BIOCHEMICAL EFFECT OF EMBLICA OFFICINALIS ON EXPERIMENTALLY INDUCED TUMOR IN FEMALE MICE

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A B S T R A C T

This study was carried out on 150 Australian female mice; 12-14 weeks old and weighted 25-30 gm. Mice were classified into two main large experiments. Experiment A: Non-tumor bearing mice (NTB) Included 60 of animals and divided into three groups each one comprised 20 mice. Group 1: NTB-control saline treated. Group 2: NTB-treated with Emblica officinalis orally (500 mg/kg/every alternate day) for 4 weeks. Group 3: NTB-treated with Emblica officinalis orally (1250 mg/kg/ every alternate day) for 4 weeks. . Experiment B: Tumor bearing (TB) mice. Included 90 of animals and divided into three groups each one comprised 30 mice. Group 1: TBM-control saline treated. Group 2: TBM-treated with Emblica officinalis orally (500 mg/kg/every alternate day 24 hours), after inoculation of ascetic fluid for 4weeks. Group 3: TBM-treated with Emblica officinalis orally (1250 mg/kg/ every alternate day) , 24 hours after inoculation of ascetic fluid for 4 weeks. Blood samples were collected from all animals groups after 1, 2 and 3 weeks from treatment. Serum blood were separated and processed directly for (AST and ALT) activities, (bilirubin, and creatinine) concentrations and antioxidant enzymes activities (catalase ,superoxide dismutase SOD, reduced glutathione GSH). The obtained results revealed significant increases in serum AST, ALT, ALP, bilirubin and creatinine concentrations. On contrary, significant decreases in serum GSH, SOD and catalase activity were observed in tumor bearing mice when compared with control. The results of this study indicated that Emblica officinalis treatment has potential benefits in cancer treatment in female mice.

Key Words: Chemoprotective, Emblica officinalis, Tumor, Mice.

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1. INTRODUCTION

Cancer is a disease of misguided cells which have high potential of excess proliferation without apparent relation to the physiological demand of the process. It is the second largest single cause of death in both men and women, claiming over six million lives each year worldwide. In modern medicine, chemotherapy, radiotherapy and surgery are the three major existing modes of treatment [1]. Phyllanthus emblica is a medicinal plant belonging to the Euphorbiaceae family, described in Ayurveda and is a constituent of several poly herbal formulations. The fruit is the most commonly used part of this plant and is the richest natural source of vitamin C. Emblica officinals (Amla) is highly

nutritious and dietary source of minerals and amino acids. It contains calcium, carbohydrates, fats, gallic acid, glutamic acid, magnesium, protein, sulphur, and tannins. It is used for treating bleeding, hemorrhoids, anemia, diabetes, inflammation, hiccoughs, hepatitis B, conjunctivitis and various other uses. Reported biological activities are anti-mutagenic activity, anti-carcinogenic activity, anti-tumor activity, anti-metastatic activity, chemo prevention activity, cytoprotective activity, anti-oxidant activity and various other activities [5, 6, 7].

Recent studies indicate that many plants used by herbal healers have been scientifically shown to possess antiviral, cancer preventive and of therapeutic value [1, 2].

Accordingly, the purpose of the present study was to investigate the possible protective effect of treatment by Emblica officinalis in experimentally induced tumor in female mice.

2. MATERIALS AND METHODS

2.1. Animals.

A total number of 160 Australian female albino mice of 12-14 weeks old age and weighting 25-30 g 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-libitum through specific nipple. Mice were kept at a constant environmental and nutritional condition throughout the period of the experiment.

2.2. Tumor induction.

Ehrlich ascites carcinoma (EAC) cells were aspirated from the tumor bearing mice aseptically. The cell were diluted with phosphate buffer saline (PBS) and 2.5×10^6 EAC cell in a 0.3 ml phosphate buffer were injected intraperitoneally (single suspension injection) to obtain ascetic tumor in mice. The tumor developed in all injected animals at 5-7 days post - tumor inoculation.

2.3. Experimental design

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

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

Included 60 of female mice divided into three groups, each one consisting of 20 animals placed in separate metal cages and classified as follows:

Group1: Non tumor bearing control (NTB-C) administered with 0.2 ml of normal saline.

Group 2: Non tumor bearing (NTB) treated with Emblica officinalis orally administered at a dose level of (500 mg/kg) for 3 weeks.

Group 3: Non tumor bearing (NTB) treated with Emblica officinalis orally administered at a dose level of (1250 mg/kg) for 3 weeks.

