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Efficacy of Curcumin and/or Chlorambucil on Ehrlich Ascitic Carcinoma in Mice

Nasr A.M. Nasr Eldeen[1*] and Haytham A. Ali[2]
[1]Clinical Pathology Department, Faculty of Veterinary Medicine, Zagazig University, 44511, Egypt
[2]Biochemistry Department, Faculty of Veterinary Medicine, Zagazig University, 44511, Egypt

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Abstract

Cancer is the most common cause for human death following cardiovascular disorders. The present study was carried out to investigate the efficacy of curcumin and/or chlorambucil on modulating cancer induced by Ehrlich ascitic cells (EAC) in mice. One hundred and twenty five femal mice were equally divided into five groups. Group (1) was the normal control, groups (2-5) were injected intraperitoneally with EAC (1.4×10^6/0.2 ml). Group (2) was left without treatment (positive control). While groups (3-5) were treated orally with curcumin (20 mg/kg BW), chlorambucil (0.2mg/kg BW) and curcumin (20 mg/kg BW) plus chlorambucil (0.2mg/kg BW), respectively, after 48 hours from transplantation of Ehrlich cells. Blood samples and specimens from peritoneum, liver and kidney were collected from 15 mice in each group at the tenth day of the experiment. Survival analysis was carried out on the remaining animals. The obtained results revealed a significant decrease in body weight, ascetic fluids, total number of Ehrlich cells, number of live cells, serum levels of aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, lactate dehydrogenase, creatinine, blood urea nitrogen, hepatic malondialdehyde and hepatic P53 and Bax gene expression in all treated groups compared with EAC bearing mice. Moreover, the mean survival time, life span, serum levels of total proteins, albumin, albumin globulins ratio, hepatic activities of antioxidants (SOD and CAT) and hepatic Bcl-2 gene expression were increased in curcumin and/or chlorambucil treated mice compared with the non treated animals. The histopathological examination showed that the least degree of changes was in the 5th group. In conclusion there is a synergistic good impact for using curamin as an adjuvant in combination with standard chemotherapy such as chlorambucil.

Keywords: Curcumin, Chlorambucil, Ehrlich Carcinoma, Mice

Introduction

In the last decades, there is an increase in the number of patients suffering from cancers. Neoplasm means a group of cells show abnormal proliferation, invasion and sometimes metastasis [1]. Cancer is the most common cause for human death after cardiovascular disorders [2,3]. Chlorambucil is an alkylating agent that retards or stops the growth of cancer cells [4]. It is used in the treatment of chronic lymphocytic leukemia, ovarian cancer and malignant lymphomas [5]. Chlorambucil has a response rate approximately 40 to 80% in previously untreated patients [6]. It has the ability to make covalent conjugation of DNA to create DNA cross-linking adducts in tumour cells [7]. This binding to DNA-reading peptides is the site of alkylation reaction to adenine-rich DNA sequences and to its cytotoxic effect on tumour cells [8].

Although great efforts have been done in the development of treatment regims using chemotherapies, they have a major adverse effect on health, therefore, extensive attempts have been devoted to search for new therapeutic approaches. The recent goal all over the world is the exploration for natural plant products that possess anticarcinogenic effects to overcome the adverse consequences of chemotherapies. Plants used in traditional medicine are now accepted as one of the main sources for anticancer agents with acondition of safety ,availability and economic impact [9, 10].

***Corresponding author e-mail: (nanasreldin@zu.edu.eg), Clinical Pathology Department, Faculty of Veterinary Medicine, Zagazig University, 44511, Egypt**

Turmeric, derived from the rhizome of the herb Curcuma longa, has been used for centuries as a dietary spice in Asia. Diferuloylmethane is an active principle present in curcumin, which is the main active ingredient of turmeric [11]. It is nontoxic and has been used to treat various diseases, including cancers and exhibits antioxidant, anti‑inflammatory, antiviral, antibacterial and antifungal activities [12]. Rodent tumours are used in screening operations for most anticarcinogenic drugs. The transplantation of certain carcinogenic cells in rodent provided a good tool for studying different cancers. Ehrlich ascitic carcinoma(EAC) is chemosensitive, so it is used in determining whether the tumour is responding to therapy or not [3].

