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Evaluation of Antioxidant & Antitumor Activities of *Moringa* Extract in Mice

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CANCER is a severe metabolic syndrome and one of mortality leading causes worldwide. Radiation has important therapeutic benefits as radiotherapy in malignant tumor, however it is also associated with serious adverse effects and thus reflecting the need to create or aid safer treatment. *Moringa oleifera* (MO) is a promising plant that has a wide range of medicinal applications. Therefore, the present study is conducted to evaluate the effect of *Moringa* extract on implanted Ehrlich›s solid tumor in mice either alone or in association with radiotherapy.

Fifty male Swiss albino mice were used in this study implanted intramuscularly with Ehrlich ascites carcinoma (EAC) and were divided into five groups; group 1 (control) orally administred1 ml saline (3 days/week for 4 weeks) ,group 2 Ehrlich ascites carcinoma bearings mice (EAC), group3 EAC-bearing mice exposed to radio therapeutic dose two times/week for 4 weeks with dose (2GY), group 4: mice bearing EAC were orally administrated *Moringa* extract (500 mg/kg b.w 3 days/week for 4 weeks) after one week of tumor implantation, group5 mice bearing EAC were exposed to radio therapeutic dose and orally administered *Moringa oleifera* extract after one week of tumor transplantation. Radiotherapy of EAC-mice with *Moringa* extract exhibited inhibition of tumor markers Her-2 and increased P53 also, improved blood parameters, lipid profile, oxidants and antioxidants levels, liver and kidney functions in EAC bearing mice. It could be concluded that *Moringa oleifera extract* is potentially capable of exerting antitumor effect.

Keywords: Ehrlich Tumor, Gamma radiation, *Moringa oleifera,* P53, Her-2.

Introduction

Cancer is one of the vital causes of morbidity and mortality worldwide and recognized as the second most leading cause of death. The incidence of cancer has been consistently increasing due to lifestyle changes and increasing environmental pollution. The availability of treatment regimens in modern therapy has not significantly reduced the cancer burden in society. The cost of cancer treatment has seen a phenomenal increase in recent years due to the approval of high-cost oncology drugs (Simoens et al., 2017). Despite the advancement in understanding the molecular basis, detection and treatment of cancer, mortality is still high and there is still not a proper treatment to eradicate the growth of tumors (Das et al., 2019).

A few strategies are accessible to treat the disease such as a medical procedure, chemotherapy, radiation treatment, immunotherapy, and monoclonal immunizer treatment. The decision of treatment relies on the area of the growth, grade of the cancer and the phase of the infection as well as the overall condition of the patient (Abdul Hayeea et al., 2022).

Experimental tumors have extraordinary significance for the reasons for modeling, and Ehrlich carcinoma is one of the most common (Abd Eldaim et al., 2019a ; Aldubayan et al., 2019). Ehrlich ascites carcinoma (EAC) is mentioned as a spontaneous murine mammary adenocarcinoma, originally hyperdiploid and

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can transplant intraperitoneally passages on all mouse strains (Mutar et al., 2020).

Radiotherapy is regarded as one of the most important therapeutic modalities for the treatment of malignant tumors. A benefit of ionizing radiation as a therapeutic tool is the possibility to apply it locoregionally, thereby preventing systemic toxicity. However, similar to chemotherapeutic agents, ionizing radiation can lead to severe side effects in the surrounding tissues after the therapy. In addition, there are large numbers of human malignant tumor cells that respond poorly to ionizing radiation (Hanafi & Mansour, 2010). Recently, there is an obvious increase in the usage of complementary or / and alternative medication (Tousson et al., 2019, 2020; Abd Eldaim et al., 2019b).

Medicinal plant research and applications are expanding each day due to therapeutic phytochemicals, which can stimulate the progress of novel medicines. Most plant-based phytochemicals, e.g., carotenoids, phenolic acids, flavonoids, tannins, saponins, alkaloids, and glucosinolates, have beneficial effects on well-being and avoidance of malignancy (Venugopal & Liu, 2012). Phytochemicals are secondary aromatic plant metabolites that prevent disease and are extensively present in plants. They are widely recognized for preventing and reducing chronic diseases risk (e.g., cancer cardiovascular, and neurological) and for beneficial mediation in treating these diseases (Shahidi & Ambigaipalan, 2015; Kaur Kala et al., 2016).

