.11	81.98	88.18	94.20
Normal Cell Lines	11.32	11.32	10.96	10.69	8.51	6.61	7.07

including the evaluation of the cytotoxicity of both ginger and olive leaf extracts together with the mixture of these two extracts at a ratio of 1:1 against RD cell lines and normal cells, in addition, to evaluating the oxidative stress in terms of MDA and SOD in cell culture of RD cell lines before and after treatment with all the studied extracts within a wide range of concentrations.

#### **Materials and methods**

#### Preparation of plant extracts

Both extracts of ginger and olive leaves were prepared using soxhlet as reported elsewhere. <sup>12</sup> The third extract was prepared by mixing equal amounts for each of the ginger and olive leaf extracts within the ratio of 1:1. The cytotoxicity of these extracts was measured against RD cell lines and the levels of SOD with MDA were evaluated as best markers for antioxidants and oxidative stress, respectively.

#### Cell culture

The human cancer cell lines, RD (Rhabdomyosar-coma)cell lines, and normal cell lines were obtained from the Center of Biotechnology/Al-Nahrain University. RD cell lines were grown and maintained in minimum essential medium and incubated at 37 °C with carbon dioxide. Medium components included sodium bicarbonate (0.07%), non-essential amino acids (1%), glutamine (2 mM/L), penicillin (100 U/mL), and streptomycin (100 mg/mL).

#### MTT assay

The assessment of cell viability (inhibition rate percentage) was conducted by the 3-(4, 5-dimethyl thiazol-2-yl) - 2, 5-diphenyl-tetrazolium bromide (MTT) as reported in literature. <sup>22</sup> Hundred (100  $\mu$ L) of RD cell lines were transferred into the well plate, and then, different seven concentrations for each of the applied extracts include (12.5, 25, 50, 100, 200, 400, and 800  $\mu$ g/mL) dissolved in DMSO, were used to determine their cytotoxic effect on RD cell lines. RD cell lines with the applied concentrations of the studied extracts were incubated in triplicates at

37 °C in a carbon dioxide environment for 72 hrs. After the incubation, maintenance media was removed and an appropriate amount of MTT solution (25  $\mu$ L) was added to each well of RD cell lines and incubated at 37 °C for 2 hrs. The yellow color of MTT was converted to purple formazan which is indicated to cell viability. Then, the appropriate amount of DMSO (75  $\mu$ L) was added after removing of MTT solution. Finally, a microplate reader was used to read the optical density for plates at 490 nm wavelength. Levels of superoxide dismutase (SOD) and malondialdehyde (MDA) were evaluated according to kit procedures, which were purchased from Cohesion Bioscience – China.

#### Statistical analysis

Information for all the studied factors in the present study was identified by mean  $\pm$  SD. Measurements were assessed statistically by analysis of variance [ANOVA] using SPSS program version 17. The value of P  $\leq$  0.05 was taken as a significant difference.

#### Results and discussion

Three types of plant extracts were used in this study, including olive leaves, ginger, and olive with ginger extracts mixture 1:1. Table 1 shows the results of ginger extract effects on both RD cell lines and normal cell lines. Seven different concentrations of ginger extract were applied against RD cell lines and normal cell lines including (12.5, 25, 50, 100, 200, 400, and  $800 \,\mu \text{g/ml}$ ). Inhibition percentages for RD cell lines, which correspond to the approved ginger extract concentrations, were found to be (39.81, 52.25, 63.70, 76.11, 81.98, 88.18, 94.20). Whereas, normal cell line inhibition percentages that corresponded to the approved ginger extract concentrations were found to be much less than RD cell lines (11.32, 11.32, 10.96, 10.69, 8.51, 6.61, 7.07) as recorded in Table 1, and illustrated in Fig. 1.

The results of olive leaves extract effect on RD and normal cell lines were recorded in Table 2 and illustrated in Fig. 2. The results showed that the inhibition rate (IR%) of this type of plant extract was increased with increasing extract concentrations for seven

Table 2. Effect of olive leaves extract on RD and normal cell lines.

Cell line type	Extract concentrations in µg/Ml						
	12.5	25	50	100	200	400	800
	Inhibiti	Inhibition rate (IR%)					
RD Cell Lines	39.66	52.03	61.04	74.41	80.24	86.63	92.95
Normal Cell Lines	13.59	13.41	10.69	10.24	10.69	6.61	9.15

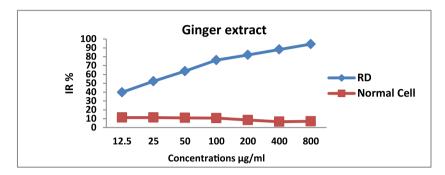


Fig. 1. Ginger extract effect on RD and normal cell lines.