The planned goal of this study was to evaluate the efficacy of curcumin and/or chlorambucil on modulating cancer induced by Ehrlich cells in mice in order to enhance new tumour therapy. For this purpose a number of parameters such as cell growth inhibition, ascetic fluid volume and survival time of EAC cell bearing mice were studied. In addition to the the rectifying biochemical, molecular and histopathological changes.

Material and Methods

Experimental animals

One hundred and twenty five female Swiss albino mice (18-23 g) were obtained from Laboratory Animal Farm in Helwan, Egypt. All mice were housed in stainless steel wire mesh cages and kept under standard hygienic conditions with dry pellet diet and water *ad libitum*. The animals were acclimatized to the laboratory conditions for 2 weeks before the begining of the experiment. The study was approved by the Committee of Animal Welfare and Research Ethics, Faculty of Veterinary Medicine, Zagazig University, Egypt.

Ehrlich ascitic carcinoma cells

Carcinogenic mice with Ehrlich ascitic carcinoma were obtained from the National Cancer Institute, Cairo, Egypt. The tumour was maintained by serial intraperitoneal transplantation (1.4×10^5 tumour cells/0.2 ml) in mice [13].

Curcumin (Sigma chemicals, street Louis, Missouri): It was used in a dose of 20mg/kg BW [14]. Chlorambucil - Leukeran tablet (Heumann Pharma GmbH for Glaxo Wellcome GmbH and Co. Bad Oldesloe, Germany): Each tablet contains 2mg Chlorambucil. The recomended dose for mice is 0.2mg/kg BW [15].

Experimental design

One hundred and twenty five female Swiss mice were equally divided into five groups for therapeutic evaluations. Group (1) was kept as normal control. Groups (2-5) were inoculated intraperitoneally by EAC cells (1.4×10^5 cells/mouse) then treated orally with curcumin (20 mg/kg BW) ; chlorambucil (0.2 mg/kg BW and curcumin plus chlorambucil in Groups 3,4,and 5 repectively 48 hours post transplantation.The oral administration of treatments was done using a bent stainless steel tube.

Blood sampling and biochemical investigations

At the end of the experimental period (8 days), all mice were subjected to overnight fasting. Fifteen mice from each group were used for collection of 5 blood sample pools (3per each) from the retro-orbital venous plexus after using ether vapour anaesthesia. The blood samples were taken without anticoagulant in sterile test tubes and allowed to clot, then centrifuged at 3000rpm for ten minutes for separation of serum for estimation of aspartate (AST) and alanine aminotransferases (ALT) [16], alkaline phosphatase (ALP) [17] and lactate dehydrogenase (LDH) activities [18] .The serum total proteins (TP) [19] and serum albumin (Alb) levels [20] were measured. Serum globulins (Glb) level was calculated by subtracting the albumin from the total proteins [21]. The blood urea nitrogen and serum creatinine levels were also determined colorimetrically [22, 23].The previous biochemical parameters were measured using Bio-diagnostic kits.

Tissue specimens for biochemical investigations

Liver samples were collected after washing with 0.9% NaCl then were homogenized in potassium phosphate buffer solution (10% W/V). The homogenates were centrifuged at 4500 rpm for 15 min at 4°C and the supernatants were collected and stored at -40°C until tissue malondialdehyde (MDA) [24], superoxoide dismutase (SOD) [25] and catalase (CAT) [26] assays were performed using Bio-diagnostic kits.

Survival analysis

Ten animals from each group were observed daily for deaths. The end point of this study was reached by death of all mice in the diseased groups (groups 2-5). The percentage of increased life span (ILS %) = (mean survival time of treated animals [T] - mean survival time of positive control × 100. If T/C is exceeding 125% and ILS exceeding 25% this indicates a significant anti-tumour activity for the drug [27].

