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Synergistic effect of avenanthramides and cisplatin co-treatment in Ehrlich ascites carcinoma-bearing mice

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Abstract

Chemotherapeutic drugs for cancer treatment cause several side effects. Natural-derived constituents as anticancer agents are effective against various cancer types. This study addressed the synergistic effect of the co-treatment with avenanthramides (AVNS) and cisplatin (Cis) in Ehrlich ascites carcinoma-bearing mice. Seventy mice were divided into seven groups (n = 10) as follows: Gp1 was used as a negative control, from Gp2 to Gp7 were inoculated with 1×10^6 EAC-cells/mouse, then Gp2 left as EAC-bearing mice, Gp3 was injected with Cis (2 mg/kg), Gp4 was injected with AVNS (50 ng/kg), Gp5 was co-treated with Cis as Gp3 and AVNS as Gp4. Gp6 was injected with a low dose of Cis (0.5 mg/kg), and Gp7 was co-treated with a low dose of Cis as Gp6 and AVNS as Gp4. The body weight change percentages (b.wt%) were calculated. On day 14, all groups were sacrificed, the ascitic fluids were harvested, and the total tumor volume, count, and live and dead tumor cells were measured. The relative expression of P53, Bcl-2, BAX, and caspase-9 genes was determined in EAC-cells by RT-PCR. Sera samples were collected for biochemical parameters assessment. Liver tissues were collected for the determination of oxidants/antioxidants biomarkers. The results showed that co-treatment of the high or low doses of Cis with AVNS led to synergistic effects via targeting apoptosis in EAC-cells that can significantly inhibit tumor growth, decrease liver dysfunctions induced by Cis, and enhance the hepatic antioxidant status.

Keywords: Avenanthramides; Cisplatin; Chemotherapy; Antitumor; Apoptosis.

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Introduction

Nearby cardiac infection and irresistible malady, cancer is one of the preeminent causes of mortality around the world. Cancer is the misfortune of the ordinary cell cycle, coming about in unregulated cell development and the need for separation, characterized as a dangerous development. Cancer can happen at any time, in any tissue or organ (Wang *et al*., 2018; Sung *et al*., 2021). The Ehrlich tumor is a spontaneous murine mammary adenocarcinoma. Not only is this type of tumor used to develop tumor models but also it is used in chemotherapy studies (Ali *et al*., 2015). As an elective to routine anticancer medications, novel drugs are being made from the auxiliary metabolites of plants; these compounds regularly show up to be less harmful and more compelling than conventional chemotherapy medicines (Al-Rasheed *et al*., 2018).

Cancer treatment has been exceedingly complex to prepare, Customary treatment approaches, such as surgery, chemotherapy, and radiotherapy, have been utilized, whereas stem cell treatment, targeted therapy, nontherapy, characteristic cancer prevention agents, and ferroptosis-based treatment (Debela *et al*., 2021). The creation of unused insurgency in neoplastic cancer or focusing on drugs depends on the pathways and characteristics of diverse tumor substances. Chemotherapy is considered the foremost compelling and broadly utilized methodology in treating a few sorts of cancers as utilized alone or in combination with other therapies, (El-Hussein *et al*., 2021). Drug resistance, a major issue with chemotherapy, could be a marvel wherein cancer cells that at first were stifled by an anti-cancer drug create resistance to the medication due to the diminished drug take-up and expanded drug efflux (Mansoori *et al*., 2017).

Cisplatin (Cis) is used for anti-neoplastic and cytotoxic effects on cancer cells. It is used as a first-line chemotherapy treatment for patients diagnosed with various types of malignancies. Oxidative stress induced by Cis has been recognized as a major factor in the toxicity of vital organs (El-Naggar and El-Said, 2020; Abdel-Latif *et al*., 2022). Treatment with a combination of quercetin and Cis ameliorates the nephrotoxicity and enhances antitumor activity (Li *et al*., 2016). Combination therapy of Cis enables an increased dose and enhances its antitumor effect by suppression of nephrotoxicity (Arita *et al*., 2021).

Combination treatment with anticancer operators improves adequacy compared to the mono-therapy approach since it targets key pathways in a characteristically synergistic way. This approach possibly decreases resistance, while at the same time giving helpful anti-cancer benefits (Bayat Mokhtari *et al*., 2017). Modern approaches, such as drugs, organic particles, and immune-mediated treatments, are being utilized for treatment indeed on the off chance that the excepted treatment level has not come to that stands up to the mortality rate and diminishes the delayed survival time for metastatic cancer (Debela *et al*., 2021). The efficiency of conventional cancer is reduced due to tumor pathology and architectural abnormality of tumor tissue blood vessels (El-Readi and Althubiti, 2019).

Avenanthramides (AVNS), the anthranilic acid amides are a group of N-cinnamoyl anthranilic acids (phenolic alkaloid compounds) that are produced in oat plants as phytoalexins, in response to pathogen attack and elicitation (Pretorius and Dubery, 2023). Numerous phytochemicals with high antioxidant, anti-inflammatory, and antiproliferative properties are produced by wild, as well as cultivated, oat plants. AVNS is one such phenolic compound identified in oats, which, due to their antioxidant, anti-inflammatory, and antiproliferative effects, are considered beneficial to health (Perrelli *et al*., 2018). These properties identify oats as a valuable food, capable of protecting against coronary heart disease, diabetes, and cancer (Wehrli *et al*., 2021). AVNS are compounds comprised of amide conjugates of

anthranilic acid or its hydroxylated derivatives and hydroxycinnamic or avenalumic acids. AVNS have an anti-inflammatory effect through the reduction in the activity of nuclear factor-kappa β (NF-κB) in NF-κB-dependent cytokine (Sur *et al*., 2008). Regulation of the transcription of DNA and the activation of genes related to inflammatory and immune responses are the responsibility of NF-κB, inhibition of NF-κB reduces cell proliferation and inflammation (Guo *et al*., 2010). The current study was conducted to investigate the anti-neoplastic activity, the curative role, biochemical, and molecular mechanism of AVNS alone or in combination with the chemotherapeutic drug Cis in Ehrlich ascites carcinoma (EAC)-bearing mice. To achieve this objective, tumor profile, hematological, biochemical, and molecular investigations were evaluated upon different treatments.