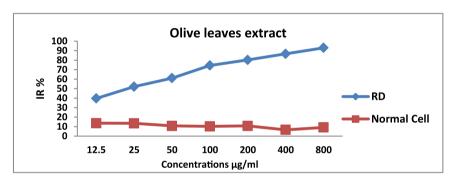


Fig. 2. Shows effect olive leaves extract on RD and normal cell lines.

different olive leaves extract concentrations against RD cell lines, which are found to be (39.66, 52. 03, 61.04, 74.41, 80,24, 86.63, 92.95). The effect of this extract on normal cell lines was recorded to be very little compared to its effect on tumor cell lines (13.59, 13.41, 10.69, 6.61, 9.15), as recorded in Table 2 and exhibited in Fig. 2.

The prepared extract from ginger and olive leaf extracts at a ratio of 1:1, was applied against RD cell lines and normal cells similarly for both ginger and olive leaf extracts, which had been used first independently. The results of this extract (ginger olive extract mixture) were recorded in Table 3. They showed a high inhibition percentage against RD cell lines including 56.31, 65.92, 76.07, 81.13, 85.75, 88.40, and 91.99) at extract concentrations of 12.5, 25, 50, 100, 200, 400, and  $800~\mu g/ml$  respectively. However, the effect of ginger and olive leaves extract on normal cells was found to be much less than its high effect on RD cell lines. Inhibition percentage of this extract at seven applied concentrations of 12.5, 25, 50,

100, 200, 400, and 800  $\mu$ g/ml were included 5.16, 6.07, 6.52, 5.62, 6.16, 7.16, and 6.88, respectively, as shown in Table 3 and illustrated in Fig. 3.

Table 4 shows the level of MDA in cell culture after being treated with both ginger and olive leaves and the mixture of ginger and olive leaf extracts. The results showed a significant decrease in MDA levels with an increase of these extract concentrations, starting with the concentration of 12.5  $\mu$ g up to a concentration of 800  $\mu$ g/ml. There are no significant differences between concentrations of 12.5 and 25  $\mu$ g/ml for these extracts. But there are significant differences between these two concentrations (12.5 and 25  $\mu$ g/ml<sup>b</sup>) and the concentrations of 50 and 100  $\mu$ g/ml<sup>c</sup> in the case of both olive leaves and ginger extracts, while the mixture of ginger and olive leaves extract was including the concentrations of 50, 100 and 200  $\mu$ g/ml<sup>c</sup> has a significant decrease in MDA level than 12.5 and 25  $\mu$ g/ml concentrations. Also, concentrations of 200, 400, and 800  $\mu$ g/ml<sup>d</sup> had significantly higher effects on the RD cell line,

Table 3. Effect of ginger and olive leaves extracts mixture (1:1) on RD and normal cell lines.

Cell line type	Extract concentrations in µg/Ml						
	12.5	25	50	100	200	400	800
	Inhibition rate (IR%)						
RD Cell Lines	56.31	65.92	76.07	81.13	85.75	88.40	91.99
Normal Cell Lines	5.16	6.07	6.52	5.62	6.16	7.16	6.88

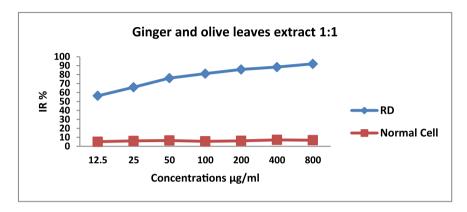


Fig. 3. Shows effect ginger and olive leaf extracts mixture on RD and normal cell lines.

than previous concentrations of olive leaf extract. Similarly, concentrations of 200 and 400  $\mu$ g/ml<sup>d</sup> had a significantly lower effect than (800  $\mu$ g/ml<sup>e</sup>) in the case of ginger extract. At the same time, the mixture of ginger and olive leaves extract at the concentrations of (400 and 800  $\mu$ g/ml<sup>d</sup>) was more effective in decreasing the MDA level than other previous concentrations of this mixture extract (12.5–200  $\mu$ g/ml). Accordingly, ginger, olive leaves, and the mixture of ginger and olive leaves extracts significantly decreased the MDA concentration (p = 0.000021, p = 0.00017 and p = 0.00016), respectively, as reported in Table 4.

Table 5 recorded the results of SOD levels in RD cell lines in the case of olive leaves, ginger, and the extract of ginger and olive leaves. The results revealed a sig-

nificant increase (p = 0.00057) in SOD level in the RD cell lines with increasing the concentrations of olive leaves extract (12.5  $\mu$ g/ml up to a concentration of 800  $\mu$ g/ml). Similarly, the results of both ginger and the mixture extracts showed a significant increase (p = 0.00042, p = 0.00075) in SOD level with increasing of these two extract concentrations 12.5–800  $\mu$ g/ml as noticed in Table 5.