Counting of Ehrlich ascitic carcinoma cells

After euthanization of mice, the ascetic fluid was aspirated. The volume of fluid was recorded. The number and percentage of life and dead cells were documented. Neubauer hemocytometer and trypan blue stain 1% were used for EAC cell count [27].The living cells did not take the stain.

Molecular analysis

Hepatic tissue samples stored in liquid nitrogen were used for determination of P53, Bax, Bcl-2 and β-actin gene expression by a semi-quantitative RT-PCR [28].

Histopathological examination

Specimens from peritoneum, liver and kidneys were collected for histopathological examination. The specimens were fixed in 10 % neutral buffered formalin and were embedded in paraffin. Sections of five micron thickness were prepared and stained with hematoxylin and eosin (H & E) [29].

Statistical analysis

The obtained data were statistically analysed using MSTAT‑C computer program [30] using one‑way analysis of variance (ANOVA) (F test). Means at the same column followed by different letters were significantly different.

Results

The results revealed a significant increase in the body weight of groups (2-5) compared with the normal control. The ascetic fluids were significantly decreased in curcumine, and/or chlorambucil treated mice (groups 3-5) compared with EAC bearing mice (Table 1). From the survival analysis the results revealed that mean survival time (MST), ILS% and T/C % were increased in curcumine, and / or chlorambucil treated mice (groups 3-5).

Table 1: The effect of curcumin and/or chlorambucil on body weight, volume of ascitic fluid and survival parameters of normal and EAC bearing mice (mean ± SE)

Groups	Treatment	Body weight/gm	Ascitic fluids/ml	MST in days	ILS%	T/C %
Gp.(1)	Normal control	21.05±0.49 ^{e}	-	-	-	-
Gp.(2)	EAC	27.75±0.71^{a}	3.82±0.19^{a}	13.40±0.64^{d}	-	-
Gp.(3)	EAC+ curcumin	26.20±0.33^{b}	2.54±0.13^{b}	19.70±0.73^{c}	47.02	147.02
Gp.(4)	EAC+ chlorambucil	24.35±0.36^{c}	1.90±0.07^{c}	23.80 ^{b}±0.77	67.61	177.61
Gp.(5)	EAC+ chlorambucil + curcumin	22.80±0.29 ^{d}	1.14±0.08^{d}	26.90±0.89 ^{a}	100.74	200.75

Means with different superscripts within the same column are significantly different at p≤0.05.EAC: Ehrlich ascites carcinoma, MST: Mean survival time, ILS%: Increased life span, T: Treated animals, C: Positive controls.

A significant decrease in the total number of EAC cells and the number and percent of live cells were recorded in infected treated groups (Table 2 and Figure 2.5) compared with EAC bearing mice(Group 2). On the other hand the number and percent of dead cells were increased significantly in the same groups compared with the second group.

The serum activity of aminotransferases (ALT & AST), alkaline phosphatase (ALP) and lactate dehydrogenase (LDH) were significantly increased in all groups compared with the normal control and the highest values were recorded in EAC bearing mice without treatment (Table 3). Also, the levels of serum total proteins, albumin and albumin globulins ratio had a significant decrease, while a significant increase in the serum levels of creatinine and blood urea nitrogen was recorded in all groups compared with the normal control. The groups (2 -5) revealed a significant increase in hepatic MDA level with a significant decrease in the activities of SOD and CAT enzymes compared with the normal control Table (3).

Table 2: The effect of curcumin and/or chlorambucil on total EAC cells, life cells (number and %) and dead cells (number and %) in EAC bearing mice (mean ± SE)

Groups	Treatment	Total EAC cells (x 10^6/µl)	Life (x10^6/µl)	%	Dead (x 10^6/µl)	%
Gp.(1)	Normal control	-	-	-	-	-
Gp.(2)	EAC	26.55±0.76^a	25.92±0.69^a	97.68±0.22^a	0.63±0.07^d	2.32±0.22^d
Gp.(3)	EAC+ curcumin	19.16±0.35^b	18.20±0.34^b	94.99±0.30^b	0.88±0.06^c	5.01±0.30^c
Gp.(4)	EAC+ chlorambucil	15.20±0.48^c	14.08±0.42^c	92.69±0.59^c	1.12±0.11^b	7.31±0.59^b
Gp.(5)	EAC+ chlorambucil + curcumin	11.54±0.50^d	10.17±0.45^d	88.17±0.85^d	1.37±0.12^a	11.83±0.85^a

Means with different superscripts within the same column are significantly different at p≤0.05, EAC: Ehrlich ascites carcinoma.

