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Rosemary and Costus Reduces Oxidative Stress and Normalize Pituitary-Thyroid Hormones in Ehrlich-Bearing Mice Treated with Cisplatin

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Abstract

Background: Medicinal plants are widely used in traditional medicine; many employed in cancer treatment or amend bad effects of cancer chemotherapy.

Aim of study: was to investigate possible ameliorating effects of rosemary (ROLE) and costus (SLRE) on lipid profile, antioxidants and thyroid toxicity in Ehrlich-bearing mice attendant with cisplatin (CP) treatment.

Materials and Methods: 60 female Swiss albino mice, ROLE/SLRE extracts and other chemicals and kits were used. Mice divided into 6 groups (n=10); one control and the others were inoculated with EAC-cells that received CP on D3-D7 then administered both extracts concurrently with CP from D3-D14, day after day. Blood and liver tissue samples were collected and prepared for biochemical analysis.

Results: phytochemical analysis showed that the extracted value of 50 g SLRE was higher than ROLE. Antioxidant activity of SLRE is potent than ROLE, but metal chelating capacity of ROLE was higher than SLRE due to high content of total phenols (mg GAE/g) in ROLE. Serum lipids (TC, LDL, vLDL, TGs) were significantly increased and HDL was decreased after induction of EAC, CP alone but treatment with CP/ROLE/SLRE improved their levels close to normal. EAC reduced GSH and SOD tissue levels and increased MDA content and these parameters were improved to normal values after treatment of EAC mice with CP followed by ROLE/SLRE. The induction of EAC increased serum levels of fT3 and decreased pituitary TSH, while treatment of EAC mice with CP/ROLE/SLRE can enhance and regulate these thyroid hormones around normal values.

Conclusion: current results suggest that giving rosemary and costus to EAC-mice treated with CP can reduce the oxidative stress induced by EAC and CP toxicity by improving lipid components, oxidative indices and thyroid function. In addition, these plants enhanced CP efficacy by reducing its side effects via their complementary antioxidant activities.

Keywords: antioxidants; cholesterol; triglycerides, thyroid; rosemary; costus

Introduction

Cancer is one of the most important health problems worldwide. According to WHO, cancer may arise due to interaction between a person's genetic and environmental factors (Blackadar, 2016). In Egypt, breast cancer (BC) is the most common cancer among women with multi-factorial susceptibility (Britt et al., 2020) with limited calculation of incidence rates (Salem et al., 2010). However, there are multiple risk factors associated with BC prevalence among nations that can partially be affected by the use of food and dietary supplements (Lacey et al., 2009).

Although several strategies have been employed in diagnosis and treatment of BC, chemotherapy is still the best of choice, which is associated with several side effects that limit its further uses (Baudino, 2015). Furthermore, adjuvant therapy such as medicinal plants or immunotherapy parallel with chemotherapy offered better results rather than alone (Harwansh & Deshmukh, 2020). Cisplatin (CP) is one of the best and first metal-based

chemotherapeutic drugs for solid tumors including BC (Yang et al., 2018; Martinho et al., 2019). Other drugs such as cyclophosphamide can alleviate the accompanied side effects of tumors (El-Bolkiny, 2006). However, CP does not show its highest potential because of its side effects and drug resistance, which limit its application and effectiveness, depending on multiple factors causing major toxicities (Ghosh, 2019).

Inside the cells, chloride facilitates CP hydrolysis producing positively charged species that react with cytoplasmic molecules such as glutathione and other cysteine-rich proteins especially DNA to form DNA adducts and toxicity (Aldossary, 2019). Furthermore, CP produces ROS with lipid peroxidation and disrupts calcium homeostasis, increasing the mitochondrial efflux of calcium interrupting cellular respiration, decreasing ATPs production and other cofactors by enzyme inhibition (Dasari and Tchounwou, 2014). Consequently, the clinical efficacy of CP is often rare due to its induction of vast organ toxicity that affects the quality of patient's life, such as nephrotoxicity, hepatotoxicity, cardiotoxicity and neurotoxicity (Shahid et al., 2018; El-bolkiny et al., 2021).