Moringa oleifera (Moringaceae) is a promising plant for biomedicine applications because it has an impressive range of medicinal purposes with high nutritional value. It contains a diverse array of functional bioactive components including vitamin A, vitamin C, proteins, alkaloids, quinines, saponins, flavonoids, tannins, steroids, glycosides, fixed oils and fats, and phytochemicals like niazinin A, niazinin B, niazimicin A, and niaziminin B (Paikra et al., 2017). Therefore, the present study aims at evaluating the antioxidant & antitumor effects of *Moringa Extract* against the Ehrlich solid tumors and in combination with the effect of ionizing radiation, as a standard widely used radiotherapeutic medication.

Material and Methods

Blood sampling

Animals

Male albino mice weighing about (20-25 gm) were obtained from the Nuclear Research Center, Atomic Energy Authority, Egypt. They were maintained under normal conditions of temperature 28ºC, air ventilation and relative humidity (60%) , a 12:12 Light/dark hours. Mice were provided a standard rodent diet and water ad libitum. The experiments were approved by the Ethics Committee of Atomic Energy Authority in accordance with the National Institutes of Health guide for care and use of laboratory animals (NIH).

Tumor inoculation

The ascitic fluid (1 ml) from mice with EAC were initially supplied by the National Center Institute, Cairo, Egypt, it was diluted with saline at a ratio of 1:10, and 0.2 ml of diluted ascitic fluid represented 2.5×10^6 EAC (Mansour & Anis 2010) ,then injected intramuscularly in the left thigh of each mouse to stimulate Ehrlich ascites carcinoma (EAC) as described by Perry (2008).

Irradiation

Animals were irradiated two times weekly for 4 weeks, each dose was (2 Gy) with a collective dose of 2x8= 16 Gy. Gamma irradiation was performed using (C137) gamma cell 40 at the National Center for Research and Radiation Technology (NCRRT).

Plant extraction

Dry *Moringa* leaves (200 g) were extracted with 1 L ethanol (70%) and shacked each 8h, and then the extract was filtered using cotton funnel. The extract was concentrated using a rotator evaporator. The concentrated extract was lyophilized and kept. The dose of *Moringa oliefera* extracts was dissolved in DMSO (Dimethylsulfoxide) and administrated by oral gavage.

Experimental design

A total number of fifty male Swiss albino mice were randomly classified into five groups (n=10) as follows:

Group1: Was assigned as control, mice were orally adminstrated 1 ml saline

Group2: Mice bearing Ehrilch Ascites carcinoma (EAC)

Group3: Mice bearing EAC were exposed to a radio therapeutic dose after one week of tumor transplantation.

Group 4: Mice bearing EAC and were orally administrated *Moringa oleifera* extract after 7 days of tumor implantation at dose 500 mg/kg/ 3 days weekly for 4 weeks (Wagdy et al., 2014)

Group5: Mice bearing EAC were exposed to a radio therapeutic dose and orally administrated *Moringa oleifera* extract after 7 days of tumor transplantation. All treatments continued for 4 weeks. Three days after the last dose of treatments, blood and livers were immediately obtained after the mice were sacrificed. At the end of the experimental period (4 weeks), rats were fasted overnight. Blood samples were collected from each rat under light ether anesthesia into 2-tubes, one part into heparinized tubes for hematological parameters determination. The 2nd part allowed clotting for 10-15 min, then centrifuged. The serum was separated and kept frozen at -20°C for biochemical estimations.

Hematological analysis

Heparinized blood samples were immediately used for the hematological parameters. CBC parameters were examined using Sysmex (KX-21) cell counter, with a kit manufactured by (Diamond, Philadelphia, USA).