Table 3: Biochemical parameters in normal and EAC bearing mice after treatment with curcumin and/or chlorambucil (mean ± SE)

Parameters	Group.(1)	Group(2)	Group(3)	Group(4)	Group(5)
AST (U/l)	39.74± 0.89^e	87.20±2.42^a	69. 80±1.36^c	75.60±3.08^b	61.80±1.16^d
ALT (U/l)	17.88±0.77^e	44.00±1.14^a	33.60±1.17^c	38.76±0.89^b	29.20 ±1.28^d
LDH (U/l)	127.80±2.52^e	176.12±2.85^a	156.60±2.25^c	166.60±2.09^b	145.06±3.41^d
ALP (U/l)	73.36± 1.75^c	109.04±3.22^a	92.80±1.93^{bc}	96.60±2.69^b	86.602.11^c
TP (gm/dl)	6.12± 0.10^a	4.10±0.06^e	4.92±0.07 ^{c}	4.60±0.09 ^{d}	5.28±0.16 ^{b}
Alb. (gm/dl)	3.40±0.11^a	1.60±0.07^e	2.38±0.05^c	2.08±0.06^d	2.62±0.03^b
Glob. (gm/dl)	2.72±0.07^a	2.50±0.05^a	2.54±0.09^a	2.52±0.08^a	2.66±0.13^a
A/G ratio	1.26±0.06^a	0.64±0.03^d	0.94±0.05^{bc}	0.83±0.04^c	0.99±0.04^b
creatinine (mg/dl)	0.71±0.04^d	1.68±0.08^a	1.30±0.02^b	1.41±0.05^b	1.03±0.02^c
BUN (mg/dl)	16.40±0.81^d	36.60±0.68^a	26.41±0.51^b	27.44±2.00^b	21.64±0.07^c
Hepatic MDA(nmol/gm)	2.34±0.18^e	7.80±0.17^a	3.24±0.18^d	6.53±0.17^b	4.78±0.22^c
Hepatic CAT(U/gm)	94.36± 2.86^a	68.72±1.45^d	84.40±1.36^b	59.60±2.56 ^{e}	78.00±1.61 ^{c}
Hepatic SOD (U/gm)	19.04±1.20^a	9.88±0.15^d	15.4±0.30^b	7.20±0.17^e	13.20±0.80^c

Means with different superscripts within the same column are significantly different at p≤0.05, EAC: Ehrlich ascites carcinoma.

The results of molecular analysis of hepatic tissues revealed a significant increase in the transcriptional levels of proapoptotic gene P53 and Bax gene in chlorambucil and/or curcumin treated groups compared with the 2nd group (EAC) and the highest values were recorded in the 5th group .On the other hand a significant decrease in the Bcl-2 gene expression was recorded in groups (3-5) compared with the 2nd group and the lowest value was seen in the 5th group (Figure1 A, B, and C).