Medicinal plants are playing an important role in the treatment of human and animal diseases worldwide as cited by Abdel-Hamid et al. (2017). However, validating and using Phytochemicals requires a great deal of basic and applied research, in order to set this resource at the same level of importance with conventional pharmaceutical products (Ng et al., 2018). Thus, a safer, more affordable and effective strategy to minimize the toxicity of CP without affecting its anti-tumor efficacy is urgently needed some novel plant-derived compounds (Ojha et al., 2016).

Rosmarinus officinalis, L. (rosemary) is an important herb belongs to family Lamiaceae and has a variety of phytomedicine uses; therapy, cooking, food preservative in the food industry, and decorative herb (Al Sereiti et al., 1999). Rosemary is accompanied by several bioactive molecules such as flavonoids, rosmarinic acid and some diterpenes (Bianchin et al., 2020). These active compounds produce pharmacological potentials; anti-inflammatory (Altinier et al., 2007; Borges et al., 2019), antitumor (Visanji, Thompson, & Padfield, 2006), antimicrobial and antioxidant (Karadag et al., 2019), anti-proliferative, and protective, inhibitory and attenuating activities (de Oliveira et al., 2017). These bioactive compounds have been reported to inhibit lipid peroxidation through peroxide radical scavenging mechanisms that neutralize ROS generation (Chraibi et al., 2020). Thus, rosemary has a therapeutic potential against hepatotoxicity due to its antioxidant activity (El-Naggar et al., 2016). Therefore, its anti-tumor effect is caused by antioxidant, anti-proliferative and anti-tumorigenic activities that can possibly be utilized in future cancer treatments (Allegra et al., 2020).

Sussuria lappa (costus) is another potential herb belonging to family Asteraceae found mostly in northern mountainous regions of Pakistan and India (Singh & Pusalkar, 2020). It is rich in active substances used in the treatment of various diseases worldwide; for instance diarrhea, tenesmus, dyspepsia, vomiting, and inflammation as quoted by Al-Bolkiny et al. (2019). In the same line, different bioactive compounds isolated from costus have inhibitory effect on inflammation in human and animals in a dose dependent manner (El-Marghani et al., 2020). Costus has a variety of sesquiterpene lactones, which exhibited medicinal bioactivities (Zhao et al., 2017) and other health problems such as hypo- and hyperthyroidism in mice (Al-Bolkiny et al., 2019). *In vivo* studies showed that *Saussurea lappa*-derived costunolide have inhibitory effect on migration, growth and metastasis of BC cells (Choi et al., 2013; Patel et al., 2020).

Based on the above, the present work was conducted to evaluate the serious side effects of CP injection to Ehrlish-bearing mice including oxidative stress biomarkers, lipid profile, and thyroid hormones and the role of mixed rosemary and costus in amelioration.

Material and Methods:

Preparation of plant extracts:

Both dry leaves of rosemary and roots of costus were purchased from the local market of Alexandria city, Egypt. The plant materials were identified and authenticated at Botany Department, Faculty of Science, Tanta University. Leaves of rosemary and root of costus were crushed and 50 g powder / each were mixed vigorously with 500 mL of 80% ethanol to get a hydro-alcoholic extract, and then filtered and the solvent were dried under air condition. Both were weighted and suspended in 0.9% sterile saline for further processing **(El-Naggar *et al*., 2016).**

Phytochemical analysis:

The phytochemical analysis of each plant extract including the metal chelation capacity (MCC), total antioxidant capacity (TAC), scavenging activity (DPPH) and total phenolic content (TPC) were carried out. The MCC assay was carried out based on **Minotti & Aust (1987).** The quantitative determination of TAC was done by spectrophotometric approach of **Prieto *et al*. (1999).** The spectrophotometric DPPH assay was done according to **Shimada *et al*. (1992)** based on absorption reduction monitoring of the DPPH radical in the presence of antioxidants. Determination of TPC was performed by **Velioglu *et al*. (1998)** using Folin-C reagents and the absorbance was taken at 725 nm compared to a gallic acid calibration curve.