The results in Tables 1 to 4 and Figs. 1 to 3 referred to the activity of the applied plant extracts in this study against RD cell lines. Extracts of olive leaves, ginger and their mixture are giving high activities in inhibition of cancer cells. The activities of the above-studied extracts can be attributed to their bioactive components like polyphenols, saponins, alkaloids, flavonoids, and terpenoids. Subsequently, the

Table 4. Levels of MDA in RD cell line under treated with extracts of olive leaves, ginger & their mixture.

	MDA % (mean $\pm$ SD) nmol/ml					
Extract Concentrations $\mu$ g/ml	Olive leaves extract	Ginger extract	Olive & ginger extracts mix			
Control	9.784 ±1.537 <sup>a</sup>	9.784 ±1.537 <sup>a</sup>	9.784+1.537 <sup>a</sup>			
12.5	$9.264 \pm 2.065^{ab}$	$8.741 \pm 1.256^{b}$	7.470 + 1.639 <sup>b</sup>			
25	$8.963 \pm 1.782^{b}$	$8.816 \pm 2.044^{b}$	$7.384 + 1.072^{b}$			
50	$6.721 \pm 1.044^{c}$	$7.350 \pm 0.948^{c}$	6.384+0.961 <sup>c</sup>			
100	$6.585 \pm 0.952^{c}$	$7.295 \pm 1.103^{c}$	$6.240 + 0.855^{c}$			
200	$5.251 \pm 0.097^{d}$	$5.748 \pm 0.847^{d}$	$6.174 + 0.795^{c}$			
400	$5.216 \pm 1.048^{d}$	$5.692 \pm 0.944^{d}$	4.862+0.733 <sup>d</sup>			
800	$5.016 \pm 1.170^{d}$	$4.986 \pm 0.850^{e}$	$4.790 + 0.806^{d}$			
LSD	0.738	0.545	0.653			
P-value	0.00017	0.000021	0.00016			

Different small letters in the same column referred to present significant differences among MDA levels ( $p \le 0.005$ ).

	SOD (mean ±SD) U/mg					
Extract Concentrations $\mu$ g/ml	Olive leaves extract	Ginger extract	Olive &ginger extracts mix.			
Control	1.125 ±0.128 <sup>a</sup>	$1.125 \pm 0.128^a$	$1.125 \pm 0.128^{a}$			
12.5	$1.263 \pm 0.144^a$	$1.295 \pm 0.129^a$	$1.306 \pm 0.095^a$			
25	$1.285 \pm 0.162^a$	$1.306 \pm 0.131^a$	$1.362 \pm 0.128^a$			
50	$1.350 \pm 0.207^a$	$2.482 \pm 0.146^{b}$	$2.644 \pm 0.316^{b}$			
100	$2.533 \pm 0.239^{b}$	$2.546 \pm 0.151^{b}$	$2.787 \pm 0.304^{b}$			
200	$3.871\pm0.318^{c}$	$4.146 \pm 0.385^{c}$	$4.210 \pm 0.513^{c}$			
400	$3.956 \pm 0.405^{c}$	$4.269 \pm 0.507^{c}$	$4.286 + 0.482^{c}$			
800	$4.121 \pm 0.482^{c}$	$4.219 \pm 0.483^{c}$	$4.338 + 0.519^{c}$			

Table 5. Levels of SOD in RD cell line under treated with extracts of olive leaves, ginger and their mixture.

Different small letters in the same column referred to present significant differences among SOD levels ( $p \le 0.005$ ).

0.604

0.00042

0.559

0.00057

extracts of medicinal plants have a large attention recently for their medical applications in many health disorders to management and treatment of chronic diseases like diabetes <sup>12</sup> and cancers <sup>5,23</sup> as active and safe therapeutic agents. The effects of these extracts against diseases may be occurred due to present active phytochemical compounds. <sup>31</sup>

LSD

P-value

In a more recent study, a new strategy to treat cancer cells was applied via selective oxycution, which is obtained from plant extracts. Selective oxycution means the selective killing of cancer cells by targeting the stress response to ROS. Common cancer drugs lack selectivity, therefore, new attempts are conducted based on certain pathways. These pathways include the effects of plant extracts on induce apoptosis, cytotoxicity, and redox balance of cancer cells environment.<sup>32</sup> Similarly, the effect of allium extract on melanoma cell lines was found to possess high activity against this type of cancer cells. This activity is elucidated by the presence of active components that include phenolic compounds and sulfur content. 33 It was found that the plant extracts inhibit cancer cells depending on DNA damage and induce apoptosis in cancer cells. This occurred due to the action of phytochemicals which revealed a significant suppression in cancer cells by applied animal model. 34

