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Biochemical Evaluation of Antitumor Activity of Irradiated Citrus Pectin: oxidants and antioxidants content

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ABSTRACT

This study was carried out to evaluate the antitumor activity of citrus pectin (CP) and irradiated citrus pectin (Irr.CP) on 60 female mice and weighting 20-25 g. the mice were divided into four equal groups of 15 mice. Group 1: Non-tumor bearing mice (NTBM).Group 2:Tumor bearing mice (TBM). Group 3:TBM-treated with citrus pectin orally (3.3g/kg.b.w./day) for 4 weeks .Group 4:TBM-treated with Irr.CP orally (3.3g/kg.b.w./day) for 4 weeks. Blood samples were collected from all animal groups after 2 and 4 weeks from the onset of treatment and processed directly for determination of SOD, GPx, and CAT activities in addition to MDA, GSH, and $NO_{(x)}$ concentrations. Also, AST , ALT and GGT activities as well as urea and creatinine concentrations were also determined in blood. The obtained results revealed a significant increase in MDA , $No_{(x)}$ level , CAT activity and in liver and kidney functions ratios, a marked significant depletion in GSH content ,GPx and SOD activity in the blood of tumor bearing mice compared to control. Contrary results obtained in TBM treated with CP and Irr.CP. So, these compounds have potential benefits in cancer treatment.

Keywords: irradiated citrus pectin, Tumor bearing, cancer, antioxidants.

1. INTRODUCTION

Cancer is considered one of the major causes of mortality in the world. Despite the recent advances in science, cancer has not been cured yet. It is estimated that by 2020 there will be 16 million new cancer cases every year [1]. The target of much research has been on the discovery of natural and synthetic compounds that can be used in the prevention and/or treatment of cancer. Natural products of either plant or animal origin that exhibited antitumor activity have been discovered [2].

Pectin is a highly complex branched polysaccharide fiber rich in galactoside residues and present in all plant cell walls [3]. Ordinary pectin isolated from citrus fruits has high molecular weight and can be modified resulting in shorter, less complex molecules. These shorter carbohydrate chains, dissolve more readily in water and are better absorbed and utilized by the body than ordinary, long-chain pectin [4]. Moreover, pectin may be modified by physical means. Such physical means include, but are not limited to heat, cold, freeze/thaw, irradiation, shear, ultra-high shear.Gamma irradiation is a useful physical treatment for depolymerizing pectin. Gamma irradiation leads degradation of polymer molecules by the formation of free radicals [5]. Irradiation induced degradation has been applied to preparing low molecular weight pectin [6] product with increased amount of monosaccharides.

Citrus Pectin and modified Citrus pectin have been found to exhibit antimutagenic activity and inhibit cancer metastasis and proliferation, with no evidence of toxicity or other serious side effects [7,8]. Accordingly, this study was performed to investigate the biochemical effect of citrus pectin and irradiated

citrus pectin in experimentally induced tumor in female mice.

2. MATERIALS AND METHODS

2.1Animals:

Female Swiss albino mice weighting 20-25 g used in this study were obtained from Laboratory Animals Research Center, Faculty of Veterinary Medicine, Zagazig University, Egypt. The animals were housed in separated metal cage 10-15 per cage and kept at a under the same constant environmental and nutritional condition throughout the period of investigation, water was supplied *ad-Libitum*, in the special lab, animal room, in Faculty of Vet. Med. Moshtohor, Benha University.

2.2 Ehrlich Ascites Carcinoma Cells:

A line of Ehrlich Ascites Carcinoma (EAC) cells was supplied from National Cancer Institute, Cancer Biology Department. Egypt.

2.3 Tumor induction:

Solid tumors were induced by intramuscular inoculation of each mice with 0.2 ml of EAC, which contained 2.5 x 10^6 viable EAC cells, in the right thigh of the lower limb of each mouse. Mice with a palpable solid tumor , its diameter was 10mm^3, that developed within 10 days after inoculation were used in the study.

2.4 Chemicals:

Citrus Pectin purchased from El-Goumhouria Co. for trading chemicals, medicines and medical appliances, Egypt. All chemical and kits purchased from Sigma (USA).