Chemicals:

Cisplatin (Cis-diamminedichloroplatinum II) was attained from El-Hekma Company, Cairo in a vial containing 50 mg Cis/50 ml solvent. DPPH (2, 2 diphenyl-1-picrylhydrazyl) was purchased from Egyptian branch of Sigma Aldrich Co. Phosphate buffer saline (PBS) was prepared in our laboratory and the pH was adjusted to 7 for different uses and washing. Absolute ethyl alcohol was purchased from El-Gomhoria Company, Egypt. Biological commercial kits used for superoxide dismutase (SOD), glutathione reductase (GSH), malondialdehyde (MDA) were obtained from Biodiagnostic Company, Egypt.

Ehrlich Ascites Carcinoma (EAC):

Ehrlich Ascites Carcinoma (EAC) cell line was originally obtained from the National Cancer Institute (Cairo University, Egypt). EAC cells were inoculated by serial IP administration of 0.25 X $l0^6$ cells / 0.2 ml saline in CD1 mice. Donor CD1 mice were left for 10 days until ascites is formed then were harvested under sterile conditions and purified to be used later for the *in vivo* study **(Geran *et al*., 1972).**

Animals:

Sixty healthy adult female Swiss albino mice (18–20 g) were obtained from the animal house of Cairo University, Egypt. Mice groups were maintained in suitable cages with standard hygienic circumstances, 12-hour L/D cycles and were frequently provided with a balanced commercial diet and free access of water. All the experiments were done in a compliance with the guiding principles for care and use of the laboratory animals at Zoology Department, Faculty of Science, Tanta University **(IACUC-SCITU-0099**).

Experimental design:

Mice were randomly divided equally into six groups (Gp/n=10). Gp 1 was kept as the negative control. Mice in Gp 2 were IP inoculated with ($0.25X10^6$ / mouse) of EAC cells and was kept as the positive control. Gp 3 was IP injected daily for 5 days with cisplatin from D_3 - D_7. Mice in Gps 4 were IP injected with EAC ($0.25 X 10^6$) followed by CP followed by two herbal extracts; rosemary and costus. Gps 5 and 6 were only administrated with rosemary and costs, respectively. The protocol description sketch of all treatments is shown in figure (1) according to **Salem *et al*. (2011)**.

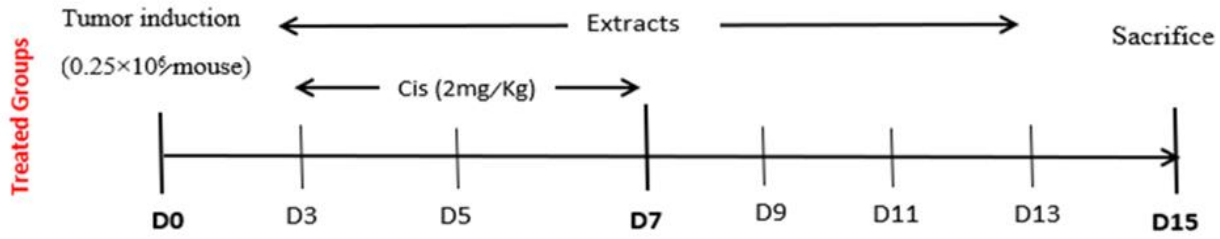

Figure 1: Experimental and treatments protocol of CP/EAC-treated groups

Based on the above protocol plan, mice inoculated with 0.25 X 10^6 EAC cells on day zero (D_0). EAC group treated every other day (3X) with cisplatin (2 mg/kg) until D_7 **(El Kholy *et al*., 2016).** In a concurrent with CP, both extracts administrated orally until D_{14} in a dose level of 100 mg/kg.