Also, medicinal plant extracts prevent the proliferation of cancer cells as a suggested mechanism to elucidate their actions. Anti-proliferative of cancer cells is considered one of the essential proposed mechanisms that are used to clarify the action of plant extracts against the mobility of tumor cells. <sup>35</sup> In a more recent study conducted by Mir SA., et al., <sup>36</sup> mechanisms of plant extract's effect on cancer were reported. The study indicated that the presence of active bio components in plant extracts like flavonoids increase the activity of plant extracts against this dis-

ease. Flavonoids possess high activity toward a wide range of diseases such as immunological, cardiovascular, and cancer diseases. In other words, flavonoids scavenge ROS and suppress cancer metastasis by blocking tumor cells and preventing nourishment to induce apoptosis. Also, flavonoids improve the immunological system <sup>37–39</sup> and antioxidant condition <sup>40</sup> in patients with cancer.

0.581

0.00075

Ginger extract has some bioactive ingredients that possess the ability to care and protect body cells against cancer. <sup>41</sup> A recent study showed anti-tumor activities of ginger against digestive tract cancers, like gastric, colorectal, liver, pancreatic, and laryngeal cancers, via a set of pathways. <sup>42</sup> In the other hand, the effect of ginger extract on breast cancer can be occurred by regulation of some pathways like proliferation, migration, and apoptosis of cancer cells through regulation of the level of TNF and IL-17 with some signaling pathways. <sup>43</sup> Similarly, the activity of ginger against colorectal cancer was evaluated. The study showed that ginger extract increases the activity against colorectal cancer by stimulating some anticancer markers. <sup>44</sup>

Extracts of olive leaves were used to treat a wide range of diseases like fever, malaria, and earaches a long time ago. The activity of olive leaves extract is due to the presence of the compound oleuropein, which possesses a high antioxidant activity. Furthermore, it was reported that oleuropein stimulates anti-proliferation and induces apoptosis in many cancer cells. Subsequently, the extract of olive leaves affects cancer cells depending on different mechanisms including antioxidant activity, inducing apoptosis, and anti-proliferation in cancer cells. Also, the activity of olive leaves extract was evaluated against both breast and lung cancer, its effect is occurred via anti-proliferation and apoptosis mechanisms. Also

The results of the malondialdehyde (MDA) level in this study showed that its level was increased in the case of RD cancer cells without treatment with any one of the studied extracts. In contrast, the level of MDA after treatment with each of the studied extracts, it is gradually decreased with increasing the concentration of the applied extract. These results of MDA were in agreement with other previous studies. <sup>29,30</sup> Also, in recent studies, it was noticed there was an increase in the level of MDA as an indicator of oxidative stress in patients with thyroid cancer. The increasing in level of MDA was adopted as a marker for disease progression, so for this reason, MDA is considered an important clinical factor for monitoring oxidative stress. <sup>47,48</sup>

In the current study, the superoxide dismutase (SOD) level was found to be less than the control before treating the RD cell lines with any one of the studied extracts. This result can be attributed to an increase in the oxidants like ROS under cancer condition and decreasing in SOD level under the same condition. Reversibly, after treatment of RD cell lines by the studied extracts, the level of SOD was increased gradually with increasing of the given extract concentration. The results of SOD in this study are consistent with other recent studies <sup>25–27</sup> and also agree with the content of more recent studies. <sup>32,33,49</sup>

In another recent study, it was reported that the bioactive components of olive leaves extract include flavonoids (polyphenols and phenolic acids), terpenodis, and oleuropein which is considered the more effective compound in olive leaves extract. Olive leaves extract was found to possess a variety of activities against oxidation, microbial disturbances, inflammation, and reconstruction of the damaged tissues. Also, olive leaves extract contains oleanolic acid that protects the body from platelet aggregation. <sup>50</sup>

#### Conclusion

Based on the obtained results, it can be concluded that the cytotoxic effects of both ginger and olive leaf extracts together with their mixture extract at the ratio of 1:1 were found to be highly active against RD cell lines but no normal cells. The effects of the studied extracts can be attributed to the active components in these extracts like flavonoids (polyphenols or phenolic acids), terpenoids, and oleuropein. Also, applied of these extracts against RD cell lines lead to an increase in the level of SOD and at the same time, decreased the level of MDA which represents the principal markers of both antioxidants and oxidants, respectively. Moreover, the active components of these extracts can affect can

cer cell lines through several mechanisms such as antioxidant, anti-proliferation, and apoptosis. The applied extract affects cancer cells but has no effect on normal cells, these results confirm the selectivity of the extract's action toward cancer cells in terms of the cancer chemoprevention concept. Further studies are required for more information that concerns with these extracts effect mechanisms against cancer cells.