Biochemical analysis

Serum levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), urea, creatinine, serum total cholesterol (TC), high density lipoprotein (HDL) and triglycerides (TG) levels were measured using kits obtained from Biodiognostic Co. Egypt. In addition, serum p53 & Her2 were determined. P53 analysis were carried out through: Rat P53/Tumor protein (P53/TP53) ELISA kit. Catalog No. CSB-E08336r CUSABIO Company. Her-2 analysis were measured using: Rat Epidermal Growth factor Receptor Extra cellular Domain (Her -2/ nue ECD) ELISA kit was used. Catalog No. MBS 728852 MyBiosource Company.

Tissue homogenate preparation

Liver tissues from experimental groups were immediately removed and saline-rinsed (NaCl 0.9 percent) to remove blood at the end of the treatment. The liver's tissues were homogenized in suitable buffer using Teflon homogenizer. The supernatant from the centrifuged homogenate was used to estimate MDA & GSH. The method developed by Ohkawa et al. (1979) was used to measure Malondialdehyde (MDA), a stable byproduct of lipid peroxidation, in the liver homogenate. The activity of the reduced glutathione (GSH) in the liver was determined in accordance with the procedures described by Beutler et al. (1963).

Statistical analysis

Data were analyzed using analysis of variance (ANOVA) test .Duncan's Range Test was used to compare between groups using SPSS. Software packageV.20.0

Results

The current study showed that the EAC-bearing mice induced a marked decrease in the levels of RBCs, HB and HCT meanwhile, the level of WBCs was significantly increased as compared to control group. Treatment EAC group with Moringa leaves extract (MO) significantly elevated the levels of RBCs, HB, HCT and reduced the level of WBCs (Table1).

The present findings revealed that the serum level of AST, ALT, Urea and creatinine were remarkably elevated in EAC-bearing mice group with comparison to control group whereas, in the EAC-suffering mice treating with MO Extract significantly decreased this elevation (Table2).

TABLE 1. Effect of *Moringa* extract on some hematological parameters in EAC-bearing mice

Groups parameters	Control	EAC	EAC+R	EAC + MOE	EAC+R+MOE
RBCs n×10¯6	6.21^{a}± 0.22	3.8^{e} ± 0.16	4.06^{c} ± 0.18	5.42 ± 0.2 ^{b}	$5.8^{a\,b}$ ± 0.22
Hb (g/dl)	12.57^{a} ± 0.16	8.27^{d} ± 0.22	8.55^{d} ± 0.17	10.65^{c} ±0.46	11.27^{b} ± 0.38
HCT (%)	37.72^{a}± 0.5	0.59 24.95^{d} ±	25.62^{d}±0.5	31.97^{c}±0.5	33.81^{b}±0.43
WBCs n×10¯3	8.05^{b} ± 0.22	15.43^{a} ± 0.24	6.6^{d} ±0.22	7.22^{c} ± 0.18	7.41^{c} ± 18

Data were expressed as mean ± SE significant at (P<0.001)

a, b, c and d: means bearing different superscripts within the same row are significantly different at P≤0.001.

TABLE 2. Effect of *Moringa* extract on the liver enzymes, urea creatinine in EAC-bearing mice

Groups parameters	Control	EAC	EAC+R	EAC + MOE	EAC+R+MOE
ALT (U/L)	77.57^{d} ±2.58	181.42^{a}±3.51	173^{a}±2.48	149.85^{b}±3.8	140.28^{c} ±3.28
AST (U/L)	83.42^{d} ±3.87	219.71^{a}± 4.32	207.85^{a} ±4.47	143.42^{b} ±4.12	122.28^{c} ± 3.94
Urea (mg/dl)	28.71^{d}± 1.32	66^{a} ±2.35	61^{a}± 2.26	48.42^{b}±2.16	40.57^{c}± 1.73
Creatinine (mg/dl)	0.62^{e} ± 0.01	1.4^{a} ± 0.05	1.28^{b} ±0.03	0.95^{c}±0.04	0.77^{d}± 0.02

Data were expressed as mean ± SE significant at (P<0.001)

a, b, c and d: means bearing different superscripts within the same row are significantly different at P≤0.001

The Present results signify a marked increase in the levels of TC &TG and a significant decrease in HDL in EAC group when compared to the control. Besides, treatment EAC with Moringa Extract ameliorated these measures and reduced the hyperlipidemia (Table 3).