Serum and tissue samples preparation:

Post-injection, 10 mice from each group used for blood sampling from retro-orbital venous plexus under anaesthesia using heparinized microhematocrit tubes **(Prasanna *et al*., 2017).** Blood samples were allowed to clot for 2 hours at room temperature or overnight at 2-8°C, and then centrifuged at approximately 1000 × g (or 3000 rpm) for 15 minutes. Serum was removed and assayed immediately or aliquot and stored at -20°C for biochemical analysis. After sacrificing of mice, liver were quickly removed, weighed and all surrounding connective tissues were removed carefully, and stored at -20°C then 10% (W/V) homogenate was prepared by splendid 0.5 g of tissue in 3ml of saline.

Biochemical analysis:

Serum level of cholesterol (CHO) was determined according to the method of Allain *et al.* (1974) using a commercial Kit of Linear Chemicals, S.L (Spain) and absorbance was spectrophotometric read at 492 nm after 10 minute incubation at 37ºC. Serum high density lipoproteins (HDL-cholesterol) level was spectrophotometric determined (at 495 nm/10 minutes incubation) by **Burstein *et al.* (1970) and Lopez-Virella (1977)** using available commercial kits (Bio diagnostic, Egypt). Determination of serum LDL-CHO level was carried out (at 492 nm/10 minutes) by enzymatic colorimetric method according to Wieland and Seidel (1983). Level of vLDL-CHO was estimated by using the empirical relationship of **Friedewald et al. (1972).** Serum triacylglycerol (TGs) level was determined by the colorimetric method (at 492 nm/10 minutes incubation) using available commercial kits (Bio diagnostic, Egypt) according to **Fossati and Prencipe (1982).**

Homogenate was used to estimate oxidative stress markers; tissue glutathione (GSH) was spectrophotometric estimated at 412 nm by the method of **Ellman (1959).** The activity of SOD (EC 1.15.1.1) in the liver homogenate was assayed spectrophotometric at 480 nm for 4 minutes by the kinetic method described by **Misra and Fridovich (1972).** Spectrophotometric estimation of malondialdehyde (MDA) was carried out at 530 nm by the method of **Placer *et al.* (1966**).

Hormonal measurements:

Serum pituitary thyrotrophic hormone (TSH) of mice was estimated by ELISA assay using Rat/Mice TSH ELISA Kit of SKU: RTF101195 from reagent Genie, Dublin, Ireland. The intra- and inter-assay precisions were CV<8% and CV<10% for 3 samples with low, middle and high levels Rat TSH were tested 20 and 8 replicates in each plate, respectively. Thyroid tri-iodotyrosine (fT_3) was assessed in the mice sera by a competitive EIA method using commercial biological kit (#3148Z) obtained from Diagnostic automation, INC. The essential reagents include, enzyme-T_3 conjugate (HRP) and native free T_3 antigen. Upon mixing reagents, a competition reaction results between fT_3 and the conjugate for a limited number of binding sites on immobilized T_3 antibody. The absorbance in each well was read at 450 nm in a microplate reader within 30 minutes of adding the stop solution.

Statistical analysis:

The obtained data were expressed as means ± standard deviation of the mean (X ± SD). Differences between groups were determined using one-way ANOVA followed by *Dunnette* test and *post-hoc* test, Student's *t* test where appropriate. Significant differences between means were indicated by *p*-values < 0.0 5. All statistical analyses were performed using SPSS statistical version 16 software package (SPSS® Inc.32., USA)**.**

Results:

Phytochemical activities of rosemary and costus:

As shown in table (1) and figure (1), the extractive value (21.432 g) of *Saussurea lappa* (SLRE) was higher than *Rosemarinus officinalis* (ROLE), which offered a least yield (16.894 g) from initial weight 50 g dry powder for both plants in hydro-ethanolic solution.

The percentages of scavenging capacity at (10 mg/ml) / tested extract of ROLE and SLRE were found to be 78.33%, 43.33%, respectively (Figure 2). These findings clearly revealed that ROLE has a potent antioxidant activity (17.38 %) than SLRE (15.26 %) as shown in figure (3). The ethanol extract of SLRE showed higher DPPH radical scavenging activity (32.25%) than ROLE (19.30%) as in figure (4).