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#### **Author's declaration**

- · Conflicts of Interest: None.
- We hereby confirm that all the Figures and Tables in the manuscript are ours. Furthermore, any Figures and images, that are not ours, have been included with the necessary permission for republication, which is attached to the manuscript.
- Authors sign on ethical consideration's approval.
- No animal studies are present in the manuscript.
- No potentially identified images or data are present in the manuscript.
- Ethical Clearance: The project was approved by the local ethical committee at University of Baghdad.

#### **Author's contribution statements**

- K.K.G.: Designing the research idea, conducting tests, interpreting the results, reviewing and auditing.
- F.M.K.: Designing the research idea, interpreting the results, reviewing and auditing.

#### References

- Al-Azzawi MA, Saleh WR, Rashid FA, Alwash BMJ. Synthesis and characterization of nanoparticles extracted from catharanthus roseus plant. Nano Hybrids Compos. 2023;38:15–24. https://doi.org/10.4028/p-z7zy4l.
- Farhan MF, Mehde AA, Mehde WA, Jassim RA, Kadhim NJ, Jasim NA. Synthesis of silver nanoparticles from leaf extract of olive and fig with silver nitrate and effect on ECTO-5-Nucleotidase (5-NT), ADA and AMPDA enzymes in sera of athrosclerosis patients. Int J Chem Sci. 2016;14(3):1805– 1817.
- Abdulhalem AG, Alwash BMJ, Ibrahim KM. Localization of saponins accumulation in yucca gloriosa variegata in intact tissues and their tissue cultures by using fluorescence

- microscopy. Ann Romanian Soc. Cell Biol. 2021;25(6):8615–8626.
- Alwash BMJ, Salman ZO, Hamad SF. Qualitative and quantitative evaluation of active constituents in callus of lavandula angustifolia plant in vitro. Baghdad Sci J. 2020;17(2 Special Issue):591–598. http://dx.doi.org/10.21123/bsj.2020. 17.2(SI).0591.
- Hamad SF, Salman ZO, Alwash BMJ. Assessment of antioxidant and cytotoxic activity of essential oil extracted from lavandola angustifolia callus leaves. Iraqi J Agric Sci. 2021;52(6):1549–1554. https://doi.org/10.36103/ijas.v52i6.1496.
- Abid Aun ZA, Habeeb HM, Jazaa LA. Morphological, anatomical and chemical study of an exotic plant jatropha integerrima JACO.1763 (Euphorbiaceae) in Iraq. Bull Iraq Nat Hist. Mus. 2022;17(1):129–140. https://doi.org/10.26842/binhm. 7.2022.17.1.0129.
- Mohammed S, Habeeb HM. Phytochemical and cytotoxic study in quisqualis indica volatile oil cultivation in iraq. Int J Health Sci. 2022;6(S4):8572–8584. https://doi.org/10. 53730/ijhs.v6nS4.10618.
- Hamid GS, Allawi AAD, Ghudhaib KK. Correlation of pentosidine with kidney diseases in iraqi patients with diabetic nephropathy. Iraqi J Sci 2021;62(10):3436–3442. https://doi.org/10.24996/ijs.2021.62.10.2.
- Ibrahim RK, Allawi AAD, Ghudhaib KK. Determining ACE-2 level and some relevant biochemical parameters and studying the effect of gender in Iraqi diabetic patients with glomeruli and renal tubules fibrosis as early prediction marker. Baghdad Sci J. 2023;20(6):2256–2264. https://doi.org/10.21123/bsj. 2023.7633.
- Salman ZA, Ghudhaib KK, Fadhil R. Association of osteopontin and alkaline phosphatase in male patients with diabetes mellitus type 2 and periodontitis. Iran J War Public Health. 2022;14(1):105–109. https://doi.org/10.24996/ijs.2021.62. 10.2.
- Ibrahim RK, Ghudhaib KK, Allawi AA. Early prediction of nephropathy in iraqi patients with diabetes type II by evaluating of some relevant biochemical factors. Baghdad Sci J. 2024;21(5):1549–1560. https://doi.org/10.21123/bsj.2023. 8008
- Ghudhaib KK, Khaleel FM. Evaluation of antioxidants, antibacterial and antidiabetic activities of aqua-alcoholic marjoram extract. Baghdad Sci J. 2024. published Online. https://doi.org/10.21123/bsj.2024.9328.
- 13. Tomasz K, Sitarek P, Skala F, Toma M, Wielanek M, Pytel D, *et al.* Induction of apoptosis by in vitro and in vivo plant extracts derived from *Menyanthes trifoliata* L. in human cancer cells. Cytotechnology 2019;71(1):165–180. https://doi.org/10.1007/s10616-018-0274-9.
- 14. Bundit P, Gutarop P, Nisana N, Anchalee T, Pacharee B, Watcharin L. Evaluation of anticancer potential of thai medicinal herb extracts against cholangiocarcinoma cell lines. PLoS One. 2019;14(5):1–16. https://doi.org/10.1371%2Fjournal.pone.0216721.
- Marei S, Basheer A, Ibrahim R, Mizied F, Anwar R. Correlation between cytotoxicity in cancer cells and free radicalscavenging activity: *In vitro* evaluation of 57 medicinal and edible plant extracts. Oncol Lett. 2019;18(6):6563–6571. https:// doi.org/10.3892/ol.2019.11054.
- Flora F, Vahideh S, Kaveh B, Elham R, Ehsan NM. Cytotoxic activity of the root of euphorbia tehranica ethanolic extract against caco-2 colorectal cancer cell line. Multidiscip Cancer Investig. 2018;2(2):1–7. http://dx.doi.org/10.30699/acadpub.mci.2.2.1.

