Clinicopathological Studies On Some Trials For Treatment Of Ehrlich Ascites Carcinoma In Swiss Mice

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ABSTRACT

One hundred female Swiss mice were used to study the effect of propolis, methotrexate on proliferation of Ehrlich ascites carcinoma cells in Swiss mice. They equally divided into fives groups: 1st was kept as negative control, the 2nd was implanted intraperitoneally with 2.5×10^6 EAC and kept as positive control and the 3rd was implanted intraperitoneally with 2.5×10^6 EAC and treated with propolis by 50 mg/kg body weight was given by gastric intubations 2 hours prior to the intraperitoneal injection of EAC, 4th was implanted with EAC and treated with methotrexate by 0.4 mg/kg body weight and 5th was implanted with EAC and treated with combination of propolis and methotrexate for eleven successive days. Survival analysis revealed increasing mean survival time (MST), increasing life span (ILS %) and treated vs. positive control (T/C %). in all treated groups. Antioxidant analysis revealed a decrease in the activities of superoxide dismutase (SOD), reduced glutathione (GSH) and catalase (CAT) and an increase in the activity of malondialdhyde (MAD) in 2nd and 4th groups, the opposite in 3rd group, while fifth group showed reverse in antioxidant activities toward the normal control group. Biochemical analysis of serum showed that implantation of EAC in Swiss mice without treatment or with methotrexate treatment revealed a significant decrease in total protein and albumin levels and a significant increase in creatinine level and ALT, AST activities, while the 3rd group that received propolis revealed an improvement in these biochemical parameters compared to the normal control group. While 5th group revealed amelioration of these parameters.

INTRODUCTION

Cancer chemotherapy began in the 1940s with the first use of nitrogen mustards and folic acid antagonist drugs. The target therapy revolution has arrived, but many of the principles and limitations of chemotherapy discovered by the early researchers still apply *(1)*.Methotrexate (MTX) is widely used as a cytotoxic chemotherapeutic agent for treatment of leukemia's and other malignancies. In addition, it has been used for the treatment of various inflammatory diseases such as psoriasis and rheumatoid arthritis. However, the efficacy of this agent in high doses has been associated with hepatotoxicity *(2)*. The basis of cancer chemotherapy lies in an understanding of biochemical abnormalities during metabolism of malignant cell. Exploitation of metabolic differences between tumour and host tissue has become one way of treating tumours effectively. Rodent tumours are a case in point where the genetic and biochemical characteristics can be studied and they have become the basis of most cancer chemotherapy screening operations. The transplantability of certain tumours in rodent has provided a useful tool for basic cancer research. The Ehrlich ascites tumour is such a tumour. It provides a reasonably homogenous sample of malignant tissue; it is available in large quantities and grows at a fairly predictable rate *(3)*. Propolis is resinous substance collected by honey bees from various plant sources and used by bees to seal holes in their honeycombs, smooth out the internal walls, and protect the entrance against intruders *(4)*.

The present work was aimed to study some survival analysis, antioxidant and biochemical effects after treatment of Ehrlich ascites carcinoma bearing mice using natural products (propolis) or synthetic products (MTX).

MATERIAL AND METHODS

1.Experimental animals

One hundred adult female Swiss albino mice (average 20 gm in weight) were obtained from the laboratory animal farm of Veterinary

Medicine at Zagazig University in Egypt. All mice were reared under strict standard hygienic conditions and were fed a balanced diet. Water was available *ad libitum.*

2. Ehrlich ascites carcinoma cells

The parent line of Ehrlich ascites carcinoma cells was kindly supplied by the National Cancer Institute of Cairo University, Egypt. The tumour line was maintained by serial intraperitoneal transplantation of Ehrlich ascites carcinoma 2.5×10^6 tumour cells/0.2 ml in female Swiss albino mice *(5)*.

3.Antineoplastic agents

a) Propolis

Obtained from an Egyptian honey bee keeper, mixed with deionised water and shaken at 95°C for 2 h. It was cooled to room temperature and centrifuged at 1500 rpm for 10 min to obtain the supernatant.

b) Trexan

Methotrexate (MTX) 2.5 mg Tablets. Orion Corporation .Finland.

