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Research Article

Ameliorative effect of curcumin and tannic acid on tumor-induced in female mice

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ABSTRACT

Curcumin, a natural polyphenol product of the plant Curcuma longa, has been shown to inhibit the growth and progression of cancer; Tannic acid, a naturally occurring dietary polyphenol, has been exerts chemopreventative activity against cancer in various animal models. This study was carried out on 220, 12-14 weeks old female mice and weighted 25-30gm. Mice were classified into two main large experiments. Experiment 1: Non-tumor bearing mice (NTB) Included 100 of animals and divided into four groups each one comprised 25 mice. Group 1: NTB-control saline treated. Group 2: NTB-treated with curcumin orally (350mg/kg/day) for 6 weeks. Group 3: NTB-treated with tannic acid orally (160mg/kg/day) for 6 weeks. Group 4: NTB-treated with curcumin and tannic acid orally at ratio (50%: 50%) for 6 weeks. Experiment 2: Tumor bearing (TB) mice. Included 120 of animals and divided into four groups each one comprised 30 mice. Group 1: TBM-control saline treated. Group 2: TBM-treated with curcumin orally (350mg/kg/day) for 6weeks. Group 3: TBM-treated with tannic acid orally (160mg/kg/day) for 6weeks. Group 4: TBM-treated with curcumin and tannic acid orally at ratio (50%: 50%) for 6 weeks. Blood samples were collected from all animals groups after 2, 4 and 6 weeks from treatment. Serum were separated and processed directly for (AST and ALT) activities, (urea, creatinine, L-MDA) concentration and catalase activity determination. The obtained results revealed that, a very highly significant increase in serum AST, ALT, urea, creatinine and L-MDA concentration. On contrary, a highly significant decrease in serum catalase activity was observed in tumor bearing mice when compared with control. The results of this study indicated that curcumin, tannic acid and their combination treatment have potential benefits in cancer treatment.

Key Word: Curcumin, tannic acid, antitumor, ALT, AST and MDA.

INTRODUCTION

Cancer is a hyper proliferative disorder marked by metastasis into the vital organs of the body through invasion and angiogenesis. Cancer chemotherapy is often associated with the side effects on immune cells. Thus, the prerequisites for anti cancer drugs are to ensure no damaging effects on the immune cells, failing which the drug may completely terminate the subsided immune response in tumor-bearing host [1]. Curcumin, a polyphenolic compound extracted from rhizomes of Curcuma species, has been shown to possess anti inflammatory and antitumor properties [2]**.** Tannic acid (TA), a glucoside of gallic acid polymer which is found, along with other condensed tannins, in several beverages including red wine, beer, coffee, black tea, green tea, and many foodstuffs. It has been shown to possess anti-bacterial, anti-enzymatic and antitumor properties [3]. Curcumin may inhibit chemotherapy-induced apoptosis in animal models of breast cancer via inhibition reactive oxygen species generation [4]. On the other hand, oral administration of tannic acid resulted in enhanced tumor inhibitory effects of doxorubicin on Ehrlich ascites carcinomas implanted in CDF1 and BDF1 mice[5]. In recent years, the role of life style and dietary behavior in reducing cancer risk has drawn widespread attention based on the geographic differences in cancer incidence and mortality. Natural compounds have been adopted increasingly in the field of chemoprevention as well as the synthetic chemicals those have been identified for inhibiting or reversing carcinogenesis [6]. Accordingly, the purpose of this experiments to investigate the

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possible protective effect of treatment curcumin and tannic acid in experimentally induced tumor in female mice.

MATERIAL AND METHODS

A total number of 220 Australian females' albino mice of 12-14 weeks old age and weighting 25-30 gm were used in the experimental investigation of this study. Mice were obtained from the Research Institutes of Ophthalmology, Giza, Egypt. Animals were housed in separate metal cages, fresh and clean drinking water was supplied ad-libtium through specific nipple. Mice were kept at a constant environmental and nutritional condition throughout the period of the experiment.

Tumor induction: The experimental induction of tumor in female mice was carried out at the National Cancer Institute Egypt. Every 1 ml of Ehrlich ascites adenocarcinoma was diluted with 4 ml of normal saline. Each mice was injected subcutaneously (S/C) in the medial aspect of the right thigh with 0.2 ml of Ehrlich ascites adenocarcinoma (2.5×10^6 tumor cells with single cell suspension) [7]. The tumor developed and become palpable in all injected animals at 5-7 days post tumor inoculation.