- Valter RM, Ivan C, Roman C. In vitro screening for cytotoxic activity of herbal extracts. Evid Based Complement Alternat Med. 2017;2017:1–9. https://doi.org/10.1155/2017/ 2675631.
- Ozra Z, Mohammed MY. Cytotoxic and anti-proliferative effects of *rosa beggeriana* schrenk extracts on human liver and breast cancer cells. Avicenna J Phytomed. 2019;9(4):386–395. https://doi.org/10.22038/ajp.2019.12780.
- Mesas C, Martínez R, Ortíz R, Galisteo M, López-Jurado M, Porres JM *et al.* Antitumor effect of the ethanolic extract from seeds of euphorbia lathyris in colorectal cancer nutrients. Nutrients. 2021;13(2):566–582. https://doi.org/10. 3390/nu13020566.
- Ko BG, An JH, Lee JH, Kim KB, Kim TH, Park SM, et al. Anticancer effect of superoxide dismutase on canine mammary gland tumor in vitro. Vet Med Sci. 2024;10(1):1–10. https://doi.org/10.1002%2Fvms3.1323.
- Hadi NA, Mahmood RI, Al-Saffar AZ. Evaluation of antioxidant enzyme activity in doxorubicin treated breast cancer patients in iraq: A molecular and cytotoxic study. Gene Rep. 2021;24:101285. https://doi.org/10.1016/ j.genrep.2021.101285.
- Ogbole O, Segun PA, Adenij AJ. In vitro cytotoxic activity of medicinal plants from nigeria ethnomedicine on rhabdomyosarcoma cancer cell line and HPLC analysis of active extracts. BMC Complement Altern Med. 2017;17:494–503. https://doi.org/10.1186/s12906-017-2005-8.
- 23. Charles-Okhe O, Odeniyi TO, Ogbole O, Akinleye TE, Adeniji AJ. Cytotoxic activity of crude extracts and fractions of african peach (nauclea latifolia smith) stem bark on two cancer cell lines. Phytomed Plus. 2022;2(1):1–9. https://doi.org/10.1016/j.phyplu.2021.100212.
- 24. Ismy J, Sugandi S, Rachmadi D, Hardjowijoto S, Mustafa A. The effect of exogenous superoxide dismutase (SOD) on caspase-3 activation and apoptosis induction in Pc-3 prostate cancer cells. Res Rep Urol. 2020;2020(12):503–508. https://doi.org/10.2147/RRU.S271203.
- 25. Yuksel B, Ozkan AD, Aydın D. Betts Z. Evaluation of the antioxidative and genotoxic effects of sodium butyrate on breast cancer cells. Saudi J Biol Sci. 2022;29(3):1394–1401. https://doi.org/10.1016/j.sibs.2021.12.061.
- Panwar A, Ruhil S, Keluskar V, Jirge VL, Kumar L, Sridhar M. Evaluation of superoxide dismutase, an antioxidant enzyme, in oral squamous cell carcinoma: A systematic review and meta-analysis. J Datta Meghe Inst Med Sci Univ. 2023;18(3):575–582. http://dx.doi.org/10.4103/jdmimsu.jdmimsu 131 22.
- Aboalhaija NH, Syaj H, Afifi F, Sunoqrot S, Al-Shalabi E, Talib W. Chemical evaluation, in vitro and in vivo anticancer activity of lavandula angustifolia grown in jordan. Molecules. 2022;27(18):1–12. https://doi.org/10.3390/molecules27185910.
- Nayila I, Sharif S, Lodhia MS, Fayyaz M, Rehman U, Amanc F. Characterization and anti-breast cancer potential of an incensole acetate nanoemulsion from catharanthus roseus essential oil; in silico, in vitro, and in vivo study. Royal Soc Chem Adv. 2023;13:32335–32362. https://doi.org/10.1039/D3RA06335F.
- 29. Cordiano R, Gioacchino M, Panzera C, Gangemi S, Mangifesta R, Minciullo PL. Malondialdehyde as a potential oxidative stress marker forallergy-oriented diseases: An update. Molecules. 2023;28(16):5979–6000. https://doi.org/10.3390/molecules28165979.
- Mustikahttps A, Fatimah N, Safitri I, Susanti N, Noor NS. Clinacanthus nutans L extracts reduce the serum tumor