Materials and Methods

Chemicals

Avenanthramide-C methyl ester (CAS number: 955382-52-2; catalog number: CAY10011336-1 mg) was purchased from Cayman Chemical (Ann Arbor, MI 48108, USA). Avenanthramides were solved in DMSO. Cisplatin (50 mg/50 mL vial) was purchased from Merk Ltd. (Cairo, Egypt). Alanine transaminases (ALT), aspartate transaminase (AST), total protein, urea, creatinine, superoxide dismutase (SOD), catalase (CAT), and malondialdehyde (MDA) kits were purchased from Bio-diagnostic Company, Egypt.

Mice and experimental design

Ehrlich ascitic carcinoma (EAC) cells were collected from the tumor-bearing mice purchased from the National Cancer Institute (NCI, Cairo, Egypt). The number of tumor cells was adjusted at 2×10^6 cells/mouse for intraperitoneal (i.p) inoculation. Seventy male Swiss albino mice (20 ± 2 g) were given drinking tap water and normal experimental pelleted animal food *ad libitium*. Mice were divided into seven groups as follows: Gp1 was used as a negative control, from Gp2 to Gp7 were inoculated with 1×10^6 EAC-cells/mouse, then Gp2 left as a positive control, Gp3 was injected with Cis (2 mg/kg) (El-Naggar et al., 2019), Gp4 was injected with AVNS (50 ng/kg) (Aldubayan *et al*., 2019), Gp5 was co-treated with Cis (2 mg/kg) and AVNS (50 ng/kg). Gp6 was injected with the low dose of Cis (0.5 mg/kg), and Gp7 was co-treated with the low dose of Cis (0.5 mg/kg) and AVNS (50 ng/kg).

All treatments were intraperitoneally (i.p) injections after 24 hours of EAC-cells inoculation for 6 consecutive days. All groups were weighted at the beginning (initial b.wt) and at the end of the experiment (final b.wt). The percentage of the change in the total body weight was calculated. On day 14, mice from all groups were sacrificed. By using 10 ml syringes, the ascitic fluids were harvested from all groups under the study. The volume of ascitic tumor fluids was measured. To determine the number of live and dead tumor cells, the trypan blue exclusion method was used. Blood samples were collected, and the sera were separated and frozen at -20 ˚C until used for the determination of biochemical parameters. Liver homogenates were used for the determination of oxidants/antioxidants biomarkers.

Biochemical analyses

Sera alanine transaminase (ALT), and aspartate aminotransferase (AST) activities were assayed according to the method of Reitman and Frankel (1957). Alkaline phosphatase was estimated according to Belfield and Goldberg, (1971). Total proteins were assessed according to Gornall *et al*. (1949). Superoxide dismutase (SOD) and catalase (CAT) activities were measured according to the methods of Nishikimi *et al*. (1972) and Aebi (1984), respectively. Reduced glutathione was assayed according to the method of Beutler *et al*. (1963). The malondialdehyde (MDA) levels were assayed according to the method of Esterbauer and Cheeseman (1990).

Statistical analysis

All data are presented as mean ± SD. One-way analysis of variance (ANOVA) was used to assess the significant differences among treatment groups. The SPSS statistics program was used for data analysis. The criterion for statistical significance was set at $p < 0.05$.

Results

Effects of the treatment with avenanthramide or/and cisplatin on the percentage of body weight changes

The percentages of bodyweight changes (% b.wt) were determined in the different groups under the study. The results obtained from the current study revealed that the % b.wt changes of the EAC-bearing mice were significantly increased ($p < 0.05$) up to 41.91% ± 2.12 when compared to the negative control group (22.75% ± 1.59). Treatment of EAC-bearing mice with the high dose of Cis (2 mg/kg b.wt) or AVNS (50 ng/kg) post 24 hours of EAC-inoculation for six consecutive days led to significant decrease in % b.wt changes to 9.91% ± 0.97, or 13.42 ± 2.13, respectively when compared to EAC-bearing mice alone (41.91% ± 2.12) **(Table 1)**. The combinatorial treatment of EAC-bearing mice with Cis (2 mg/kg b.wt)/AVNS (50 ng/kg b.wt) for 6 consecutive days led to a synergistic decrease in the % b.wt changes up to 6.29% ± 1.45 when compared to single treatments. EAC-bearing mice that were treated with the low dose of Cis (0.5 mg/kg b.wt) showed a significant decrease in % b.wt changes (15.62% ± 1.98). Moreover, the combination of low doses of Cis and AVNS also led to a synergistic effect on the decrease in the % b.wt changes due to the decrease in the ascitic tumor fluid **(Table 1 and Figure 1)**.

Table (1): Initial body weights, final body weights, % change of body weight of groups under the study

Groups	I. B. wt. (g)	F. B. wt. (g)	% change of B.wt.
Group 1	21.14 ± 1.56 [a]	25.95 ± 2.33 [a]	22.75 ± 1.59 [a]
Group 2	22.33 ± 1.47 [a]	31.69 ± 2.69 [a]	41.91± 2.12 [c]
Group 3	22.31 ± 2.37 [a]	24.52 ± 3.15 [a]	9.91 ± 0.97 [d]
Group 4	23.41 ± 1.69 [a]	26.55 ± 4.23 [a]	13.42 ± 2.13 [a,d]
Group 5	22.55 ± 1.58 [a]	23.97 ± 2.44 [a, b]	6.29 ± 1.45 [e]
Group 6	22.21 ± 2.75 [a]	25.68 ± 1.67 [a, b]	15.62 ± 1.98 [a,d]
Group 7	22.13 ± 2.33 [a]	23.97 ± 3.15 [a, b]	8.31 ± 1.24 [d]

The values represented as means ± S.D.; Group 1: Negative control; Group 2: EAC-bearing mice; Group 3: EAC/Cis (2 mg/kg b.wt); Group 4: EAC/AVNS (50 ng/kg b.wt); Group 5: EAC/Cis (2 mg/kg b.wt)/AVNS (50 ng/kg b.wt); Group 6: EAC/Cis (0.5 mg/kg b.wt); Group 7: EAC/Cis (0.5 mg/kg b.wt)/AVNS (50 ng/kg b.wt); AVNS: Avenanthramide; Cis: Cisplatin; I.B.wt: Initial body weight; F.B.wt: Final body weight. Means that do not share a letter in each column are significantly different ($p < 0.05$).