The total phenolic contents (TPC) in both extracts are presented in figure (5). Results were expressed as mg GAE/g extract using gallic acid standard curve. The amounts of phenols were 156.32 and 146.06 mg/GAE in ROLE and SLRE, respectively. Therefore, the ROLE extract have a high content of TPC than SLRE.

Serum total cholesterol (TC):

The serum levels of total cholesterol (TC) after all treatments are presented in Figure (6). Data showed that TC level was significantly increased (about 3 folds) in EAC-bearing mice group (Gp2) compared to control. This level of TC reduced significantly after treatment of tumorized mice with Cisplatin. No further decrease in TC level was obtained after treatment of EAC/Cis group with ROLE or SLRE extract. Treatment with a combined therapy of the two plant extracts exerted a further decrease in TC level compared to the corresponding control value.

The levels of cholesterol lipoprotein derivatives High-density lipoprotein (HDL), low-density lipoprotein (LDL) and low-density lipoprotein (vLDL) are shown in Table (2). Unfortunately, HDL was reduced after EAC inoculation compared to control. Treatment with Cis and other plant extracts improved HDL level and its value reaches to the naïve mice (Gp1). In divergence, EAC-inoculation in mice was significantly increased the level of LDL compared to the control. The effects of Cis and plant extracts were limited, while the combined effect of both extracts had a prominent significant decreasing effect. In concurrent with LDL level, the level of vLDL showed a high significant increase in EAC bearing mice when compared to control. In turn, its level begins to decrease during treatment with cisplatin alone or combined with ROLE and SLRE that ameliorate its level near to the corresponding control.

Serum triglycerides (TG) level:

As shown in Figure (7), EAC induction increased the serum level of triglycerides (TG) reaching about 5 folds compared to the control value (Gp1). The treatment of EAC-bearing mice with cisplatin reduced this high TG level but did not reach the control. Treatments with the plant extracts,

alone, or combined reduced TG levels to a little extent, the combined extract is more potent**.**

Oxidative stress biomarkers:

Data shown in Table (3) represent the results of the oxidative stress biomarkers; reduced glutathione (GSH), superoxide dismutase (SOD) and malondialdehyde (MDA). The level of GSH was significantly reduced in EAC-bearing mice (Gp2) compared to the control naive mice (Gp1). This reduction did not changed in Gp3 after treatment with cisplatin. GSH was improved in the other groups after treatment with ROLE or SLRE alone or combined after cisplatin injection compared to naïve control or EAC/Cis mice, the combined effect was marked and near the level of control. Also, the activity of SOD was significantly reduced in EAC-bearing mice (Gp2) compared to the control naive mice (Gp1). This reduction improved to an extent in Gp3 after treatment with cisplatin. SOD activity was further improved in the other groups after treatment with ROLE or SLRE alone or combined following cisplatin compared to naïve control or EAC/Cis mice, the combined effect was more potent. In contrary, the level of MDA in EAC-bearing mice was significantly increased compared to the control. Treatment of tumorized mice with Cisplatin alone, ROLE and SLRE alone or combined improved its level by decreasing the end product MDA compared to EAC-bearing mice.

Serum pituitary-thyroid hormones:

Data presented in Table (4) showed the serum level of thyroitropin (TSH) and free thyroid tri-iodothronine (fT_3). TSH in EAC-bearing mice was significantly decreased compared to control value in Gp1. A light improvement was resulted after treatment of EAC-mice with cisplatin. This advance was increased gradually to reach near to the normal after treating EAC-bearing mice with Cis/ROLE or Cis/SLRE or Cis/ROLE/SLRE, respectively compared to (Gp2). However, the high significant increase was observed in (Gp6) compared to (Gp3).