Experimental design: The experimental work was classified into two main large experiments as follow:

Experiment A: Non-tumor bearing mice." NTB-mice"

Included 100 of female mice divided into four groups, each one consisting of 25 animals placed in separate metal cages and classified as follows:

Group 1: Non tumor bearing control (NTB-C) administered with 0.2 ml of normal saline.

Group 2: Non tumor bearing (NTB-cur) treated with curcumin orally administered daily at adose level of (350mg/kg/day) for **6 weeks**.

Group 3: Non tumor bearing (NTB-tan) treated with tannic acid orally administered daily at adose level of (160mg/kg/day) for **6 weeks**.

Group 4: Non tumor bearing (NTB-cur+tan) treated with curcumin and tannic acid orally and daily at ratio of (50%: 50%) for **6 weeks.**

Experiment B: Tumor bearing mice."TB- mice"

A total number of 120 female TB-mice were divided into four groups, each one included 30 mice placed in separate metal cages and classified as follows:

Group 1: Tumor bearing control (TB-C) administered with 0.2 ml of normal saline.

Group 2: Tumor bearing (TB-cur) treated with curcumin orally administered daily at adose level of (350mg/kg/day) for **6 weeks**.

Group 3: Tumor bearing (TB-tan) treated with tannic acid orally administered daily at adose level of (160mg/kg/day) for **6 weeks.**

Group 4: Tumor bearing (TB-cur+tan) treated with curcumin and tannic acid orally and daily at ratio of (50%: 50%) for **6 weeks.**

Sampling: Blood samples were collected in the morning after overnight fasting from all mice by decapitation every 2, 4, 6 weeks from the onset of treatment, then obtained in dry and clean tubes and serum was separated by centrifugation at 3000 r.p.m for 15 minutes. The clear serum were aspirated by Pasteur pipette and received in dry sterile sample tube, processed directly for enzymes determination, then kept in a deep freeze at -20until used for subsequent biochemical analysis.

Biochemical analysis: Serum (AST and ALT) activity, urea, creatinine, L-MDA, catalase were analyzed colorimeterically according to the methods described by [8-12]**,** respectively.

Statistical analysis: Statistical analyses of the obtained results were carried out using T-test and student's F-test according to [13].

RESULTS AND DISCUSSION

The presented data in tables (1) revealed that, a very highly significant increase in serum (AST and ALT) activities, urea, creatinine and L-MDA concentration was observed in TB female mice. Mean while, a very highly significant decrease in plasma catalase activity in tumor-bearing female mice were observed all over the experimental period of tumor induction when compared to control. Similary**,** a rise in plasma bilirubin and hepatic enzyme activities were observed in tumor bearing rats is the results of changes in the liver indicated by the presence of tumor [14]. The recorded increase in plasma ALT and AST activities in tumor bearing mice of the present study might be due to generalized destruction of liver cells and release of AST into plasma after tumor induction. On the other hand, a very highly significant increase in tumor-bearing female mice in serum urea concentration was confirmed by the results observed by [15] who observed that, there was a highly significant increase in plasma urea concentration in tumor-bearing mice. The author attributed such increase in blood urea concentration to the increase in urea production as a result of catabolic effect of tumor. Our results demonstrated a very highly significant increase in serum creatinine concentration in tumor bearing mice were Similar results reported by [16] who observed that, serum creatinine level showed a significant increase in mice-bearing Ehrlich ascites carcinoma

due to muscle necrosis. Regarding, a very highly significant increase in serum L-MDA concentration in tumor bearing mice, the obtained results reported by [17] demonstrated that, lipid peroxidation level was significantly increased in blood, liver and tumor tissues of (EAC) mice when compared with control group. On the other hand, a very highly significant decrease in plasma catalase activity in tumor-bearing female mice. Similar results were reported by [18] have recorded that, very low catalase activities observed in tumor cells. Moreover, [19] noticed that, Very large differences are found in catalase activity among the tissues and cell lines. Most neoplastic cell lines were low in catalase activity although some possessed large amounts, like the promyelocytic leukemia cell line HL 60. The obtained results in table (2) revealed that, in TBM (cur) group showed a significant decrease in serum (AST) activity after 4 and 6 weeks as compared to control (S) group. Furthermore, a significant increase after 2 weeks followed by a significant decrease after 4 and 6 weeks as compared to (tan) and (cur+tan) treated groups. On the other hand, a significant decrease after 2 and 6 weeks as compared to control (S). Furthermore, a significant decrease observed all over the experimental period as compared to (tan) and (cur+tan) treated groups. Similar results were reported by [20] who showed that, curcumin decreased the induction of (AST and ALT) activity of rats treated with Sodium arsenite. The author attributed such decrease in transaminase enzymes to administration of curcumin preserved the structural integrity of the hepatocellular membrane. This was evident from the reduction in the enzyme activities against the arsenic induced rise in the enzyme levels in plasma. It could be suggested that the leakage of enzymes because of liver injury is prevented by the liver cell membrane stabilizing action of curcumin.