2.5 Nutraceuticals preparation:

Preparation of 4% CP:

Four grams of citrus pectin were dissolved in100ml distilled water.

Preparation of Irr.CP:

Prepare 4% citrus pectin solution in distilled water. This solution was irradiated with a dose of 5 kGy (kilo Gray) gamma radiation. The irradiation process was performed at National Center of Radiation Research and Technology (NCRRT, Cairo), Egypt.

2.6 Experimental design:

Sixty female mice were divided into 4 groups each one containing 15 mice were placed in individual cages and classified as follows:

1. **Group (1):** Served as negative control and orally received saline (NTBM: Non-tumor bearing mice).
2. **Group (2):** Tumor bearing mice without any treatment served as positive control group (TBM) for 4 weeks.
3. **Group (3):** Tumor bearing mice received citrus pectin orally at a dose level of 3.3gm /Kg body weight/day ($TBM_{(CP)}$) for 4 weeks
4. **Group (4):** Tumor bearing mice received Irr.CP orally at a dose level of 3.3gm /Kg body weight /day ($TBM_{(Irr.CP)}$) for 4 weeks

2.7 Blood sampling:

Directly, after animals were anaesthetized using diethyl ether, heparinized blood samples were collected from all animal groups after 2 and 4 weeks from the heart for determination of the following Biochemical parameters:

SOD [9], GPx [10,11] and CAT [12] (in packed RBCs) activities and plasma MDA [13], GSH(in packed RBCs) [14] and plasma NO(x) [15] concentrations. Plasma AST, ALT[16] and GGT [17] activities (as liver function tests), urea [18] and creatinine [19] concentrations (as kidney function tests).

2.8 Statistical analysis:

Statistical analysis was done using SPSS software version 15. The inter-group variation was measured by one way analysis of variance (ANOVA) followed by Post Hoc LSD test. Results were expressed as mean ± SEM. The mean difference is significant at the 0.05 level [20].

3. RESULTS AND DISCUSSION

3.1 Antioxidant parameters:

The presented data in table (1) revealed that, a highly significant decrease in SOD activity ($p<0.01$) after 2 weeks, a very highly significant decrease ($p<0.001$) in CAT and GPx activity and GSH content and a very highly significant increase in $NO_{(x)}$ and MDA concentration after 2 and 4 weeks in TBM group when compared to control group (NTBM).

These findings were in agreement with [21] who found that, the presence of tumor caused disequilibria of the antioxidant defense system. Moreover [22] demonstrated that, lipid peroxidation level was significantly increased in blood, liver and tumor tissues of EAC mice when compared with control group.

Also, our findings are in accordance with [23] who demonstrated that a decrease in blood GSH in circulation has been reported in several diseases including malignancies. Decline in SOD activity recorded in mice bearing Ehrlich carcinoma was also reported earlier by [24].They postulated that the loss of Mn-SOD activity could be due to the loss of mitochondria which leads to a decrease in total SOD activity in different tissues of the tumor host.

It seems that oxidative damage caused by decreased capacity for H_2O_2 elimination is related to suppressed activity of CAT, as well as to suppressed direct antioxidant action of GSH. This is in agreement with the previous findings that CAT has a more significant role than GPx in protecting erythrocytes against oxidative stress [25-26].

Table 1: Effect of treatment with citrus pectin and irradiated citrus pectin on SOD, GPx, CAT activities, GSH , MDA and NO (x) (μM/L) concentrations in Ehrlich carcinoma bearing female mice and their control during 4 weeks.