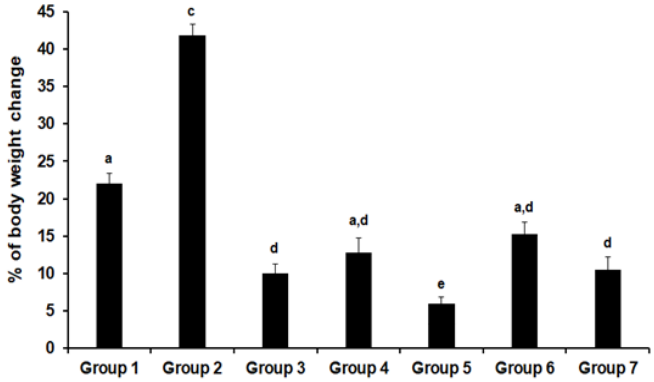

Figure (1): The percentages of body weight changes in the different groups under the study. The values represented as means ± S.D.; Group 1: Negative control; Group 2: EAC-bearing mice; Group 3: EAC/Cis (2 mg/kg b.wt); Group 4: EAC/AVNS (50 ng/kg b.wt); Group 5: EAC/Cis (2 mg/kg b.wt)/AVNS (50 ng/kg b.wt); Group 6: EAC/Cis (0.5 mg/kg b.wt); Group 7: EAC/Cis (0.5 mg/kg b.wt)/AVNS (50 ng/kg b.wt). Means that do not share a letter are significantly different ($p < 0.05$).

Total tumor volume, viable and dead EAC-cells post different treatments in the different EAC-bearing mice's groups.

The data obtained from the present study demonstrated that there were significant decreases ($p < 0.05$) in the total tumor volume of EAC-bearing mice that were co-treated with the Cis or/and AVNS (2.5 mL ± 0.32, 4.1 mL ± 0.54, or 1.3 mL ± 0.12) when compared to EAC-bearing mice alone (10.0 mL ± 0.58). EAC-bearing mice that were treated with the low dose of Cis showed a significant reduction ($p < 0.05$) in the total tumor volume (3.9 mL ± 0.33) when compared to its value in EAC-bearing mice alone (10.0 mL ± 0.58). The co-treatment of EAC-bearing mice with the low dose of Cis and AVNS for six consecutive days led to a synergistic effect on the total tumor volume that represented 2.2 mL ± 0.27 when compared to the EAC-bearing mice that was treated with the low dose of Cis **(Table 2 and Figure 2)**. The treatment with Cis (2 mg/kg/6 days) led to a significant decrease ($p < 0.01$) in the total tumor cell counts (T.T.C) (59 $\times 10^6$/mouse ± 2.6). Also, the treatment of EAC-bearing mice with AVNS led to a significant decrease in the T.T.C (235 $\times 10^6$/mouse ± 8.1) when compared to EAC-bearing mice alone (634 $\times 10^6$/mouse ± 27.8). Co-treatment with the Cis/AVNS caused a significant reduction in the T.T.C to 32 $\times 10^6$/mouse ± 2.7 when compared to a single treatment. Treatment with a low dose of Cis or in combination with AVNS caused a marked decrease in the T.T.C **(Table 2)**.

Effect of the treatment with Cis or/and AVNS on the hematological parameters of EAC-bearing mice

The hematological analysis showed that the total red blood cells (RBCs) count, hemoglobin (Hb) level, hematocrit percentages (Hct %), and platelets count were significantly decreased ($p < 0.01$) in EAC-bearing mice when compared to the negative control group. However, the treatment of EAC-bearing mice with high or low doses of Cis alone or in combination with AVNS (50 ng/kg) did restore these hematological parameters when compared to their values in EAC-bearing mice **(Table 3)**. The total white blood cells (WBCs) count was significantly increased ($p < 0.001$) in the EAC-bearing mice (24.8 x 10^3 /µL ± 3.23) when compared to the naïve mice (7.8 x 10^3 /µL ± 0.87). In EAC-bearing mice treated with Cis (2 mg/kg), and AVNS (50 ng/kg) for six consecutive days, the total WBCs counts were increased to 12.4 x 10^3 /µL ± 1.6 and 17.5 x 10^3 /µL ± 2.1, respectively. However, the co-treatment of EAC-bearing mice with Cis/AVNS for six consecutive days restored the total WBCs count close to the normal control (10.5 x 10^3 /µL ± 2.4) **(Table 4)**. The treatment with the low dose of Cis alone or in combination with AVNS led to significant improvement in the WBCs count when compared to their values in EAC-bearing mice. EAC-bearing mice that were treated with Cis alone or in combination with AVNS did alter the differential leucocyte's percentages **(Table 4)**.

Table (2): Total volume, viable and dead EAC-cells in the different groups of EAC-bearing mice.