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

A total number of 90 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) mice treated with Emblica officinalis orally administered at a dose level of (500 mg/kg) for 3 weeks.

Group 3: Tumor bearing (TB) treated with Emblica officinalis orally administered at a dose level of (1250 mg/kg) for 3 weeks.

2.4. 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 rpm for 15 minutes. The clear sera were aspirated by Pasteur pipette and received in dry sterile sample tube, processed directly for enzymes determination, then kept in a deep freeze at -20°C until used for subsequent biochemical analysis.

2.5. Biochemical analysis:

Serum (AST and ALT) activity, bilirubin, creatinine, catalase, superoxide dismutase (SOD) and reduced glutathione (GSH) were analyzed colorimeterically according to the methods described by Reitman and Frankel [4], Walter et al. [9], Rock et al. [10], Aebi, H., Beulter et al. and Nishikimi et al. [11,12], respectively.

2.6. Statistical analysis:

The obtained results were Statistical analysis was done by using SPSS computer program (version 10). One-way analysis of variance (ANOVA) was done to study test of significance within treated groups, within Scheffe's posthoc test. Significance was considered when P values were less than 0.05.

3. RESULTS

The presented data in table (1) revealed significant increases in serum (AST and ALT) activities, bilirubin, creatinine and alkaline phosphatase concentration in TB female mice. Meanwhile, significant decreases in serum catalase activity, superoxide dismutase and reduced glutathione were observed in tumor-bearing female mice during the experimental period as compared with control. The results showed that serum transaminases (SGPT and SGOT), alkaline phosphatase and bilirubin levels were elevated in tumor-bearing female mice at the different time intervals (table 2).

DISCUSSION

The presented data in table (1) revealed significant increases in serum (AST and ALT) activities, bilirubin, creatinine and alkaline phosphatase concentration in TB female mice. Meanwhile, significant decreases in serum catalase activity, superoxide dismutase and reduced glutathione were observed in tumor-bearing female mice during the experimental period as compared with control. Similarly, Rafei et al. [18]recorded that 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. This study examined the effect of Ehrlich ascites carcinoma (EAC) tumor on these liver functions parameters and the effect of Emblica officinalis treatment on these parameters in non-tumor bearing and tumor bearing mice. The results showed that serum transaminases (SGPT and SGOT), alkaline

phosphatase and bilirubin levels were elevated in tumor-bearing female mice at the different time intervals (1, 2 and 3 weeks after inoculation of ascitic tumour cells) in comparison with non-tumor bearing mice. These findings are in agreement with Salem, F.S, Badr, M.O., Neamat-Allah, A.N. (2011) [13] who reported significant elevations of hepatic enzymes in EAC tumor bearing mice. This could be due to the hepatic damage resulting from tumor cell invasion Moreover, Tofani et al., (2002)[15], indicated that larger tumor masses and the associated liver necrosis are considered as metabolic overload of the liver leading to elevation of liver enzymes. Moreover, EAC caused significant deterioration of liver morphology

Treatment of non-tumor bearing mice with EO caused no significant changes in SGPT, SGOT, alkaline phosphatase and bilirubin. On the other hand, treatment of tumor-bearing mice caused significant reduction in the activity of liver enzymes (SGPT, SGOT and alkaline phosphatase) and serum bilirubin after 1, 2 and 3 weeks of experiment . These findings suggest that EO has hepatoprotective effect in EAC-induced mice. Similar results demonstrated the hepatoprotective effect of Emblica officinalis in other models of hepatic injury (Chen TS, Liou SY, Chang YL.., 2011 [26]; Tasduq SA, Kaisar P, Gupta DK, Kapahi BK, Maheshwari HS, Jyotsna S. 2005 [27] . Serum creatinine concentration revealed a very highly significant increase after 1, 2, and 3 weeks of tumor induction in saline treated groups in comparison with their normal group. The observed increase in serum creatinine level in tumor bearing mice are similar to the results reported by (Abd El-Salam, I.M; Abdel-Wahab, S; El-aeser, A and El-merazabani, M (1992) [16] ; Salem, F.S, Badr, M.O., Neamat-Allah, A.N. (2011) [13] who observed that serum creatinine level showed a significant increase in mice-bearing Ehrlich ascites carcinoma. Moreover, the increase in plasma creatinine concentration in tumor bearing mice recorded by (Hussein,, S.A., 2003[17]) attributed such increase due to muscle necrosis. As confirmed by (Kawaguchi, H., Itoh, K., Mori, H., Hayashi Y and Makino, S., 1991 [28]) observed that, creatinine was decreased in tumor-bearing rats as the glomerular lesions progressed, associated with a rise in serum creatinine level. Also, this elevation in serum creatinine could be attributed to tumor cell invasion to kidney tissues. Serum GSH, SOD and catalase activities revealed a significant decrease in tumor bearing female mice along the periods of experiment in saline-treated group in comparison with its normal control. Similar results were reported by previous studies such as Bozzi, A., Mavelli, J., Finazzi Agro, A., Strom, R., Wolf, A .M., Mondovi, B and Rotilio .G(1976) [20] who demonstrated very low catalase activities in tumor cells. Moreover, Stefan L .Marklund, N .Gunnar Westman, Erik Lundgren, and Goran Roos.(1982)[21] noticed a very large difference 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. Effect of Emblica officinalis on Survival rate and percentage: Table (3) shows the number of survived and dead mice and percentage of survived tumour bearing mice. In each group, we started with 30 mice 2 mice dead in the first week, 4 dead in the second week and 6 dead in the third week in saline treated group. So, the total