Group / Parameters	weeks	NTBM	TBM	$TBM_{(CP)}$	$TBM_{(Irr.CP)}$
SOD(U/ml)	2	3.34±0.09	2.58 ' ±0.15	2.91^{d} ±0.12	3.04^{d} ±0.17
	4	3.22±0.28	2.60±0.06	2.70^{c} ±0.41	3.89^{ab}±0.46
GPx(mg/dl)	2	0.78± 0.005	0.64 ''' ± 0.007	0.62^{d}± 0.008	0.64 ± 0.008
	4	0.78 ±0.007	0.61 ''' ± 0.010	0.67^{b}± 0.011	0.65^{b}±0.016
GSH(mg/dl)	2	137.74±1.64	109.96 '''±2.13	145.42^{b}± 3.25	153.49^{b}±5.58
	4	140.67±4.22	109.96'''±6.14	154.43^{b}± 4.63	154.11^{b}±3.68
CAT (uM/ml)	2	0.595±0.014	0.350'' ± 0.017	0.462^{b}± 0.037	0.433^{b} ± 0.022
	4	0.610±0.007	0.541''' ± 0.004	0.479^{bc} ±0.004	0.574^{ab}± 0.018
MDA(uM/ml)	2	91.740±1.680	137.73 '''±4.878	120.405^{b}± 4.029	128.905 ±7.360
	4	92.655±2.479	123.988'''±5.314	108.408^{bc}± 3.947	88.072^{ab} ± 3.931
$NO_{(x)}$(uM/L)	2	20.960±0.768	31.515 '''±0.816	31.675^{cd} ± 0.692	28.045^{ab}± 0.799
	4	22.073±0.545	33.000'''±0.606	18.980^{b}±1.022	18.243^{b}± 1.802

Non-significant (N.S): p>0.05; Significant: *p<0.05; highly significant: ** p<0.01:; very highly significant: ***p<0.001 from NTBM.
a, significant from TBM group p<0.05. b, significant from TBM(CP) group p<0.05. c, significant from TBM(Irr.CP) group p<0.05

Some investigators have reported a higher $NO^{\cdot}$ synthase activity in tumors**,** while some have reported a lower activity [27]**.** The obtained result supports the general observation that some malignancies are associated with an increased level of nitric oxide. In contrary [28] suggested that, there is a decrease rate of lipid peroxidation in liver tumor cell than normal liver cells.

Treatment with CP showed a non-significant increase in SOD activity and significant increase after 4 weeks in GPx activity and after 2 and 4 weeks in GSH content and CAT activity compared to TBM group. Furthermore, a significant decrease in MDA concentration was observed after 4 weeks. Also, significant decrease in $NO_{(x)}$ concentration was reported after 2 and 4 weeks when compared with TBM group.

But, treatment with Irr.CP revealed significant increase in SOD activity after 4weeks compared to TBM and CP treated group, in GPx activity after4 weeks , in GSH content after2 and 4 weeks compared to TBM group. Also, showed significant increase in CAT activity after 2and 4weeks compared to TBM group and after 4weeks compared to CP treated group.

Moreover, it indicated significant decrease in MDA concentration after 4weeks compared to TBM group and CP treated group. Also, a significant decrease in $NO_{(x)}$ concentration was observed after 2 and 4weeks compared to TBM and after 2 weeks compared to CP.

Our results are in harmony with [29], who reported that pectin could reduce MDA levels and increase SOD in aorta tissue in high fat diet fed rats. Also**,** [30] reported that, the addition of pectin to the cystine diet counteracted the activities of the total and Cu,Zn-superoxide dismutase, and of catalase in liver. Moreover [31], reported thatpectin extracted from citrus and grapefruit peel in laying hens diet increases blood serum SOD activity.

Our results disagree with [32] who reported that, erythrocyte SOD activity was not affected by pectin treatment in hypercholesterolemic rabbits. Moreover, our results are in agreement with [33], the bioactivity of SOD and GSH-Px increased in all MCP-fed groups and the level of MDA decreased markedly in hyperlipidemic rats. It has been suggested that pectin interacts directly with oxidants and free radicals [34]. The antioxidant activity in pectin could be related to the high galacturonic acid content. It has been reported that a relatively low molecular weight and a high uronic acid content in polysaccharides appeared to increase the antioxidant activity[35]and this express the high antioxidant activity of modified citrus pectin (pectin degraded by irradiation) more than citrus pectin without degradation.

3.2 Liver and kidney functions:

The obtained results in table (2) showed highly significant increase after 4 weeks in ALT activity and after2 and 4 weeks in AST activity, furthermore, it revealed very highly significant increase in GGT activity in TBM group compared to control group. Meanwhile, a highly significant increase in urea and creatinine concentrations in TBM group compared to NTBM group.