Groups	T.T.V (ml)	T.T.C (×10⁶/mouse)	T.L.C (×10⁶/mouse)	T.D.C (×10⁶/mouse)
Group 2	10.0 ± 0.58 [a]	634 ± 27.8 [a]	605 ± 25.8 [a]	29 ± 1.9 [a]
Group 3	2.5 ± 0.32 [b]	59 ± 2.6 [b]	15 ± 2.1 [b]	44 ± 2.7 [a]
Group 4	4.1 ± 0.54 [c]	235 ± 8.1 [c]	102 ± 7.3 [c]	133 ± 6.5 [c]
Group 5	1.3 ± 0.12 [b]	32 ± 2.7 [b, e]	10 ± 0.9 [e]	22 ± 1.1 [a]
Group 6	3.9 ± 0.33 [c]	97 ± 3.9 [d]	23 ± 1.2 [d]	74 ± 2.3 [a]
Group 7	2.2 ± 0.27 [b,c]	45 ± 2.8 [b]	9 ± 0.8 [b]	36 ± 1.7 [a]

The values represented mean ± SD. T.T.V: Total tumor volume, T.T.C: Total tumor count, T.L.C: Total live cells, T.D.C: Total dead cells. Group 1: Negative control; Group 2: EAC-bearing mice; Group 3: EAC/Cis (2 mg/kg b.wt); Group 4: EAC/AVNS (50 ng/kg b.wt); Group 5: EAC/Cis (2 mg/kg b.wt)/AVNS (50 ng/kg b.wt); Group 6: EAC/Cis (0.5 mg/kg b.wt); Group 7: EAC/Cis (0.5 mg/kg b.wt)/AVNS (50 ng/kg b.wt); AVNS: Avenanthramide; Cis: Cisplatin. Means that do not share a letter in each column are significantly different ($p < 0.05$).

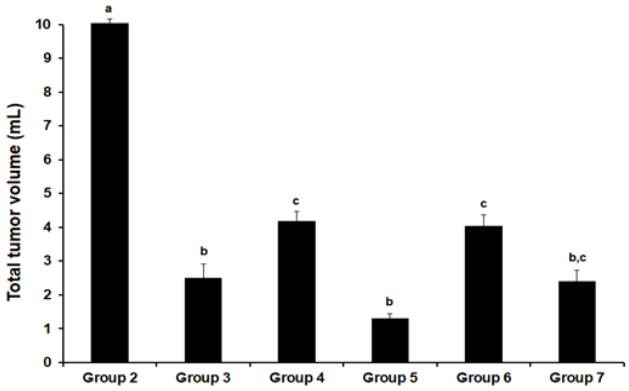

Figure (2): Total tumor volume of EAC-bearing groups under the study. Group 1: Negative control; Group 2: EAC-bearing mice; Group 3: EAC/Cis (2 mg/kg b.wt); Group 4: EAC/AVNS (50 ng/kg b.wt); Group 5: EAC/Cis (2 mg/kg b.wt)/AVNS (50 ng/kg b.wt); Group 6: EAC/Cis (0.5 mg/kg b.wt); Group 7: EAC/Cis (0.5 mg/kg b.wt)/AVNS (50 ng/kg b.wt). Means that do not share a letter are significantly different ($p < 0.05$).

Table (3): The hematological parameters in different groups under the study.

Groups	RBCs ($x10^6$ /µL)	Hb (g/dL)	Hct (%)	Platelets ($x10^3$ /µL)
Group 1	9.6 ± 1.55 [a]	12.9 ± 1.67 [a]	44.3 ± 3.15 [a, b]	732.5 ± 113.5 [b]
Group 2	5.7 ± 0.57 [b]	8.3 ± 0.75 [b]	30.2 ± 1.44 [c]	687.1 ± 108.5 [c]
Group 3	7.4 ±1.08 [b, c]	10.5 ±1.21 [b, c]	38.0 ± 1.78[c]	707 ± 110.4 [b, c]
Group 4	6.9 ± 0.65 [b, c]	9.7 ± 0.79 [b, c]	35.5± 2.34 [c]	696.8 ± 114.5 [b, c]
Group 5	8.5 ± 0.75 [a, c]	11.7 ± 0.87 [a, c]	41.5± 2.34 [a, b]	716.8 ± 94.5 [b, c]
Group 6	7.8 ± 0.55 [a,b, c]	9.1 ± 0.93 [a,b, c]	37.5 ± 3.23 [a,b, c]	700.2 ± 100.6 [b, c]
Group 7	8.2 ± 0.69 [a, c]	11.3 ± 1.44 [a, c]	40.2 ± 3.6 [a, b]	720.3 ± 100.5 [b, c]

The values represented as means ± S.D.; Group 1: Negative control; Group 2: EAC-bearing mice; Group 3: EAC/Cis (2 mg/kg b.wt); Group 4: EAC/AVNS (50 ng/kg b.wt); Group 5: EAC/Cis (2 mg/kg b.wt)/AVNS (50 ng/kg b.wt); Group 6: EAC/Cis (0.5 mg/kg b.wt); Group 7: EAC/Cis (0.5 mg/kg b.wt)/AVNS (50 ng/kg b.wt); AVNS: Avenanthramide; Cis: Cisplatin. Means that do not share a letter in each column are significantly different ($p < 0.05$).

Table (4): The percentages of the leucocytes and their differential in the different groups under the study

Groups	WBCs ($x10^3$ /µL)	Lymphocytes (%)	Neutrophiles (%)	Monocytes (%)
Group 1	7.8 ± 0.87 [a]	64.43 ± 6.78 [b]	19.4 ± 1.33 [b]	9.1 ± 0.74 [b]
Group 2	24.8 ± 3.23 [b]	75.76 ± 7.44 [a, b]	20.3 ± 2.8 [b]	6.3 ± 0.83 [a, b]
Group 3	12.4 ± 1.6 [c]	67.31 ± 8.27 [b]	16.5 ± 2.16 [c]	10.5 ± 1.30 [b]
Group 4	17.5 ± 2.1 [c]	69.18 ± 9.54 [b]	19.5 ± 3.44 [b]	9.4 ± 1.21 [b]
Group 5	10.5 ± 2.4 [a, c]	68.43 ± 7.15 [b]	18.7 ± 2.76 [b]	8.9 ± 2.03 [b]
Group 6	16.7 ± 1.5 [c]	71.21 ± 6.50 [a, b]	25.3 ± 2.5 [a, b]	4.8 ± 0.62 [e]
Group 7	12.4 ± 1.9 [c]	68.39 ± 5.45 [a, b]	26.4 ± 2.7 [a, b]	7.7 ± 1.01 [b]

The values represented as means ± S.D.; Group 1: Negative control; Group 2: EAC-bearing mice; Group 3: EAC/Cis (2 mg/kg b.wt); Group 4: EAC/AVNS (50 ng/kg b.wt); Group 5: EAC/Cis (2 mg/kg b.wt)/AVNS (50 ng/kg b.wt); Group 6: EAC/Cis (0.5 mg/kg b.wt); Group 7: EAC/Cis (0.5 mg/kg b.wt)/AVNS (50 ng/kg b.wt); AVNS: Avenanthramide; Cis: Cisplatin. Means that do not share a letter in each column are significantly different ($p < 0.05$).