Administration of (tan) to TBM showed a significant decrease in serum (AST) activity after 2 weeks as compared to control (S) group. Furthermore, a significant decrease after 2 and 4 weeks as compared to (DMSO) group. Moreover, a significant decrease after 2 weeks followed by a significant increase after 4 and 6 weeks as compared to (cur) treated group. Also, a significant increase after 6 weeks as compared to (cur+ tan) treated group. On the other hand, a significant increase in serum (ALT) activity after 4 weeks as compared to control (S) group. Furthermore, a significant increase after 2 and 4 weeks as compared to (DMSO) group. Furthermore, a significant increase observed all over the experimental period as compared to (cur) treated group. Moreover, a significant increase after 4 and 6 weeks when compared to (cur+tan) treated group. Similar results were reported by [21] who observed that**,** Treatment of tannic acid significantly reduced the increased plasma levels of (ALT) and (AST) levels to normal in Azido thymidine (AZT) treated mice, indicating its hepatoprotective effect.

Administration of (cur) to TBM showed a significant decrease in serum urea concentration was observed all over the experimental period as compared to control (S), (DMSO) and (tan) treated groups. Moreover, a significant decrease after 2 and 4 weeks while a significant increase after 6 weeks as compared to (cur+tan) treated group. Similar results were reported by [22] showed in studies with cyclosporine that, treatment with curcumin was significantly decreased the level of urea and creatinine because of its role as potent antioxidant. These suggestions was confirmed by [23] who found that, the preventive effect of curcumin on the gentamicin-induced decrease in the activity of glutathione peroxidase (GSHPx) and CAT could contribute to the restoration of markers of renal tubular injury. It seems reasonable to assume that curcumin is able to suppress nephrotoxicity in kidney, as it was demonstrated in studies with adriamycin [24] Our results in (TBM-tan) group showed a significant decrease in serum urea concentration was observed all over the experimental period as compared to control (S) group. Mean while, a significant increase all over the experimental period as compared to (cur) treated group. Moreover, a significant increase after 6 weeks when compared to (cur+tan) treated group. Similar results were reported by [25] who found that, There was significant reduction in the elevated levels of marker parameters of kidney toxicity, BUN and serum creatinine by treatment with Terminalia chebula extract due to presence of tannic acid. The author attributed this reduction to the highly antioxidant effect of tannic acid.

Administration of (cur) to TBM showed a significant decrease in serum creatinine concentration was observed all over the experimental period as compared to control (S), (DMSO) and (tan) treated groups. Furthermore, a significant decrease after 2 and 4 weeks followed by a significant increase after 6 weeks as compared to (cur+tan) treated group. Similar results were reported by [20] who showed that, treatment with curcumin alone caused a significant decrease creatinine level due to The renoprotective effect of curcumin via scavenging of ROS is well documented. Administration of (tan) to TBM showed a significant decrease in serum creatinine concentration was observed all over the experimental period as compared to control (S) and (DMSO) groups. Mean while, a significant increase all over the experimental period as compared to

(cur) and (cur+tan) treated groups. Similar results were reported by [26]who reported that, Tannins of herba ephedra could improve renal function in adenine-induced chronic renal failure in rats, that causing highly significant decreased blood urea nitrogen(BUN) by 37%, creatinine (Cr) 35%.