The results of the present work agreed with previous studies which have demonstrated that the level of the liver enzymes increased in serum of EAC-bearing mice indicating general toxicity that occurred due to cancer development [36]**.** The same results were observed by[37]in their study on the liver function in the assessment of head and neck cancer patients. The observed increase in serum urea level in tumor bearing mice are in agreement withthe results reported by [38]who observed that, there was a significant increase in plasma urea concentration in tumor-bearing mice, he attributed such increase to the increase in urea production as a result of catabolic effect of tumor. As confirmed by [39] who suggested that, creatinine was decreased in tumor-bearing rats as the glomerular

lesions progressed, associated with a rise in serum creatinine level. Also, liver and kidney toxicity induced during tumor growth may be due to the excessive production of ROS that leads to oxidative damage [40].

Table 2: Effect of treatment with citrus pectin and irradiated citrus pectin on blood ALT, AST, GGT activities, Urea and Creatinine concentrations in Ehrlich carcinoma bearing female mice and their control during 4 weeks.

Parameters / Group	ALT(U/L)		AST(U/L)		GGT(U/L)		Urea(mg/dl)		Creatinine(mg/d)	
	2wks	4wks	2wks	4wks	2wks	4wks	2wks	4wks	2wks	4wks
NTBM	50.51 ±2.80	51.64 ±0.71	96.50 ± 4.99	102.32 ±2.64	31.01 ±2.22	31.81 ±0.78	29.65 ± 2.52	29.10 ±0.67	0.79 ±0.07	0.78 ±.017
TBM	56.45 ±4.76	68.43** ±5.14	130.45*** ±6.16	123.94** ±4.64	52.62*** ±0.73	57.56*** ±0.39	28.85 ±2.84	33.20** ±1.24	0.77 ±0.08	0.89** ±.032
$TBM_{(CP)}$	71.07^{a} ±1.97	60.74 ±3.19	100.39^{a} ±1.42	119.53 ±1.37	52.72 ±1.78	55.05^{c} ±1.05	26.15 ±0.35	26.15^{a} ±0.35	0.70 ±0.09	0.78^{a} ±.013
$TBM_{(Irr.CP)}$	69.14^{a} ±0.88	69.89 ±3.07	118.30 ±11.30	120.10 ±6.91	50.80 ±1.77	50.09^{ab} ±2.14	28.90 ±1.515	28.10^{a} ±0.84	0.77 ±0.04	0.75^{a} ±.023

Non-significant (N.S): p>0.05; Significant: *p<0.05; highly significant: ** p<0.01:; very highly significant: ***p<0.001 from NTBM. a, significant from TBM group p<0.05. b, significant from TBM(CP) group p<0.05. c, significant from TBM(Irr.CP) group p<0.05

It was previously observed that oxidative damage which appeared as increased lipid peroxidation and inhibition of GSH content, catalase and SOD activity, led to liver and kidney dysfunctions [41]. Our results revealed that treatment with CP revealed significant decrease in ALT and AST activities after 2 weeks and in urea and creatinine concentration after 4 weeks compared to TBM group.

Meanwhile, treatment with Irr.CP showed significant decrease in ALT activity after 2 weeks compared to TBM group and in GGT activity after 4 weeks compared to TBM group and CP treated group. Also, it showed significant increase in urea and creatinine concentration after 4 weeks compared to TBM group. [41] similarly reported that, ALT, AST, GGT activities, serum urea and creatinine were significantly increased in positive control rat groups administrated lead acetate (LA). Low and high esterified pectin significantly decreased the effect of LA on the tested parameters. Who added that, histopathological examination clearly indicated that high or low esterified pectin eliminated the harmful effect of LA on liver, kidney and brain tissues and showed no significant difference in serum ALT, AST, GGT activities, serum urea and creatinine in treated groups with high and low esterified pectin compared to normal control group.

4. CONCLUSION

From the obtained results, it could be concluded that, citrus pectin and irradiated citrus pectin have high antioxidant activity and protect liver and kidney from the harmful effect of tumor and oxidative damage. So we recommend using citrus pectin and irradiated citrus pectin in our food as prophylactic and preventive for many diseases and as adjuvant therapy in cancer thereby.

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