Co-treatment with Cis and AVNS alleviated the liver injury in EAC-bearing mice.

Liver transaminase enzymes (ALT and AST), and alkaline phosphatase (ALP) levels were determined in the serum of the different groups under the study. The results showed that in EAC-bearing mice, there were significant increases ($p < 0.05$) in the sera levels of ALT, AST, and ALP enzymes due to liver injury (185.2 ± 9.5 U/L, 210.5 ± 9.4 U/L, and 222.27 ± 10.5 U/L, respectively) when compared to the normal control group (35.5 ± 2.3 U/L, 75.5 ± 5.5 U/L, and 84.24 ± 7.4 U/L, respectively) **(Table 5)**. The data of the present study indicated that the treatment of EAC-bearing mice

with high or low doses of Cis resulted in significant maintenance of the liver functions by a significant decrease ($p < 0.05$) in the sera ALT, AST, and ALP levels when compared to the group of EAC-bearing mice alone. However, the co-treatment of EAC-bearing mice with AVNS and high or low doses of Cis led to significant alleviation of liver injury much more than single treatments and a significant decrease in the sera activities of ALT, AST, and ALP **(Table 5)**. The results demonstrated that the EAC-bearing mice showed a significant decrease ($p < 0.05$) in their sera total protein levels when compared to the negative control group. However, the treatment of EAC-bearing mice with high or low doses of Cis led to a significant increase in the sera protein levels. Co-treatment with AVNS and Cis (high or low doses) resulted in significant alleviation of liver injury much more than single treatments by a significant increase in the total protein levels **(Table 5)**.

Co-treatment with Cis and AVNS alleviated the kidneys dysfunction in EAC-bearing mice.

Urea and creatinine levels were evaluated as kidney function biomarkers, the results showed that there were significant increases in the urea and creatinine levels of EAC-bearing mice up to 60.3 mg/dL ± 2.2 and 1.44 mg/dL ± 0.08, respectively ($p < 0.05$) when compared to the normal control group (29.1 mg/dL ± 1.9 and 0.57 mg/dL ± 0.05). However, the treatment with high or low doses of Cis improved kidney functions of EAC-bearing mice indicated by the reduction of sera urea and creatinine levels when compared to the EAC-bearing mice alone. The co-treatment of Cis and AVNS enhanced the antitumor activity with much more improvement of kidney functions of EAC-bearing mice when compared to treatment with Cis alone **(Figure 3)**.

Table (5): Serum activities of ALT, AST, ALP, and the total protein levels in the different groups under the study

Groups	ALT (U/L)	AST (U/L)	ALP (U/L)	T. protein (g/dL)
Group 1	35.5 ± 2.3 ^{a}	75.5 ± 5.5 ^{a}	84.24 ± 7.4 ^{a}	8.21 ± 0.35 ^{a}
Group 2	185.2 ± 9.5 ^{c}	210.5 ± 9.4 ^{b}	222.27 ± 10.5 ^{b}	4.56 ± 0.21 ^{b}
Group 3	110.5 ± 7.4 ^{b}	140.2 ± 7.5 ^{c}	172.84 ± 8.5 ^{c}	5.21 ± 0.25 ^{c}
Group 4	95.3 ± 6.7 ^{b,d}	135.0 ± 6.3 ^{c}	164.32 ± 7.2 ^{c}	5.88 ± 0.28 ^{c}
Group 5	85.2 ± 5.5 ^{e}	100.7± 8.1 ^{d}	130.33 ± 7.2 ^{e}	6.24 ± 0.20 ^{a,c}
Group 6	120.5 ± 7.1 ^{b}	165.2 ± 8.3 $^{c, e}$	202.45 ± 10.1 ^{b,d}	4.75 ± 0.12 ^{b,c}
Group 7	98.7 ± 6.5 ^{b,d}	136.2 ± 6.5 ^{c}	192.54 ± 8.3 ^{c,d}	5.81 ± 0.13 ^{a,c}

The values represented as means ± S.D.; Group 1: Negative control; Group 2: EAC-bearing mice; Group 3: EAC/Cis (2 mg/kg b.wt); Group 4: EAC/AVNS (50 ng/kg b.wt); Group 5: EAC/Cis (2 mg/kg b.wt)/AVNS (50 ng/kg b.wt); Group 6: EAC/Cis (0.5 mg/kg b.wt); Group 7: EAC/Cis (0.5 mg/kg b.wt)/AVNS (50 ng/kg b.wt); AVNS: Avenanthramide; Cis: Cisplatin. ALT: Alanine transaminase; AST: Aspartate transaminase; ALP: Alkaline phosphatase; T. protein: Total proteins. Means that do not share a letter in each column are significantly different ($p < 0.05$).