The obtained findings exhibited that the level of liver MDA was meaningfully raised; but the level of GSH was considerably reduced in the EAC group as compared to the control group. Conversely, treatment with either *Moringa* extract alone or MO & radiotherapy considerably declined the level of MDA and raised the level of GSH.. Data for tumor markers in EAC groupshowed a significant increase in Her2 associated with a significant reduction in p53 level in comparison to the control group. On the other hand, treatment of the EAC group with either MO or radiotherapy and MO resulted in a significant increase in P53 level accompanied by a significant decrease in Her2 level (Table4).

Discussion

Cancer is a disease of misguided cells that have a high potential of excess proliferation without any apparent relation to the physiological demand. It is the second largest cause of death in the world. Of all the available anticancer drugs were natural products or natural product derived (Nidhi, 2012). Hence, there is a great potential for the development of anticancer drugs from the essential plant kingdom (Greenwell & Rahman, 2015). Ehrlich tumor is very aggressive and readily grows more quickly with extremely destructive behavior that can develop in mice strains (Portilho et al., 2011). *Moringa oleifera* (Moringaceae) is a promising plant for biomedicine applications because it has an impressive range of medicinal purposes with high nutritional value. Therefore, the authors investigated the therapeutic effect of *Moringa oleifera* leaf extract (MOLE) on Ehrlich's solid tumor implanted mice (EST-mice).

TABLE 3. Effect of *Moringa Extract* on lipid profile in EAC-bearing mice

Groups parameters	Control	EAC	EAC+R	EAC + MOE	EAC+R+MOE
TC (mg/dl)	121.42^{d}±2.1	153.57^{a}±2.4	136.57^{b}± 3.2	134^{cb}±3.4	127^{cd}± 2.8
(mg/dl) TG	135^{c}±2.25	166^{a}±3.13	147.28^{b}±2.51	143^{b}±2.51	140.57^{bc}±2.44
(mg/dl) HDL	61.42^{a}±1.84	41^{d}± 1.39	42.28^{d}±1.34	49.42^{c}±1.55	54.28^{b}±1.61

Data were expressed as mean ± SE significant at (P<0.001)

a, b, c and d: means bearing different superscripts within the same row are significantly different at P≤0.001

TABLE 4. Effect of *Moringa* extract on MDA, GSH, P53 and Her2

Groups parameters	Control	EAC	EAC+R	EAC + MOE	EAC+R+MOE
MDA nmol/g	26.08^{c}±0.6	55.28^{a}±2.9	53.25^{a}±2.46	41^{b}±1.63	38.26^{b}±1.89
GSH mg/g	15.4^{a}±0.24	10.31^{c}±0.21	10.68^{c}±0.15	12.71^{b}±0.3	13.11^{b}±0.31
P53 mg/dl	20.28^{b}± 0.58	11.14^{d}±0.4	16.28^{c}± 0.5	28.71^{a}±0.42	31.42^{a}±0.43
Her2 ng/ml	17^{b}± 2.98	36.2^{a}± 4.8	13.14^{c}± 3.9	9.71^{d}± 4.4	$7.57s^{d}$± 4.1

Data were expressed as mean ± SE significant at (P<0.001)

a, b, c and d: means bearing different superscripts within the same row are significantly different at P≤0.001.