Administration of (cur) to TBM showed a significant decrease in serum L-Malondialdehyde concentration was observed all over the experimental period as compared to control (S) group. Furthermore, a significant decrease after 6 weeks as compared to (tan) treated group. Similar results were reported by [27]**,** who found that, Pretreatment of curcumin to β-irradiated hepatocytes resulted in decreased lipid peroxidation and improved antioxidant status preventing the damage to the hepatocytes. This may be due to the antioxidant sparing action of curcumin. The presence of π conjugation in curcumin makes it more hydrophobic. As a result curcumin get localized in the lipid bilayer membrane. Curcumin, being lipid soluble, reacts with the lipid peroxyl radicals and acts as a chain terminating antioxidant. A significant decrease in serum L-MDA concentration was observed in TBM (tan) group after 4 and 6 weeks as compared to control (S) and (DMSO) groups. Furthermore, a significant increase after 6 weeks as compared to (cur) treated group. Moreover, a significant increase after 4 and 6 weeks as compared to (cur+tan) treated group. Similar results were reported by [28] were found that, there was a significant decrease in lipid peroxides , nitric oxide levels and the activity of catalase in the aluminium chloride treated rats. However, tannic acid could play a prophylactic role in reducing the oxidative damage in the brain tissue of aluminium chloride exposed rats that induce cancer. Also, [29] reported that, tannic acid has been reported to quench lipid peroxidation, prevent DNA oxidative damage and scavenge hydroxyl radical. Administration of (cur) to TBM showed a significant increase in plasma catalase activity was observed all over the experimental period as compared to control (S), (DMSO) and (tan) treated groups. Furthermore, a significant increase after 2 and 4 weeks followed by a significant decrease after 6 weeks as compared to (cur+tan) treated group. Similar results were reported by [30] who found that, Curcumin (300 mg/kg) pretreatment for 20 days in isoproterenol treated rats significantly increased the levels of myocardial endogenous antioxidants (superoxide dismutase, catalase, and tissue glutathione) as compared to pathogenic control rats**.**

TBM (tan) group showed a significant increase in plasma catalase activity after 4 and 6 weeks as compared to control (S) and (DMSO) groups. Furthermore, moreover, a significant decrease was observed all over the experimental period as compared to (cur) treated group. Furthermore, asignificant decrease after 6 weeks as compared to (cur+tan) treated group. Similar results were reported by [31] investigated the effect of tannic acid on some biochemical parameters in Swiss albino mice and reported that, The administration of 20 mg tannic acid/kg body weight three times a week every other day for three weeks, enhanced the endogenous antioxidant capacity of the cells by increasing the activities of antioxidant enzymes (SOD, CAT, GSH-R, GST), GSH content and serum Cu^{2+} and Zn^{2+} levels Compared to the lead acetate-exposed group.

CONCLUSION & RECOMMENDATION

Curcumin has potent chemopreventative activity against awide avarity of tumors and has great potential in the prevention and treatment of cancer, also prevent LDL oxidation. In addition, tannic acid exerts chemopreventative activity against cancer by its poly phenols which have antioxidant and free radicals scavenging activity and trapping of activated metabolites of carcinogene. So we recommended by using curcumin in our food as proflactic and preventive for many diseases. Also, drinking tannic acid after food by times to take it is benefit and alone.

Table (1): Mean values of serum AST (U/ml) , ALT (U/ml) activities, urea concentration (mg/dl), creatinine concentration (mg/dl),L-MDA concentration (nmol/ml) and catalase activity(nmol/ml) of experimentlly induced tumor in female mice and their control.

Parameters	2 weeks		4 weeks		6 weeks	
	NTB	TBM	NTB	TBM	NTB	TBM
S. AST (U/ml)	27.00 ±1.26	53.40*** ± 2.20	17.20 ± 1.20	58.20***± 3.20	33.50 ± 1.82	81.20***± 3.18
S. ALT (U/ml)	37.80 ±1.71	40.40± 2.20	38.80 ± 2.55	36.80 ± 2.41	43.40 ± 2.01	84.80***± 2.17
S. Urea concentration (mg/dl)	26.14± 0.31	48.66***± 1.07	26.82± 0.38	54.33***± 0.81	27.41± 0.90	52.82***± 1.13
S. Cretinine concentrtion (mg/dl)	1.29 ± 0.032	2.38***± 0.021	1.56 ± 0.046	3.24***± 0.046	1.75 ± 0.034	3.76***± 0.032
S. L-MDA concentration (nmol/ml)	7.99 ± 0.56	12.36**± 0.65	9.74 ± 0.45	17.69***± 0.80	10.00 ± 0.78	23.55***± 1.18
S. Catalase activity (nmol/ml)	1.43± 0.015	1.28**± 0.022	1.28 ± 0.010	1.13*± 0.041	1.29 ± 0.024	1.03***± 0.025

Data are presented as (mean ± S.E) & S.E. = standard error.
*** = a significant after p < 0.05**
**** = a highly significant after p < 0.01**
*****= a very highly significant after p < 0.001**

Table (2): Effect of curcumin, tannic acid alone or in combination on serum AST (U/ml) , ALT (U/ml) activities, urea (mg/dl), creatinine (mg/dl),L-MDA concentrations and catalase activity (nmol/ml) in NTB and TBM.