number of dead mice in this group was 12 and 18 survived. While in E.O (500 mg) treated group, 1 mouse dead in the first week, 3 dead in the second week and 3 dead in the third week. So, the total number of died mice in this group was 7 and 23 survived. In E.O (1250 mg) treated group, 2 mice dead in the second week and 2 dead in the third week. So, the total number of dead mice in this group was 4 and 26 survived.

Effect of Emblica Officinals Treatment on ascetic volume and cell count: Table (4) shows the volume of ascitic fluid and cell count per ml in different groups. Treatment with EO (500 mg) significantly attenuated the cell count and volume at different interval times (day 8, day 15 and day 23 after injection of ascitic fluid) ($p< 0.05$) when compared to saline treated group. Increasing the dose to 1250 mg caused more significant attenuation in the cell count and number compared to EO (500 mg) group ($p< 0.05$). Recent studies indicate that he immense potential of Emblica officinalis in cancer prevention and treatment. However, gaps in the studies conducted are apparent which need to be bridged for EO to be of regular use in cancer treatment and prevention. Moreover, the mechanism of action of the herbal drugs and their extract preparations differs in many respects from that of synthetic drugs. Also, antioxidant principles showed cytotoxicity towards tumor cells and antitumor activity in experimental animal. So, in this study we investigated the effects of anticancer activity of EO and its effects on some biochemical parameters such as liver enzymes, serum bilirubin, serum creatinine and antioxidants such as GSH, SOD and catalase in normal healthy and EAC tumor mice. The anticancer potential of EO was assessed by change in number and percentage of survived mice and total volume of ascitic fluid and tumor cell count parameters. The Emblica officinalis treated animals at doses of 500 mg and 1250 mg /kg body weight showed significant reduction in the tumor volume and tumor cell count, and also prolongation in survival time and increase in number and percentage of survived animals in a dose –dependent manner when compared with EAC tumor bearing mice. These findings are in agreement with Lemma, A.V. (2011) [23] who reported significant prolongation in survival time of EAC-mice treated with Terminalia chebula, Terminalia bellerica and Phyllanthus emblica. The ascitic fluid is the direct nutritional source for tumor cells, and the faster increase in ascitic fluid with tumor growth could possibly be a means to meet the nutritional requirements of tumor cells. The reliable criteria for judging the value of any anticancer drug are the prolongation of lifespan of the animal and control of tumour growth and size. Emblica officinalis decreased the ascitic fluid volume, and thereby decreasing the nutritional fluid volume and arresting the tumor growth, so it increased the number of survived EAC-bearing mice. Table no.4 depicts the beneficial effect of EO on the number and percentage of survived of EAC-bearing mice. In the EAC- saline treated mice, the number of survived mice was 18 mice out of 30 (60%) which increased to 23 of 30

(76.66%) with EO at a dose of 500 mg/kg and to 26 of 30 (86.66%) with EO at a dose of 1250 mg/kg. A regular rapid increase in ascitic tumour volume was noted in tumour bearing mice. The increase in percentage of survived mice of tumor-bearing mice by the treatment is a positive result and supports the anti-cancer effect of Emblica officinals.

Table (1): Mean values of serum AST (U/ml) , ALT (U/ml) activities bilirubin concentration (mg/dl), creatinine concentration (mg/dl), ALP concentration (U/ml) , catalase activity (U/ml), GSH activity (mg/dl) and SOD activity (U/ml) of experimentlly induced tumor in female mice and their control.