On contrary to TSH, the level of free T_3 in EAC-bearing mice showed a high significant increase (about 5 folds) compared to Gp1**.** While the level of fT_3 in EAC-bearing mice that treated with Cis, Cis/ROLE and Cis/SLRE showed a significant gradual decrease compared to (Gp2). As compared to Gp3, fT_3 level in EAC/Cis/ROLE/SLRE showed a significant decrease level near to the corresponding value in GP3.

Discussion:

Breast cancer (BC) is the most common cancer in women worldwide; most patients become better with therapy as the breast tissue is not essential for survival **(Alam *et al*., 2018; Seidler & Huber, 2020)**. There are multiple clinical approaches of chemotherapy **(Miller et al., 2016)** to control BC including cisplatin (CP)**.** Since treatment of BC with CP have a number of bad side effects due to collective toxicity **(Sorsa *et al*., 1985; El-Bokiny et al., 2021),** the oxidative stress is approved as a major factor of CP-toxicity **(El-Sawalhi & Ahmed, 2014)**. Therefore, we aimed to investigate the role of *Rosemarinus officinalis* (ROLE) and *Saussurea lappa* (SLRE) in the protection against CP-induced collective toxicity and to enhance CP efficacy in EAC-mice model.

Present data showed that SLRE has marked antioxidant properties with higher extractive value than ROLE, whereas the percentage of metal scavenging capacity of ROLE was better than SLRE. Our findings revealed that SLRE has a potent antioxidant activity than ROLE as formerly showed by **Al-Bolkiny *et al* (2019)** and ROLE has better scavenging capacity **(El-Naggar *et al*., 2016)**. Moreover, SLRE showed radical scavenging activity (DPPH) more than ROLE, which has a total phenolic content (TPC) more than SLRE. These levels of the antioxidant properties of both ROLE and SLRE are complementary and consistent with **Perez-Fons et al. (2010).** On phytochemical investigation, SLRE contains flavonoids and phenolic compounds in high concentrations, which might be active principles responsible for the organ's-protection against toxicity induced by chemotherapy that agrees with **Saleem et al. (2013).**

The results showed that a significant increase of cholesterol (TC) in EAC-bearing mice group (Gp2) compared to control. These results seem to be similar with many authors **(Habib et al., 2010 ; Khanam et al., 2010)** who showed changes in biochemical parameters in EAC untreated mice. Also, this result agrees with **Abdel-Maksoud et al. (2014)** and **Alotaibi et al. (2021)** who reported that EAC induced elevation in lipid profiles. The present study showed a significant increase in serum TGs, TC, LDL cholesterol levels, but a decrease in serum HDL-cholesterol in tumor bearing mice. These changes in the lipid components may be related to abnormal lipid metabolism, associated with excess lipogenesis leading to the pathogenesis of malignancies **(Tania et al., 2010).** In addition, the above alterations may be due to metabolic disturbances of tumor cells as confirmed previously by **Obeid and Emary (1993).** Decreased serum TC, LDL-/vLDL-cholesterol levels and a decrease in serum TG levels associated with increased serum HDL-cholesterol in all groups treated with either ROLE or SLRE or combined, as compared to cancer bearing group were consistent with **Boghdady (2013).**

According to **Blade et al. (2010),** polyphenols and proanthocyanidins containing diet significantly decreased plasma total cholesterol, TGs and LDL-cholesterol and increased HDL-cholesterol in animals, which are also attributed to their inhibition of both LDL oxidation and oxidative stress **(Natella et al., 2002)**. Thus, the ameliorative effect of rosemary extract in lipid-lowering could be due to the potential of abundant phenolics in the extract, which suppressed the activity of hepatic LDL receptor sites. A slight reduction of plasma TG was observed, which was associated with a decreased LDL fraction. A similar trend was revealed in TC level **(Harnafi et al., 2013; Shen et al., 2020).** The hypolipidemic effects of rosemary extract in our study were similar to formerly reported by **Ozkol et al. (2013)**. In the same line, administration of rosemary oil to rats fed high fat diet modulated the elevation of lipid parameters **(Abdel Gawad et al., 2021)**.