4.Experimental design

One hundred female Swiss mice were equally divided randomly into fives groups (20 mice per group). 1st was kept as negative control, 2nd was were implanted intraperitoneally with 2.5×10^6 EAC and kept as positive control and, 3rd was implanted with EAC and treated with propolis by 50 mg/kg body weight dose was given by gastric intubations 2 hours prior to the intraperitoneal injection of EAC,4th was implanted with EAC and treated with MTX by 0.4 mg/kg body weight dose and 5th was implanted with EAC and treated with combination of propolis and MTX with the same previously mentioned for eleven successive days.

5.Blood sampling

Fifteen mice in each group were used for blood collection from the retro-orbital venous plexus. Samples were taken without anticoagulant in a sterile test tube for separation of serum which was used to measure biochemical parameters.

6.Survival analysis

Five mice from each group were kept under daily observation for survival analysis. Endpoint of experiment was determined by spontaneous death of animals. Results are expressed as percent of mean survival time of treated animals over mean survival time of the control group (treated vs. positive control, T/C %). The percentage of increased life span (ILS) was calculated according the formula:

$$ILS\ \% = (T-C)/C \times 100$$

Where T represents mean survival time of treated animals; C represents mean survival time of the positive control group.

By NCI criteria, T/C exceeding 125% and ILS exceeding 25% indicate that the drug has a significant anti-tumour activity *(6)*.

7.Antioxidant enzymatic activities in tissue homogenate.

The supernatant obtained after centrifugation of liver homogenates was used for the determination of enzyme activities. Superoxide dismutase SOD *(7)*, reduced glutathione GSH *(8)*,Catalase CAT *(9)* and lipid peroxidase expressed by Malondialdhyde MAD *(10)* were determined colorimetrically.

8-Biochemical studies

The serum total protein (TP) and albumin levels were measured *(11,12)*. The activities of aspartate (AST) and alanine aminotransferase (ALT) *(13)*, and serum creatinine level *(14)* were determined colorimetrically.

9.Statistical analysis

The data obtained from this investigation were statistically analysed using F test *(15)*. Means at the same column followed by different letters were significantly different and the highest value was represented with the letter (a).

RESULTS AND DISCUSSION

Survival analysis results (Table 1), shows that 3rd , 4th and 5th groups which treated by propolis, methotrexate and combination, revealed increasing of MST, ILS % in 3rd group this could be due to interfere with the growth of

EAC cells directly during early phase of treatment leading to a considerable elimination of these cells *(16)* and also may be due to treatment with the immune-stimulants resist in various degrees subsequent inoculation of tumour cells as evidenced by the reduced "tumour take", slowed growth of the tumours ,and prolonged survival of recipients *(17)*. While in 4th group may be as a result of inhibition of EAC proliferation *(18)* but 5th group which revealed the best result in survival analysis that could be due to improve cellular immune response *(19)* and antioxidant system *(20)* that maximize their anti-tumour activity when using water soluble of propolis combined with chemotherapeutic agents *(21)*.

Table 1.Effect of propolis and methotrexate (50 mg/kg body weight, 0.4mg/kg body weight) on MST, ILS% and T/C% in Ehrlich ascites carcinoma bearing mice

Groups	Parameters			
	Range of survival time	MST	ILS (%)	T/C (%)
(2)mice bearing EAC	11–14	12.5	–	–
(3)propolis treated group	17-24	20.5	64	164
(4)Methotrexate treated group	15-23	19	52	152
(5)Combination treated group	22-31	26.5	112	212

MST mean survival time
ILS percentage of increasing life span (day)
T/C percentage of treated animals vs. positive control