- necrosis factor- $\alpha$ , malondialdehyde, and interleukin-6 levels and improve the langerhans islet area in diabetic rat models. Clin Med Insights Endocrinol Diabetes. 2023;16:1–9. https://doi.org/10.1177/11795514231196462.
- 31. Hegde MM, Lakshman K. Role of polyphenols and flavonoids as anti-cancer drug candidates: A review. Pharmacogn Res. 2023;15(2):206–216. http://dx.doi.org/10.5530/pres. 15.2.022.
- Jara-Gutiérrez C, Mercado L, Paz-Araos M, Howard C, Parraga M, Escobar C., et al. Oxidative stress promotes cytotoxicity in human cancer cell lines exposed to Escallonia spp. Extracts. BMC Complement Med Ther. 2024;24:38–50. https://doi.org/ 10.1186/s12906-024-04341-4.
- Shirani M, Aghazadeh E, Yousefsani B, Shirani K. Anticancer activity of allium jesdianum extract: A potential therapeutic approach for melanoma. Jundishapur J Nat Pharm Prod. 2024;19(1):1–9. https://doi.org/10.5812/jjnpp-139022.
- 34. Chandra S, Gahlot M, Choudhary AN, Palai S, Almeida RS, Vasconcelos JL, *et al.* Scientific evidences of anticancer potential of medicinal plants. Food Chem Adv. 2023;2:1–19. https://doi.org/10.1016/j.focha.2023.100239.
- Ahmad GM, Abu Serie MM, Abdel-Latif MS, Ghoneem T, Ghareeb DA, Yacout GA. Potential anti-proliferative activity of Salix mucronata and Triticum spelta plant extracts on liver and colorectal cancer cell lines. Sci Rep. 2023;13:3815–3830. https://doi.org/10.1038/s41598-023-30845-z.
- 36. Mir SA, Dar A, Hamid L, Nisar N, Malik JA, Ali T, *et al.* Flavonoids as promising molecules in the cancer therapy: An insight. Curr Res Pharmacol Drug Discov. 2024;6:1–14. https://doi.org/10.1016/j.crphar.2023.100167.
- Luna FC, Ferreira WA, Casseb SM, Oliveira EH. Anticancer potential of flavonoids: An overview with an emphasis on Tangeretin. Pharmaceut. 2023;16(9):1229–1249. https://doi. org/10.3390/ph16091229.
- 38. Slika A, Mansour H, Wehbe N, Nasser SA, Iratni R, Nasrallah G, *et al.* Therapeutic potential of flavonoids in cancer: ROS-mediated mechanisms. Biomed Pharmacother. 2022;146:1–18. https://doi.org/10.1016/j.biopha.2021.112442.
- 39. Magdalena M, Alessandro B, Corrado C. Effects of Flavonoids on Cancer, Cardiovascular and Neurodegenerative Diseases: Role of NF-κB Signaling Pathway. Int J Mol Sci. 2023;24(11):9236–9256. https://doi.org/10.3390/ijms24119236.
- 40. Hosseinzadeh A, Poursoleiman F, Biregani AN, Poursoleiman F, Esmailzadeh A. Flavonoids target different molecules of