Parameters	1 weeks		2 weeks		3 weeks	
	NTB	TBM	NTB	TBM	NTB	TBM
AST (U/ml)	28.10 ±3.71	211.27 ± 5.58	28.53 ± 4.05	264.67± 4.60	28.83 ± 4.07	354.77± 12.58
ALT (U/ml)	35.83 ±3.39	257± 12.25	35.67 ± 3.78	348.33 ±17.21	36.33 ± 4.29	531.17± 17.30
Bilirubin(mg/dl)	0.72± 0.04	2.58± 0.03	0.71± 0.05	2.30± 0.04	0.72± 0.04	2.00± 0.06
Creatinine concentration	0.59 ± 0.02	2.32± 0.86	0.63 ± 0.04	2.78± 0.28	0.67 ± 0.04	2.98± 0.52
Alkaline phosphatase	15.05 ±0.66	58.98± 0.76	14.58 ± 0.64	54.53± 1.17	14.70 ± 0.72	47.86± 2.259
Catalase activity (U/ml)	0.86± 0.007	0.65± 0.026	0.85 ± 0.012	0.44± 0.008	0.85±0.007	0.17± 0.014
Reduced glutathione	51.31±1.87	2.22± 0.59	50.90 ±1.70	3.83± 0.64	50.85 ± 1.73	7.87± 0.74
Superoxidase	28.37± 0.62	4.66± 0.26	28.47± 0.62	6.82± 0.19	28.42 ±0.58	9.95± 0.27

Data are presented as (mean ± S.E.M) & S.E.M = standard error of mean .

Table (2): Effect of Emblica officinalis on serum AST (U/ml) , ALT (U/ml) activities, bilirubin (mg/dl), creatinine (mg/dl), ALP concentration ((U/ml) ,SOD concentrations , catalase activity (nmol/ml) and GSH activity (mg/dl) in TBM.

Parameters	*	TBM C(s)	TBM (500mg)	TBM (1250mg)
ALT (U/ml)	1	257.00±12.25	207.83±6.13	173.17±2.61
	2	348.33±17.21	159.83±1.30	156.50 ±1.23
	3	531.17±17.30	146.50±1.17	137.83±2.15
AST (U/ml)	1	211.27±5.85	165.77±5.16	125.03 ±4.82
	2	264.67 ±4.60	107.20 ±1.33	88.53 ±2.45
	3	354.77±12.58	88.27±1.11	66.40±3.18
Bilirubin(mg/dl)	1	2.58±0.03	1.32±0.04	1.05±0.02
	2	2.30 ±0.04	1.38±0.03	1.27 ±0.02
	3	2.00±0.06	1.63±0.03	1.50 ±0.03
Creatinine concentration (mg/dl)	1	2.32±0.86	2.10±0.38	1.95 ±0.07
	2	2.78±0.28	1.96±0.18	1.68±0.13
	3	2.98±0.52	1.72±0.76	1.38±0.15
Alkaline phosphatase (U/ml)	1	58.98±0.76	23.60±0.93	19.67±0.66
	2	54.53±1.17	27.93±0.50	24.41±0.63
	3	47.86±2.59	34.88±1.65	29.10±0.72
Catalase activity (nmol/ml)	1	0.65±0.026	0.79±0.015	086±0.015
	2	0.44± 0.008	0.89±0.006	0.95±0.01
	3	0.17±0.014	1.12±0.097	1.46±0.055
Reduced glutathione (mg/dl)	1	2.22±0.59	25.08±0.47	48.73±1.31
	2	3.83± 0.64	18.68±0.77	35.83±1.31
	3	7.87± 0.74	13.58±0.63	18.09±1.06
Superoxidase dismutase (U/ml)	1	4.66±0.26	20.29±0.36	23.02±0.25
	2	6.82±0.19	15.69±0.23	20.00±0.26
	3	9.95±0.27	13.76±1.71	34.19±6.67

Data are presented as (mean ± S.E.M) & S.E.M = standard error of mean.