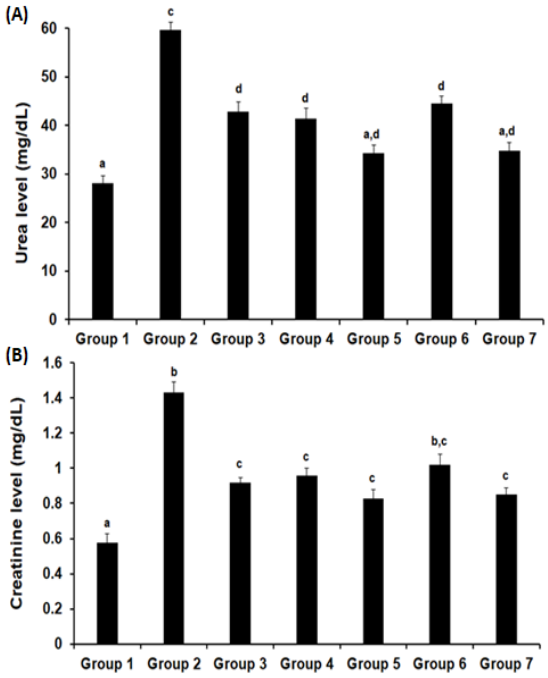

Figure (3): Sera urea **(A)** and creatinine **(B)** levels in the different groups under the study. The values represented as means ± S.D.; Group 1: Negative control; Group 2: EAC-bearing mice; Group 3: EAC/Cis (2 mg/kg b.wt); Group 4: EAC/AVNS (50 ng/kg b.wt); Group 5: EAC/Cis (2 mg/kg b.wt)/AVNS (50 ng/kg b.wt); Group 6: EAC/Cis (0.5 mg/kg b.wt); Group 7: EAC/Cis (0.5 mg/kg b.wt)/AVNS (50 ng/kg b.wt). Means that do not share a letter are significantly different ($p < 0.05$).

Co-treatment of EAC-bearing mice with Cis and AVNS improved hepatic antioxidants/oxidants hemostasis.

The result showed that EAC-bearing mice showed significant decreased ($p < 0.05$) in the hepatic SOD, CAT, and GSH levels to 2.16 ± 0.12 U/mg protein, 35.34 ± 2.5 U/mg protein, and 2.24 ± 0.21 µmol/g tissue, respectively when compared to the negative control group that represented 6.23 ± 0.68 U/mg protein, 74.67 ± 4.2 U/mg protein, and 7.15 ± 0.46 µmol/g tissue, respectively. In contrast, the levels of MDA in the liver tissues of EAC-bearing mice were significantly increased ($p < 0.05$) up to 77.21 ± 2.3 nmol/g tissue when compared to the normal control group that represented 37.43 ± 1.8 nmol/g tissue **(Figures 4)**. The results obtained from the present study revealed that treatment of EAC-bearing mice with high or low doses of Cis led to significant improvement in the antioxidants/oxidants status that was evidenced by the increase in SOD, CAT, and GSH levels, in contrast, there was significant decrease in the levels of MDA in the liver tissues homogenates. Moreover, the treatment of EAC-bearing mice with Cis and AVNS showed much more improvement in their antioxidant capacity **(Figure 4)**.

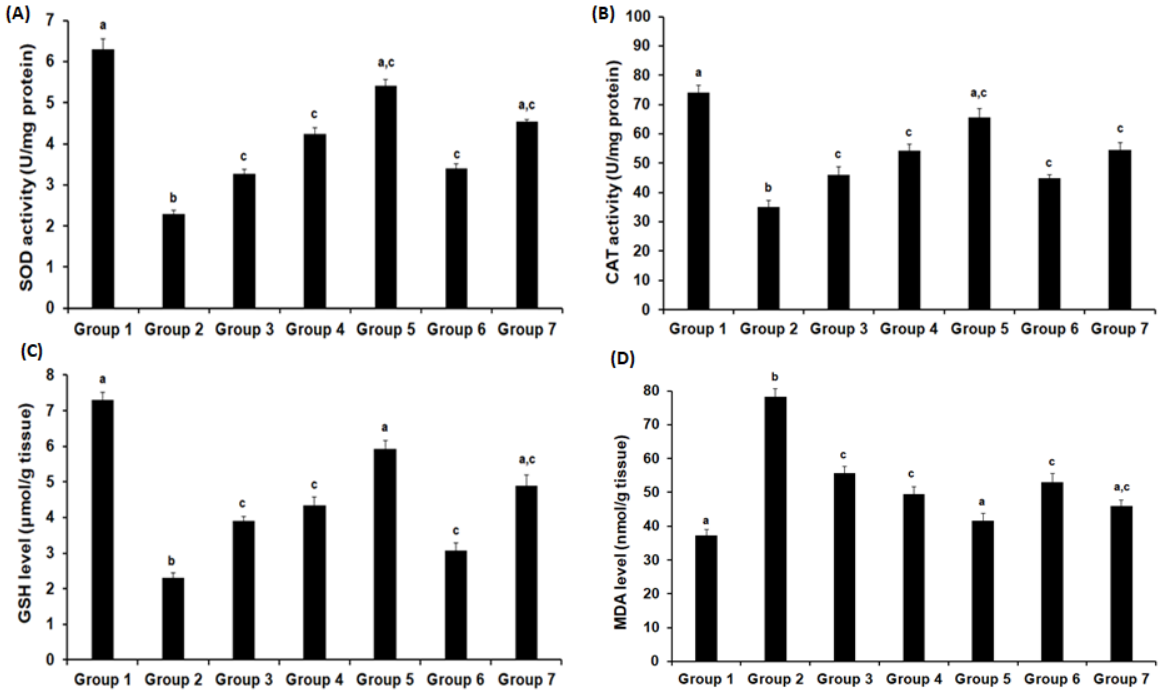

Figure (4): Hepatic superoxide dismutase (SOD) activity **(A)**, catalase (CAT) activity **(B)**, reduced glutathione (GSH) level **(C)**, and malondialdehyde (MDA) **(D)** level in the different groups under the study. The values represented as means ± S.D.; Group 1: Negative control; Group 2: EAC-bearing mice; Group 3: EAC/Cis (2 mg/kg b.wt); Group 4: EAC/AVNS (50 ng/kg b.wt); Group 5: EAC/Cis (2 mg/kg b.wt)/AVNS (50 ng/kg b.wt); Group 6: EAC/Cis (0.5 mg/kg b.wt); Group 7: EAC/Cis (0.5 mg/kg b.wt)/AVNS (50 ng/kg b.wt). Means that do not share a letter are significantly different ($p < 0.05$).