Cancer cells can generate large amounts of H_2O_2, which may contribute to mutate, damage normal tissues and invade other tissues. So, there is a direct correlation between changes in the rate of cancer cell proliferation and changes in the antioxidant machinery, an explanation which is in accordance with **Gupta and Ghatak (1967).** Reduced glutathione (GSH), superoxide dismutase (SOD) and elevated malondialdehyde (MDA) in hepatic tissue as indicators for oxidative stress and cell damage was performed in this study. The GSH is an antioxidant tripeptide plays an important role in cellular defense against cytotoxicity and scavenging the reactive species **(Dröge, 2002)**. Current decreased GSH in EAC- bearing mice might be ascribed to an enhanced rate of oxidation and consumption of GSH in the process of H_2O_2 removal.

Moreover, SOD converts the highly reactive superoxide anion to H_2O_2 and subsequently, catalase is responsible for their conversion into water and molecular oxygen **(Duggina et al., 2015)**. As cited by **Kalaiselvi et al. (2013),** SOD acts as a prime player in the defense against ROS, the present reduction in SOD activity in EAC-mice relative to the control group could be linked to augmented circulating lipid peroxides produced by metabolic activation. These results also were in agreement with **Raju and Arockiasamy (2013)** who reported that the consumption of free amino acids for building proteins of rapidly dividing tumor cells might result in the disturbance of the enzyme activity in the liver **(Abu-Sinna et al., 2003)**.

Furthermore, MDA the lipid peroxidation end product, is an indicator of oxidative damage resulted from ROS generation **(Oyagbemi et al., 2016)**. Our study showed that the activity of SOD was significantly decreased accompanied with reduction of GSH level and increased MDA level in EAC alone when compared to control. On treatment of EAC/CP-mice with ROLE and SLRE alone or combined, SOD, GSH and MDA levels were improved near to the control. These results are in agreement with previous studies **(Abou Zaid et al., 2015; Al-Bolkiny et al., 2019; El-Masry et al., 2019).** A similar study conducted by **Kotebagilu et al. (2014)** reported that *Costus speciosus* have antioxidant potency that able to inhibit cellular oxidation and microsomes due to its content of flavonoid and polyphenol ingredients. The decline in SOD activity in EAC- mice might be due to the loss of Mn-SOD

activity in EAC cells and the loss of mitochondria (**Sun et al., 2012)** leading to a decrease in total SOD activity as reported by **Abou-Bedair et al., (2002).** The tumor promotion and progression that associated with systemic dysfunction in cancer bearing mice may be due to formation of ROS and other free radicals **(Trush and Kensler, 1991),** which are cured by current use of natural antioxidants.

Certainly, the growth of tumor cells and invasion cause a disturbance in antioxidant indices through generation of high amounts of free radicals, which may contribute to mutate and damage the normal tissues. Present MDA, as an end product of lipid peroxidation, usually used to estimate the extent of lipid peroxidation **(Sahna et al., 2006; Oyouni et al., 2019)**.. The obtained results exhibited high increased MDA in EAC-mice compared to the healthy mice, but it was improved in mice given ROLE/SLRE alone or combined. Current results are similar to **El-Moselhy et al. (2014)** that there is a positive correlation between changes in antioxidant mechanisms and proliferation of cancer cells. In the same line, there was a diminish in GSH/GSSG during growth of cancer cells in Ehrlich ascites as a result of increasing the oxidative stress **(Navarro et al., 1999)**. However, SOD and CAT are important key enzymes in blocking or inhibiting ROS, thus during tumor growth, oxidative damage occurs resulting in degradation of ROS scavenging enzymes **(Ratnam et al., 2006; Medhat et al., 2017)**.