Table (3): Effects of Emblica Officinals on survival rate and percentage of TB- mice:

Group	Number of Mice	Number of survived mice	Number of dead mice	Percentage of survival rate
a) Saline group	30	18	12	60 %
b)Emblica offiicinalis 500 mg/Kg	30	23	7	76.66 %
c) Emblica offiicinalis 1250 mg/Kg	30	26	4	86.66 %

Table (4): Effects of Emblica Officinals on volume of ascetic fluid and count of tumor cells per ml in T-bearing mice:

Group	Day 8	Day 15	Day 23
Saline group Volume Cell count (x 10^6)	6.67 ± 0.65 128.25 ±13.87	7.23 ±0.265 124.26±10.27	14.76 ± 0.78 120.85 ±20.45
Emblica offiicinalis (500mg) group Volume Cell count (x 10^6)	4.56* ± 0.21 86.23*±11.79	5.23* ± 0.44 92.13*±13.56	4.87* ± 0.29 86.27*±17.56
Emblica offiicinalis (1250mg) group Volume Cell count (x 10^6)	3.21*# ± 0.24 65.29*#±12.97	3.32*# ±0.43 64.56*# ±15.23	2.93*# ± 0.27 62.43*# ±14.92

All data were expressed as mean ± SE.
One-way ANOVA with Scheffe's posthoc test.
* significant versus saline group,
# significant versus Emblica officinalis (500 mg) group.

4.CONCLUSION

Emblica officinalis has apotent chemopreventive activity against a wide variety of tumors and has great potential in the prevention and treatment of cancer in female mice. In addition, Emblica officinalis exerts chemopreventative activity against cancer due to its content of polyphenols and vitamin C which has antioxidant and free radicals scavenging activity and trapping of activated metabolites of carcinogen. Therefore, we recommend using Emblica officinalis in our food as aprophylactic and preventive for many diseases.

5. REFERENCES

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التأثير الكيميائي الحيوي للسرطان المحدث تجريبيا على اناث الجرذان.

حسين عبد المقصود على وياقوت عبد الفتاح السنوسي ومحمد محمود عبد الستار البيلي
قسم الفارماكولوجيا كلية الطب البيطري بمشتهر جامعة بنها –القليوبية –مصر

الملخص العربي

أجريت هذه الدراسة بهدف البحث عن أدويه تمنع انقسام الخلايا السرطانية بدون أثار جانبيه. قسمت حيوانات التجارب محل الدراسة (150 فأر من الاناث) الى تجربتين: التجربة الأولى: فئران لا تحمل أي ورم وتحتوي على (60) فأر والتي قسمت الى (3) مجموعات. المجموعة الأولى: وتحتوي على (20) فأر تم تجريعها بالمحلول الملحي. المجموعة الثانية: وتحتوي على (20) فأر تم تجريعها بالأملج (500 مجم / كجم) لمدة 3 أسابيع. المجموعة الثالثة: وتحتوي على (20) فأر تم تجريعها بالأملج (1250 مجم / كجم /يوم بعد يوم). التجربة الثانية: وهي التي تم زراعة السرطان بها وتحتوي على (90) فأر والتي قسمت الى (3) مجموعات أيضا: المجموعة الأولى: وتحتوي على (30) فأر من الفئران الحاملة للورم والتي تم تجريعها بالمحلول الملحي وهي مجموعه ضابطه مسرطنه. المجموعة الثانية: وتحتوي على (30) من الفئران الحاملة للورم والتي تم تجريعها بالأملج (500 مجم/ كجم/ يوم بعد يوم) لمدة (3) أسابيع. المجموعة الثالثة: وتحتوي على (30) من الفئران الحاملة للورم والتي تم تجريعها بالأملج (1250مجم/كجم يوم بعد يوم) لمدة (3) أسابيع. تم تجميع عينات الدم بعد وقياس كلا من انزيمات الكبد AST & ALT الذبح ونسبة الصفراء، الكيرياتنين، الفوسفاتيز القلوي وبعض الانزيمات المضادة للأكسدة مثل الكاتلاز، انزيم المحول للفوق اكسيد الأكسجين، ومستوى الجلوناثيون المختزل. وقد تم تجميع عينات من السائل البروتيني للفئران المصابة بالسرطان اليوم الثامن والرابع عشر والثالث والعشرون وقياس حجم السائل وعدد الخلايا السرطانية بالسائل. ويتضح أن نبات الأملج الجرعات المختلفة لا يؤثر على انزيمات الكبد ونسبة الصفراء والكرياتنين فى الفئران الطبيعية ولكنه يزيد من مستوى مضادات الاكسدة فالدم فى الفئران الطبيعية وعند استخدامه فى الفئران المصابة بالسرطان تبين انه يقلل من حجم الورم وعدد الخلايا السرطانية مع تحسن فى فترة العمر التي يعشيها الفئران المصابة بالسرطان هذا وقد وجد ايضا انه يحسن من انزيمات الكبد ويقلل من نسبة الصفراء والكرياتنين بالدم مع تحسن ملحوظ فى مستوى مضادات الاكسدة بالدم.

(مجلة بنها للعلوم الطبية البيطرية: عدد 25(1):192–200, سبتمبر 2013)