- autophagic and metastatic pathways in cancer cells. Cancer Cell Int. 2023;23(114):1–14. https://doi.org/10.1186/s12935-023-02960-4.
- Mao QQ, Xu XY, Cao SY, Gan RY, Corke H, Beta T, et al. Bioactive compounds and bioactivities of ginger (Zingiber Officinale Roscoe). Foods. 2019;8(6):185–205. https://doi.org/10.3390/foods8060185.
- 42. Chen GQ. Nan Y, Huang SC, Ning N, Du YH, Lu DD, *et al.* Research progress of ginger in the treatment of gastrointestinal tumors. World J Gastrointest Oncol. 2023;15(11):1835–1851. http://dx.doi.org/10.4251/wjgo.v15.i11.1835.
- 43. Luo A, Chen Y, Ma Q, Huang Y, Hong T, Shu K, et al. Exploring the mechanism of an active ingredient of ginger, dihydrocapsaicin, on triple negative breast cancer based on network pharmacology and in vitro experiments. Oncol Lett. 2023;25:195–208. https://doi.org/10.3892/ol.2023.13781.
- Nachvak SM, Soleimani DS, Rahimi M, Azizi A, Moradinazar M, Rouhani MH, et al. Ginger as an Anti-colorectal Cancer Spice: A systematic review of in vitro to clinical evidence. Food Sci Nutr. 2023;11(2):651–660. https://doi.org/10.1002/fsn3.3153.
- 45. Rishmawi S, Haddad F, Dokmak G, Karaman R. Comprehensive review on the anti-cancer effects of Oleuropein. Life. 2022;12(8):1140–1157. https://doi.org/10.3390%2Flife12081140.
- 46. Bal Y, Sürmeli Y, Mohamed GS. Antiproliferative and apoptotic effects of olive leaf extract microcapsules on MCF-7 and A549 cancer cells. Amer Chem Soc Omega. 2023;8(32):28984–28993. https://doi.org/10.1021/acsomega.3c01493.
- Buczyńska A, Sidorkiewicz I, Kościuszko M, Adamska A, Siewko K, DzieciołJ, *et al.* Clinical significance of oxidative stress markers as angioinvasion and metastasis indicators in papillary. Thyroid Cancer. Sci Rep. 2023;13:1–10. https://doi. org/10.1038/s41598-023-40898-9.
- Dharmajaya R, Sari DK. Malondialdehyde value as radical oxidative marker and endogenous antioxidant value analysis in brain tumor. Ann Med Surg. 2022;77:1–5. https://doi.org/ 10.1016/j.amsu.2021.103231.
- Zheng M, Liu Y, Zhang G, Yang Z, Xu W, Chen Q. The applications and mechanisms of superoxide dismutase in medicine, food, and cosmetics. Antioxidants 2023;12(9):1675–1694. https://doi.org/10.3390/antiox12091675.
- 50. Markhali FS, Teixeira JA, Rocha CMR. Olive Tree Leaves—A Source of Valuable Active Compounds. Processes. 2020;8:1177–1194. https://doi.org/10.3390/pr8091177.

# تقييم مستويات SOD و MDA مع السمية الخلوية لبعض المستخلصات النباتية تجاه خطوط خلايا السرطان العضلية البشرية

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#### الخلاصة

حظيت السمية الخلوية للمستخلصات النباتية باهتمام كبير في السنوات الأخيرة لتقييم نشاطها ضد الأورام. تركز الوقاية الكيميائية من السرطان على السمية الخلوية للمستخلصات النباتية لتكون سامة تجاه خطوط الخلايا السرطانية دون أن تؤثر على الخلايا الطبيعية. الهدف من هذه الدراسة هو تقييم السمية الخلوية لمستخلصي الزنجبيل و أوراق الزيتون ومزيجهما بنسبة 1: 1 تجاه خطوط خلايا QS وتقدير فعالية هذه المستخلصات ضد الاجهاد التاكسدي بدلالة QSO و MDA و ملصول على المستخلصات الكحولية لكل من الزنجبيل وأوراق الزيتون بطريقة السوكسيلت. وحضر المستخلص الثالث بخلط كميات متساوية من مستخلص أوراق الزيتون الزنجبيل بنسبة 1: 1. تم تحضير اطباق من خطوط الخلايا QS وخطوط الخلايا الاعتيادية وتم اختبار تراكيز مختلفة من المستخلصات المحضرة (2.5 - 800 ميكروغرام / مل) لتسجيل فعالياتهم تجاه كل من QS وخطوط الخلايا الطبيعية . كذلك ، تم الخلايا الطبيعية وجود فرق معنوي بين السمية الخلوية للتراكيز المدروسة لكل مستخلص تجاه خطوط الخلايا الطبيعية . كذلك بينت النتائج وجود فرق معنوي بين السمية المستخلصات ، وفي نفس الوقت لم تؤثر تلك المستخلصات على خطوط الخلايا الطبيعية . كذلك بينت النتائج ازدياد مستويات OSO مع زيادة تركيز المستخلصات على عكس مستويات ADM التي انخفضت مع زيادة تراكيز المستخلص. يمكن الاستنتاج بان نشاط المستخلصات المحضرة تجاه خطوط الخلايا QR دون التأثير على خطوط الخلايا الطبيعية يعزز مبادئ المنع الكيميائي للسرطان ، المستخلصات المحضرة في جسم الانسان.

الكلمات المفتاحية: انزيم مضاد الاكسدة، مستخلص درنات الزنجبيل، مستخلص اوراق الزيتون، واسم الاكسدة الفوقية، خطوط خلايا الساركوما.