The complete blood picture (CBC) and different hematological measurements are used as an extensive screening assessment to monitor many health problems such as immunodeficiencies, cancer prognosis and progression (Omuse et al., 2018). Anemia is the most common hematological conditions in cancer patients, and their prevalence rises with routine treatments such as chemotherapy/ radiotherapy, which has a damaging effect on the bone marrow (Madeddu et al., 2018). The current study revealed that EAC induced anemia, manifested by significant reductions in RBCs count, hemoglobin concentration, and hematocrit. Similar findings were reported by Hashem et al. (2020a). This may be due to the suppressive effect of EAC on erythropoiesis that could result from iron deficiency, hemolytic or myelopathic illnesses reported in cancer (Hogland, 1982). Moreover, the anemia observed in the EAC-bearing mice could be attributed, in part, to the deficiency of folic acid that may be due to EAC-induced thiamine deficiency, which is essential for folic acid metabolism (Koheil et al., 2011). Consistent with the obtained results, Badr et al. (2011) found that the EAC-bearing mice exhibited significant increases in white blood cells count that may be due to the acute inflammatory response and/or oxidative stress mediated by the proliferation of Ehrlich cells. Recovery of the HB content, hematocrit and ameliorating RBCs count and WBCs almost near the normal values, was prominent in the treated mice with Moringa extracts (MOE) indicating that Moringa extract can normalize the levels of hematological parameters, which may be due to the presence of antioxidant phytochemicals (e.g., isothiocyanates, niazimicin, niaziminin and quercetin) in the plant leaves (Tiloke et al., 2013). Based on the current study the authors hypothesized that Moringa could enhance the efficacy of radiotherapy on hematological parameters (Table1). The findings of other researchers support this result (Berkovich et al., 2013).

Studies demonstrated that the cancer cells interrupted the metabolism of the normal liver cell which elevated the activity of serum enzymes, the destruction of hepatocytes by the invasion of cancer cells result in the release of AST and ALT into the plasma and subsequently the elevation of these liver enzymes (Saravanan et al., 2006). In line with previous studies, it was found that the serum level of AST and ALT was significantly increased in mice bearing EAC, indicating that EAC induce organ dysfunction and metabolic disturbance (Abu-Sinna et al., 2003). However, treatment with MO significantly reduced the level of ALT & AST (Table 2). It could be concluded that the tumor cells induce hepatotoxicity and the radiotherapy cannot stop this damage which was partly prevented by MO supplementation (Muhammad et al., 2011).

The current study proved the impairment of kidney functions in EAC-bearing mice, which was indicated by the elevated serum urea and creatinine levels (Table 2). These findings are in line with those recorded by Donia et al. (2018) and Hashem et al. (2020b). Subsequently, the elevation of kidney function could be attributed to the tumor's catabolic effect and the increase in urea production. Additionally, the renal damage induced by the tumor metastasis resulting in the impairment of the glomerular filtration rate and the reduction in urea and creatinine excretion; thus, increasing their blood levels (Adedara et al., 2012). Treatment of the EAC-bearing mice with *Moringa* leaves extract ameliorated their levels by reducing the oxidative stress which induce renal tissue damage (Arafat et al., 2018).

The development of hyperlipidemia in experimental animals with carcinoma has been previously reported (Silverstein et al., 1988). In the present study, serum cholesterol, triglyceride were significantly increased meanwhile, serum HDL decreased in EAC-bearing mice group in comparison to the control group (Table 3). These findings are parallel to those of Aldubayan et al. (2019), who found that the reduction in body weight in cancer patient is due to exhaustion of body fat. Triglyceride, the major storage form of fat, is converted to glycerol and free fatty acids (FFA) by hydrolytic metabolism. This causes hyperlipidemia and is associated with some tumors. In certain cancers, there is an association between weight loss and reduction of enzyme activity. When compared to a normal person, cancer patient's body energy requirements are provided by fat which are mobilized and oxidized in a greater extent which leads to low HDL and high triglycerides. Conversely, EAC and *Moringa* treatment modulated reverse changes in lipid profiles (Table 3). In the present study, cholesterol reduction by *Moringa* is thought to occur through lowering plasma concentrations of LDL by B-Sitosterol, a bioactive phyto-constituent of *M. oleifera* (Senthilkumar et al., 2018).

The hypolipidemic effect of *Moringa* was manifested by Al Juhaimi et al. (2017), who reported that *Moringa* contains phytosterols among which are campesterol, stigmasterol, and β-sitosterol. β-Sitosterol is a plant sterol with a structure similar to that of cholesterol. It could lower cholesterol by lowering plasma concentrations.