Discussion

Cancer is a highly heterogeneous disease that refers to several distinct pathologies affecting many different tissues and cell types. However, all forms of cancer are characterized by abnormal cell growth resulting from inherited or environmentally induced genomic instability and mutations (Sonugür and Akbulut, 2019). Conventional chemotherapy is an effective approach to cancer treatment. However, chemotherapy treatment led to wide side effects on some vital organs and induced resistance of the tumor cells to the treatment. Cisplatin (Cis) is an effective chemotherapeutic agent that is used to treat different types of cancer; however, it has toxic effects on the kidney, liver, and other organs (El-Naggar and El-Said, 2020). Resistance to chemotherapy and its adverse effects remain the major problems in its cancer treatment regimen Therefore, decreasing its resistance and adverse effects without any limitation to its anticancer efficacy is necessary (Bukowski *et al*., 2020).

Natural products, with remarkable chemical diversity, have been extensively investigated for their anticancer potential for more than a half-century (Huang *et al*., 2021). Oat (*Avena sativa* L.) is an eco-friendly grain trim, with different wholesome, therapeutic, and pharmaceutical applications. Various phytochemicals with antioxidant, anti-inflammatory, and antiproliferative properties are created by wild, as well as developed, oat plants. These include flavonoids, phenolics, saponins, tocopherols, and tocotrienols. Among the cultivated species, white oat (*A. sativa*) and

red oat (*A. byzantina*) are the only grain crops that synthesize avenanthramides (AVNS). These AVNS compounds have thus been regarded as signature compounds in oats. Here, the biosynthesis and structural and functional diversity of these unique oat phytochemicals (Pretorius and Dubery, 2023). Physiologically active constitutes of oats incorporate vitamin E, carotenoids, anthocyanins, lignans, phytic corrosive, phenolics, phytosterol, and AVNS, which could be a phenol display as it were in oats (Raguindin *et al*., 2020).

The development of a new class of anticancer drugs that lacks the toxicity of conventional chemotherapeutic agents unaffected by common mechanisms of chemoresistance would be a major advance in cancer therapeutics. Several important chemotherapy agents were originally identified from natural sources. Therefore, new biologically active compounds obtained from natural products will certainly continue to offer vast opportunities as sources of new anticancer therapeutic leads (Cabral *et al*., 2018). The current study was conducted to investigate the anti-neoplastic activity, the curative role, biochemical, and molecular mechanism of AVNS alone or in combination with the chemotherapeutic drug Cis in Ehrlich ascites carcinoma (EAC)-bearing mice through assessing tumor profile, hematological, biochemical, and molecular investigations after different treatments.

Due to cancer diseases, cachexia, in which there is a progressive loss of fat and muscle mass leading to overwhelming weakness; is attributed to weight loss (Bachmann *et al*., 2008). This study showed that inoculation of EAC-cells led to an increase in the percentage of body weight change, and this could be due to the proliferation of EAC-cells inside the peritoneal cavity of mice. This could be due to the rapid and gradual accumulation of tumor cells. This finding agreed with a previous study by El-Naggar *et al*. (2019), who reported that there was a significant increase in the total body weight change in EAC-bearing mice compared to naïve mice. Treatment of EAC-bearing mice with Cis or AVNS led to a significant decrease in the percentage of body weight change. This finding was following previous studies (El-Naggar and El-Said, 2020; Ibrahim *et al*., 2022). Furthermore, combinatorial treatment with high or low doses of Cis and AVNS increases the reduction in the percentage of body weight change this could be due to the inhibition of EAC-cells growth in the peritoneal cavity of mice and decrease in tumor growth compared to single treatments. Previous studies reported that co-treatment of Cis with natural products enhanced the antitumor activity and resulted in a reduction in the percentage of body weight change of EAC-bearing mice (El-Naggar *et al*., 2019; Amuthan *et al*., 2021; Saleh *et al*., 2022).

Our data suggests that AVNS treatment might represent an excellent therapeutic tool as an anticancer agent in agreement with Aldubayan *et al*. (2019) who reported the antineoplastic activity and curative role of avenanthramides against the growth of Ehrlich solid tumors in mice, diminished the size and weight of the tumors removed from the treated mice, and induced apoptosis. In the present study, the treatment of EAC-bearing mice with high or low doses of Cis, AVNS led to a significant reduction in the total tumor volume, total tumor count, and total live tumor cells, however, the low dose of Cis or AVNS did not completely treat the EAC-bearing mice. This could be due to the low doses of Cis not enough to eliminate or stop the tumor cells completely. Co-treatment with AVNS increased the efficacy of the low doses of Cis as anticancer agents in EAC-bearing mice. This finding was supported by the decrease in the total volume, total tumor count, total live tumor cells, and increase in the total dead tumor cells. These results were in line with a previous study reported that the antitumor efficacy of a low dose of Cis could be enhanced in EAC-bearing mice by increasing

the percentages of dead tumor cells (El-Naggar *et al*., 2019; Aldubayan *et al*., 2019; Hashem *et al*., 2021; Morsi *et al*., 2022).

In EAC-bearing mice, anemia is mainly due to iron deficiency by hemolytic conditions accompanied by a decrease in RBCs' count (Sreelatha *et al.,* 2011). Hemoglobin (Hb) showed a significant decrease in the treated group compared with normal control This decrease could be due oppressive effect of EAC on erythropoiesis (Degowin and Gibson, 1978). The total RBCs count, Hb level, and Hct % were decreased in EAC-bearing mice. Propagation of EAC cells may be attributed to this increase due to inflammatory reactions or stress (Badr *et al*., 2011). Lymphopenia in all groups compared to negative control may attributed to drug immunosuppressive. The total WBCs count was increased in EAC-bearing mice, while the treatment with Cis or/and AVNS resulted in a decrease in the WBCs count. This effect could be due to the protection of the hematopoietic system (Nafie *et al*., 2020; Fawzy *et al*., 2023).