Antioxidant results (Table 2) revealed a significant increase in MAD level in liver homogenate of EAC bearing mice which could be due to cancer is as a multifactor disease, where oxidative stress may be involved in both initiation and promotion of multi step carcinogenesis, reactive oxygen species can accelerate DNA damage, stimulate pro-carcinogenesis, initiate lipid peroxidation (MAD), inactivate antioxidant enzyme systems and thus can modulate the expression of genes related to tumour promotion *(22)* and malondialdehyde (MDA) the end product of lipid peroxidation, are seen to be higher in cancer tissues than in non diseased organ *(23)* ,while decreased in other antioxidant levels SOD,GSH and CAT may be as a result of tumour growth and emergence of the malignancy *(24,25)*. where in 4th group treated only with MTX revealed the same result but a higher elevation in MAD or diminution in SOD, GSH and CAT that may be as a result of significantly altered the oxidant/antioxidant balance, oxidative stress or oxidative cellular damage with its dual of free radical generation and profound lipid peroxidation are hallmarks of MTX toxicity *(2)*. Actually, the decrease in liver GSH content promoted by MTX represents an alteration in the cellular redox state, suggesting that the cells could be more sensitive to reactive oxygen metabolites *(26)* and leads to a reduction in the effectiveness of the antioxidant enzyme defense system *(27)*. While in propolis treated groups showed more increase SOD, GSH, CAT and decrease MAD activities than positive and negative control and MTX treated group that perhaps due to propolis, protects tissues from reactive oxygene species mediated oxidative stress in toxic injuries *(28)* and improvement of antioxidant system one of suggested role of propolis against cancer is preventing oxidative damage *(29)*.

Table 2. Effect of propolis, methotrexate (50 mg/kg body weight, 0.4mg/kg body weight) on SOD, GSH, CAT and MAD activities on liver homogenate (mean values ±SE) in mice bearing EAC

Parameters / Group	SOD U/gm	GSH mg/gm	CAT U/gm	MAD nmol/gm
(1)Control	27.93^{b}±1.07	1.09^{b}±0.09	118.28^{a}±3.93	141.40^{d}±1.50
(2)Mice bearing EAC	١٧,٥١^{d}±1.40	0.81^{c}±0.02	70.18^{c}±1.47	180.20^{b}±1.98
(3)Propolis group	32.74^{a}±0.20	1.65^{a}±0.05	122.86^{a}±2.54	١٠١,٤٠^{c}±3.82
(4)Methotrexate group	10.67^{e}±0.59	0.60^{d}±0.03	54.74^{d}±3.81	207.80^{a}±6.25
(5)Combination group	20.34^{c}±0.33	1.10^{b}±0.03	80.85^{b}±2.31	153.60^{c}±3.15
F test	**	**	**	**
LSD	2.52	0.16	8.69	11.05

** highly significant difference at $p \leq 0.01$
LSD least significant difference

Biochemical result (Table 3) revealed a decrease in the total proteins and albumin levels in 2nd group; this may be attributed to increased mitotic division of tumour cells with high bloody fluid withdrawal and the capillary permeability, which permit the escape of plasma proteins into the peritoneal cavity *(30)*. Furthermore, hypoproteinemia and hypoalbuminemia may be due to excessive nephritis, certain cases of massive ascites and associated with liver disease *(31)* which confirmed with the result of increased ALT and AST activities with increased of creatinine level in this group which may be attributed to hepatic and renal damage as a result of cancer cells invasion *(32)*. While in 3rd group displayed amelioration of these parameters toward the normal control group levels which reflects a protective effect of propolis against organ dysfunction and cellular injury *(33)*.where in group treated with MTX revealed dysfunction of liver and kidney more than other groups that appeared by decrease in total proteins, albumin and increase in ALT, AST and cretinine which could be due to liver damage, mild necrosis and inflammation *(34)* and renal damage as a result of MTX therapy *(35)* while in 5th group protected by propolis revealed an amelioration toward the normal control group as propolis have marked hepatoprotective potential because of its composition of minerals, flavonoids *(36)* that able to restore the hepatic damage *(37)*.

Table 3. Effect of propolis, methotrexate (50 mg/kg body weight, 0.4mg/kg body weight) on total proteins, albumin, ALT, AST and creatinine (mean values ±SE) in mice bearing EAC.