Oxidative stress is one of the most important factors in the initiation and progression of cancer through increasing mutations and damage in DNA, genome variation, and inhibition of cell multiplying, etc. (Visconti & Grieco, 2009). Lipid peroxidation, is known to be associated with pathological conditions of a cell. Malondialdehyde (MDA), the end product of lipid peroxidation, was reported to be high in cancer tissues (Yagi, 1987). Glutathione, a potent inhibitor of the neoplastic process, plays an important role in the endogenous antioxidant system. It is found in particularly high concentration in the liver and is known to have a key function in the protective process. Excessive production of free radicals resulted in oxidative stress, which leads to damage of macromolecules (Sinclair et al., 1990).

Studies shows that antioxidants agents especially those extracted from natural products are potentially able to interfere with carcinogenesis and preserve human beings from damages of oxidative stress (Almutairi et al., 2021).

The obtained findings recorded a significant rise in the activity of liver MDA along with a decrease in the endogenous antioxidants GSH in in EAC- bearing mice. These results indicated that the status of oxidative stress occurred in these animals. It is well known that the development of cancer is linked with the generation of free radicals resulting in lipid peroxidation and DNA damage, chromosomal aberration and mutations consequently the tissue damage and disorganization (Abdel-Wahhab et al., 2012) Radiotherapy alone cannot improve the oxidant and antioxidant status. As stated in the current study, *Moringa* leaf extract successfully restored the antioxidative power in EAC- bearing mice group by promoting the levels of GSH as well as inhibiting the level of MDA, which may be attributed to its potent free radical scavenging and antioxidant properties. Furthermore, *Moringa* leaves act as a respectable source of natural antioxidant due to the presence of several kinds of antioxidant compounds such as ascorbic acid, phenolics, flavonoids, and carotenoids (Anwar et al., 2007). Polyphenols have been known to have powerful antioxidant activity in vitro. They prevent lipid peroxidation by acting as chain-breaking peroxyl radical scavengers and can protect LDL from oxidation (O'Byme et al., 2002).

Apoptosis or programmed cell death is well known as one of the key factors in various phases of a living organism's biological evolution and which in case of irregular and abnormal activity; it results in various serious diseases (Sankari et al., 2012). Apoptosis inhibition is one of the main routes in tumorigenesis and cancers which is essential for cancer cells to continue their uncontrollable proliferating. Hence, induction and elevation of apoptosis is a standard target to discover new anticancer agents (Koff et al., 2015).

p53 is a tumor suppressor protein and its functional inactivation is frequently observed in a wide range of human malignancies (Candelaria et al., 1997). Functional p53 protein is also required for the efficient activation of apoptosis following irradiation or treatment with chemotherapeutic compounds (Zamai et al., 2002). Thus, the lack of p53 function leads to a dramatic increase in cellular resistance to these agents.

Her2 is the protein which promotes the growth of cancer cells. p53 & Her2 are two of the most important onco-related protein that involved in tumor progression.

According to data presented in Table 4, the level of serum p53 was significantly decreased whereas the level of Her2 was increased in EAC-bearing mice when compared to the control. In the current study, it is evident that the radiotherapy had beneficial effect on these two parameters indicating the successful effect of radiation on this induced cancer. Treatment of the Ehrlich group with *Moringa* leaf extract ameliorated their levels, this might be due to MO possesses an antiapoptotic function. It acts as a free radical quencher against the ROS generated in various tumor cells (Galuppo et al., 2014). Moreover, the leaves extract: phenolic compounds (thymol and ascorbic acid), long chain fatty acids (myristic acid, palmitic acid, and linoleic acid), and retinol which is known as a cancer treatment. Future studies are required to separate the bioactive compound from the leaves of *Moringa oleifera* in order to identify

the exact anticancer compounds. These results will contribute to developing anticancer drug from natural compounds (Doldo et al., 2015). From the present study, it is evident that the additional effect of *Moringa* when used plus radiotherapy, whether this effect is restricted only to this type of induced tumor or we can safely use in various tumor cells, needs further investigations.

Conclusion

From the aforementioned results, it can be supposed that the combination of *Moringa* leaf extract with γ-radiation exposure resulted in super-additive cytotoxic effects on cancer cells and super-relieving effect on hematological, hepatic & renal testing parameters in addition to its hypolipidemic effect and improvement of oxidant-antioxidant status .

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