Liver damage induced by tumor cells typically indicates disruption of normal liver cell metabolism, resulting in changes in the activity of serum enzymes. The progression of EAC-tumor in mice led to liver dysfunctions evidenced by the increase of liver transaminase enzymes (ALT and AST). ALT and AST levels were elevated in the serum of EAC–bearing mice because the inoculation of Ehrlich cells induced organ dysfunction and metabolic disturbance (Bhattacharyya, 2007). The elevation levels of ALT and AST in EAC tumor-bearing mice is an index of deterioration of hepatic functions due to cancer proliferation as observed in the EAC group and it suggested a functional impairment of hepatic cell membranes and a cellular leakage which demonstrated that EAC-induced liver injury (El-Naggar *et al*., 2019; Tousson *et al*., 2020). The results explained the deleterious effect of Cis on liver cells. A previous study revealed the protective antioxidant effects of natural constitutes in Cis-induced hepatotoxicity in experimental animals (Abd Rashid *et al*., 2021). In the present study, the protective and antitumor effects of AVNS were addressed in EAC-bearing mice. Elevation of ALT and AST enzymes in EAC-bearing mice may be due to the cytotoxic effect of EAC tumors which led to damage of liver cells and canaliculi. Cis increased ALT and AST in EAC-bearing mice (El-Naggar *et al*., 2019). The results showed that AVNS had antitumor effects *in vivo* studies. In addition, the co-treatment with AVNS could protect liver tissues against Cis toxicity. These results agreed with the previous studies that reported the efficacies of the co-treatment with Cis and natural products (Nafie *et al*., 2020; Abd Rashid *et al*., 2022). The present study showed that the levels of ALT, AST, ALP, and total proteins in the group of EAC-bearing mice that were treated with Cis were significantly decreased when compared to the EAC-bearing mice alone. This finding was in line with a previous study by El-Naggar *et al*. (2019).

Combinatorial treatment with Cis/AVNS led to a significant decrease in the levels of these transaminases in EAC-bearing mice compared to single injections which indicates the ameliorative effects of AVNS on hepatotoxicity. The ALT, AST, and ALP enzymes were decreased in the group of EAC-bearing mice treated with a combination of Cis/AVNS. Decreasing the hepatic toxicity upon treatment with this combination indicates that the AVNS has a protective effect against liver dysfunction and cellular injury of the liver. Nephrotoxicity and kidney damage are characterized by a marked increase in the serum levels of urea and creatinine. The results showed that urea and creatinine levels were increased in EAC-bearing mice which could be attributed to the catabolic effect of the tumor and the elevation in urea production and nephritis (Ibrahim *et al*., 2022). In the meantime, increased creatinine levels in the EAC-bearing mice might be due to muscle necrosis. Co-treatment of EAC-bearing mice with Cis/AVNS reduced the levels of

serum urea and creatinine compared to EAC-bearing mice. This finding agreed with the previous reports of Thulfiqar and Tousson (2020) and Nafie *et al*. (2020).

Oxidative stress is considered key in the development of numerous health conditions including cardiovascular diseases and obesity. The evidence also indicates it has a major role in tumorigenesis (Boz, 2015). Studies of diverse cereal components find that the antioxidant activity of AVNS is 10–30 times greater than ferulic acid, gentisic acid, phydroxybenzoic acid, protocatechuic acid, syringic acid, vanillic acid, and vanillin (Yang *et al*., 2014). These radicals can encourage tumor growth and metastasis by promoting the tumor cells' invasive, angiogenic, and migratory abilities (Abd El-Aziz *et al*., 2014). The present study reported that AVNS induced significant improvement in reversing the alterations in the hepatic antioxidant/oxidant hemostasis of EAC-bearing mice as it can inhibit lipid peroxidation and prevent oxidative stress. Treatment with AVNS significantly reversed the oxidative stress-associated changes, which could be due to the improvement of the antioxidant defense system in agreement with Aldubayan *et al*. (2019) who found that the enhancement of antioxidant status resulted in a significant decrease in MDA level as well as a significant increase in SOD level. The current study indicated that due to the tumor growth CAT and SOD activities were inhibited, AVNS could modulate antioxidant enzyme activity by promoting the levels of CAT, GSH, and SOD, as well as inhibiting the level of MDA. These results agree with a previous study that detected elevated levels of MDA in breast cancer. The findings here are consistent with those of Abd El-Aziz *et al*. (2014) who reported that Ehrlich tumors exhibited significant increases in MDA and considerable decreases in CAT and SOD. Furthermore, it has been proposed that the diminished levels of SOD levels may arise from the altered antioxidant status initiated by carcinogenesis (Moselhi and Al Mslmani, 2008). Macromolecules such as lipids become damaged through oxidative stress brought on by the excessive production of free radicals; *in vivo*, this can induce lipid peroxidation. The reduced levels of GSH found in tumor-bearing mice might be due to the transformation rate of GSH to oxidized GSH increasing to reduce the intracellular concentration of hydrogen peroxide (Abd El-Dayem *et al*., 2010).

Conclusion

The co-treatment with Avns with Cis leads to enhancement of the antitumor efficacy of Cis by inducing apoptosis and decreasing its toxic effects on the liver tissues via improvement of liver functions and antioxidant status. Avns exerted its antitumor effect mainly by blocking reactive oxygen species due to its antioxidant potential. Moreover, they exhibit potential therapeutic activity through the modulation of different pathways including the activation of apoptosis, and the block of cell proliferation. Avns are promising chemo-preventive and anticancer phytochemicals, which need further clinical trials and toxicological studies to define their efficacy in preventing and reducing the burden of cancer diseases.

Conflict of interest

All authors declared that there were no conflicts of interest.

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