Parameters		TBM C(s)	TBM (DMSO)	TBM (cur)	TBM (tan)	TBM (cur+tan)
S. AST (U/ml)	2	$53.40^{a}\pm2.20$	$54.80^{a}\pm1.95$	$51.80^{a}\pm2.31$	$43.40^{b}\pm2.76$	$44.20^{b}\pm 1.74$
	4	$58.20^{a,b}\pm3.2$	$62.40^{a}\pm3.80$	$33.20^{c}\pm1.82$	$48.40^{b}\pm4.66$	$54.40^{b}\pm 3.84$
	6	$81.20^{a}\pm3.18$	$79.40^{a}\pm4.24$	$13.60^{c}\pm1.74$	$77.80^{a}\pm2.03$	$35.00^{b}\pm 1.87$
S. ALT (U/ml)	2	$40.40^{a,b}\pm1.7$	$37.00^{b,c}\pm1.22$	$33.00^{c}\pm1.87$	$44.20^{a}\pm1.71$	$42.40^{a}\pm 1.66$
	4	$36.80^{c}\pm$	$35.00^{c}\pm2.23$	$36.60^{c}\pm3.18$	$79.60^{a}\pm2.22$	$56.00^{b}\pm1.87$
	6	$84.80^{a}\pm2.17$	$84.60^{a}\pm3.23$	$21.80^{c}\pm1.49$	$82.60^{a}\pm2.46$	$35.00^{b}\pm 1.87$
S. Urea concentration (mg/dl)	2	$48.66^{b}\pm1.07$	$55.74^{a}\pm1.40$	$43.74^{c}\pm1.48$	$31.99^{d}\pm0.44$	$41.90^{c}\pm 0.63$
	4	$54.33^{a}\pm0.81$	$55.91^{a}\pm0.48$	$32.24^{b}\pm0.46$	$27.16^{c}\pm0.40$	$31.24^{b}\pm 1.10$
	6	$52.82^{b}\pm1.13$	$60.83^{a}\pm0.45$	$40.49^{c}\pm0.62$	$33.41^{d}\pm0.59$	$25.73^{e}\pm0.35$
S. Cretinine concentrtion (mg/dl)	2	$2.38^{a}\pm0.021$	$2.44^{a}\pm0.019$	$1.23^{d}\pm0.037$	$2.27^{b}\pm0.017$	$1.92^{c}\pm 0.06$
	4	$3.24^{b}\pm0.046$	$3.46^{a}\pm0.036$	$1.40^{e}\pm0.032$	$1.96^{c}\pm0.029$	$1.67^{d}\pm 0.04$
	6	$3.76^{a}\pm0.032$	$3.84^{a}\pm0.066$	$1.58^{e}\pm0.023$	$1.91^{b}\pm0.43$	$1.28^{d}\pm0.03$
S. L-MDA concentration (nmol/m	2	$12.36^{a,b}\pm0.6$	$12.88^{a}\pm0.69$	$9.09^{e}\pm0.45$	$11.19^{a,b,c}\pm1.0$	$10.39^{b,c}\pm0.61$
	4	$17.69^{a}\pm0.80$	$18.09^{a}\pm0.63$	$12.23^{b,c}\pm0.8$	$13.13^{b}\pm0.69$	$10.26^{c}\pm0.43$
	6	$23.55^{a}\pm1.18$	$24.85^{a}\pm0.48$	$13.52^{c}\pm0.60$	$18.09^{b}\pm0.63$	$11.71^{c}\pm0.61$
S. Catalase activity (nmol/ml)	2	$1.28^{b}\pm0.022$	$1.20^{c}\pm0.019$	$1.41^{a}\pm0.020$	$1.22^{b,c}\pm0.021$	$1.25^{b,c}\pm 0.024$
	4	$1.13^{c}\pm 0.041$	$1.00^{d}\pm0.038$	$1.77^{a}\pm0.030$	$1.44^{b}\pm0.022$	$1.54^{b}\pm 0.025$
	6	$1.03^{d}\pm0.025$	$0.94^{d}\pm0.050$	$2.02^{b}\pm0.049$	$1.85^{c}\pm0.030$	$2.26^{a}\pm 0.012$

Mean values with different super script letters in the same raws are significantly different at ($p < 0.05$).

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