Parameters / Groups	Total proteins (g/dl)	Albumin (g/dl)	ALT Unit/l	AST Unit/l	Creatinine (mg/dl)
(1)Control	6.98^{a}± 0.16	3.80^{a}±0.20	19.78^{e}±0.92	32.27^{c}±1.02	0.47^{d}±0.03
(2)Mice bearing EAC	4.96 ^{c}± 0.07	1.84^{c}±0.10	30.28^{b}±1.12	55.29^{b}±1.63	1.34^{b}±0.07
(3)Propolis group	5.50^{b}± 0.21	2.76^{b}± 0.13	22.59^{d}±0.36	34.91^{c}±2.15	0.56^{d}±0.06
(4)Methotrexate group	3.50^{e}± 0.17	1.86^{c}±0.19	49.40^{a}±0.41	96.01^{a}±2.78	1.87^{a}±0.03
(5)Combination group	4.40^{d}± 0.12	3.00^{b}±0.00	25.37^{c}±0.21	50.15^{b}±1.00	0.85^{c}±0.02
F test	**	**	**	**	**
LSD	0.47	0.43	2.07	5.47	0.22

** Highly significant difference at $p \leq 0.01$
LSD least significant difference

CONCLUSIONS

Treatment of Ehrlich ascites carcinoma 2.5×10^6 transplanted intraperitoneally in Swiss mice by combination of Egyptian propolis (50 mg/kg body weight) and methotrexate (0.4 mg/kg body weight) ameliorate alteration in antioxidant state and biochemical analysis of the implanted mice toward normal control and improve survival analysis.

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الملخص العربى

دراسات باثولوجية إكلينيكية على بعض المحاولات لعلاج سرطان ايرليش في الفئران السويسرية

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أجريت هذه الدراسة لمعرفة تأثيراستخدام البروبوليس(صمغ نحل العسل) وعقار الميثوتريكسات لعلاج سرطان ايرليش على الصحة العامة و التغيرات الكيميائية في الفئران السويسرية .وقد إستخدم لهذه الدراسة عدد مائة فأر سويسري (وزن ٢٠ جرام). و قد قسمت إلى خمس مجاميع متساوية. المجموعة الأولى: تركت بدون معاملة كمجموعة ضابطة، المجموعة الثانية تم حقنها في الغشاء البريتوني بخلايا سرطان ايرليش بجرعة $٢,٥\times١٠^{٦}$ وتركت حاملة للسرطان وبلا علاج بينما المجموعات(٣ ,٤ &٥): تم حقنها في الغشاء البريتوني بخلايا سرطان ايرليش بجرعة $٢,٥\times١٠^{٦}$ وعولجت بالبروبوليس (٥٠/ملليجرام/كجم) والميثوتريكسات(٠,٤/ملليجرام/كجم) والاثنين معا عن طريق الفم لمدة أحد عشر يوما علي التوالي.

لقد أظهرت كل المجموعات المعالجة زيادة في اعمار الفئران مقارنة بالمجموعة الثانية. وقد أظهرت النتائج البيوكيميائية في المجموعة الثانية والرابعة وجود أثار جانبية في الكبد والكلي (زيادة فى إنزيمات الكبد الألانين أمينوترانسفيريز و الاسبرتيت أمينوترانسفيريز مع نقص البروتين الكلي والالبيومين) مع وجود فشل كلوى (زيادة الكرياتينين) في المجموعة الثانية مع نقص فى انزيمات الاكسدة(السوبر اكسيد الديزميوتيز. الجلوتاثيون المختزل والكاتاليز) وزيادة في المالونديالدهيد. كل هذه الاثار الجانبية اتجهت ناحية المجموعة الضابطة عندما عولجت بالبروبوليس.

نستخلص من هذا البحث أن:

١- يمكن استخدام البروبوليس في علاج سرطان ايرليش
٢- استخدام البروبوليس مع الميثوتريكسات يثبط من الاثار الجانبية للأخير
٣- استخدام البروبوليس مع الميثوتريكسات يحسن كل منهما الاخر في علاج سرطان ايرليش