Accordingly, the current results revealed that ROLE or SLRE reduced the elevated levels of MDA and increased SOD and GSH activities in EAC - mice treated with CP, indicating possible complementary antioxidant and free radical scavenging properties of rosemary and Costus. Principally, the antioxidant activity of rosemary seems to be mediated by an increase in the polyphenols concentration including carnosol, rosmarinic acid that enhance the antioxidant property **(El-Naggar et al., 2016).** The antioxidant and anti-oxidative stress properties of costus are appeared in the present study as significant decrease in GSH and significant increase of MDA. However, the using of rosemary and costus in combination was synergistically better than treatment with either agent alone.

On the other side, the above results are in disagreement with **Sathuvan et al. (2012)** and **Chen et al. (2018)** who tested the antioxidant capacity of *Costus pictus* and proved its capacity to decrease MDA due to the presence of two major polysaccharides (SLT-3, SLT-4) in costus, which efficiently prevented the generation of ROS and inhibited MDA formation**.** The decreased MDA concentrations in the costus treated animals may be due to the protective effect of costus from the deleterious effect of ROS-mediated lipid peroxidation of tissue macromolecules **(Anyasor et al. 2014).** Furthermore, they stated that the increase in GSH level may be due to the enhancement of its synthesis.

Thyroid hormones are important regulators of growth, development and metabolism in higher animals and humans. They act as a major physiological regulator of mammalian tissue development through specific effect on the rate of cell differentiation and gene expression **(Baksi & Pradhan, 2021).** There is a clinical association between BC and thyroid diseases but this clinical association is still not clear yet. However, fT_3 plays an important role in the normal development of breast by stimulating ductal branching and alveolar budding **(Senthil Kumar & Reshma, 2017).** Growing and developing breasts require a coordinated action of several hormones such as estrogen (E_2), progesterone, fT_3, adrenal steroids, insulin, and prolactin **(Lai, 2002)**. In the present study, significant increase of fT_3 levels in EAC-bearing mice compared to control while its level in EAC/Cis, Cis/ROLE and Cis/SLRE showed a significant decrease compared to EAC alone (Gp2). These results are in agreement with **Saraiva et al. (2005)** where hyperthyroidism associated with BC patients is significantly higher T_3 and T_4 values and lower TSH levels suggesting a possible promoting effect caused by this hormonal pattern on the tumor growth.

Basically, the biological activity of thyroid hormones is involved in cells expressing thyroid hormone receptors, whereby hormone-receptor complexes bind to CP acting DNA elements and enhance or repress transcription of target genes **(Jensen et al., 2001)**. The results of several *in vitro* studies suggested that thyroid hormones may influence responses to chemotherapy through promotion of cancer cell proliferation, where T_3 enhanced the sensitivity of BC cells to various chemotherapies **(Huang et al., 2013)**. Conclusively, decreased thyroid hormones during chemotherapy were found in a lot of BC patients. On the other hand, thyroid hormones can enhance the efficacy of chemotherapy through gathering tumor cells in actively proliferating stage, which may provide a new adjuvant therapy for BC in future.

Accordingly, present data revealed that the level of thyroid stimulating hormone (TSH) in EAC-bearing mice showed a significant decrease compared to control as in the same line with **Abdel-Rahman et al. (2020),** while there was a significant increase in TSH level in EAC/Cis, EAC/Cis/ROLE, EAC/Cis/SLRE compared to Gp2. There was a high significant TSH increase in EAC/Cis/ROLE/SLRE (Gp6) when compared to (Gp3). in a conducted study by **Al-Bolkiny *et al*. (2019)** who concluded that costus treatment improved the thyroid hormones in mice with both hypo-, hyper-thyroidism and it decreased oxidative stress in mice with both thyroid disorders.

Conclusion

The present study suggests that medicating of rosemary and costus can ameliorate the disturbed lipid components, oxidative stress biomarkers and thyroid hormones provoked by Ehrlish solid tumor in female mice treated with cisplatin and enhances CP efficacy due to their complementary antioxidant activities, recommending their use as adjuvants with breast cancer chemotherapy.

Conflicts of interest

All authors have approved this article and declare no conflicts of interest.

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