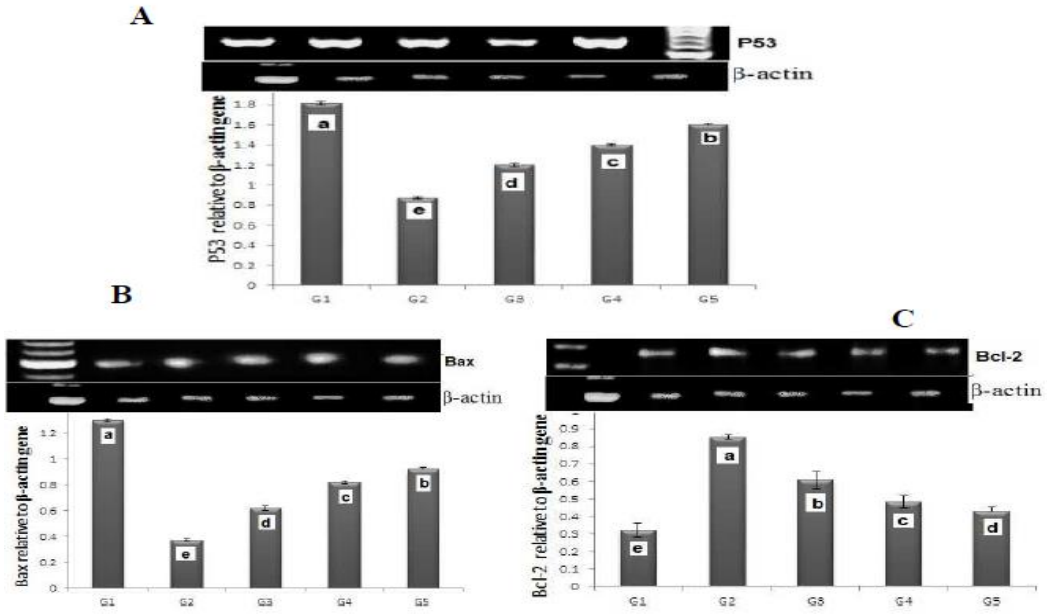

Figure 1: Relative gene expression, P53(A); Bax(B) and Bcl-2 (C) in hepatic tissues of EAC mice after treatment with curcumin and chlorambucil

Normal control animals showed no gross abnormalities nor microscopical changes in the examined organs. However,group 2 (EAC) revealed mass of cancer cells in potenium. The latter had malignant features from polymorphism, nuclear atypia, hyperchromasia and mitotic activities within the peritoneal and extended to replace the omental fat (Figure 2.1). Macroscopically the liver was severely enlarged. Microscopically, it revealed focal necrotic areas and the remaining suffered from different types of cells injury mainly hydropic degeneration (Figure2.2). Sometimes tumour emboli could be seen in the portal blood vessel (Figure 2.3). The kidneys were macroscopically enlarged. Microscopically the renal parenchyma showed nephritic changes in renal tubules (Figure 2.4).

Group 3 (EAC and curcumin): the peritoneum revealed a few scattered malignant cells within the omentum. Apoptotic changes could be seen in some of the forementioned neoplastic cells accompanied with lymphocytic infiltration in the neoplastic stroma (Figure 2.5). Macroscopically the liver was enlarged. Microscopically, it showed mild degenerative changes in the hepatic cells accompanied with interstitial lymphocytic infiltration and congestion of blood vessels and hepatic sinusoids (Figure2.6).The kidneys were macroscopically enlarged. Microscopically the majority of nephrons revealed mild reversible nephritic changes. A few glomeruli had contracted glomerular tufts and cystic dilatation of some tubules together with some regenerative attempts (Figure 2.7).

Group 4 (EAC and chlorambucil), the peritoneum showed a few neoplastic cells scattered between fat cells which usually invaded with leukocytes (Figure 2.8). Macroscopically the liver was slightly enlarged. Microscopically, focal replacement of hepatic parenchyma with few erythrocytes and leukocytes (Figure 2.9). The kidneys were macroscopically enlarged. Microscopically, contracted glomerular tufts and dilated glomerular space could be seen (Figure 2.10).

Group 5 (EAC and curcumin plus chlorambucil): the peritoneum showed a few scattered malignant cells in the omentum. Sometimes these cells suffered from programmed cell death (Figure 2.11). The liver was apparently normal. Microscopically, the liver revealed interstitial lymphocytic aggregation with dilated hepatic sinusoids and hyperplastic kuppfer cells (Figure 2.12). Macroscopically the kidneys were slightly enlarged. Microscopically, the kidney showed interstitial lymphocytic aggregation or normal renal parenchyma (Figure 2.13).

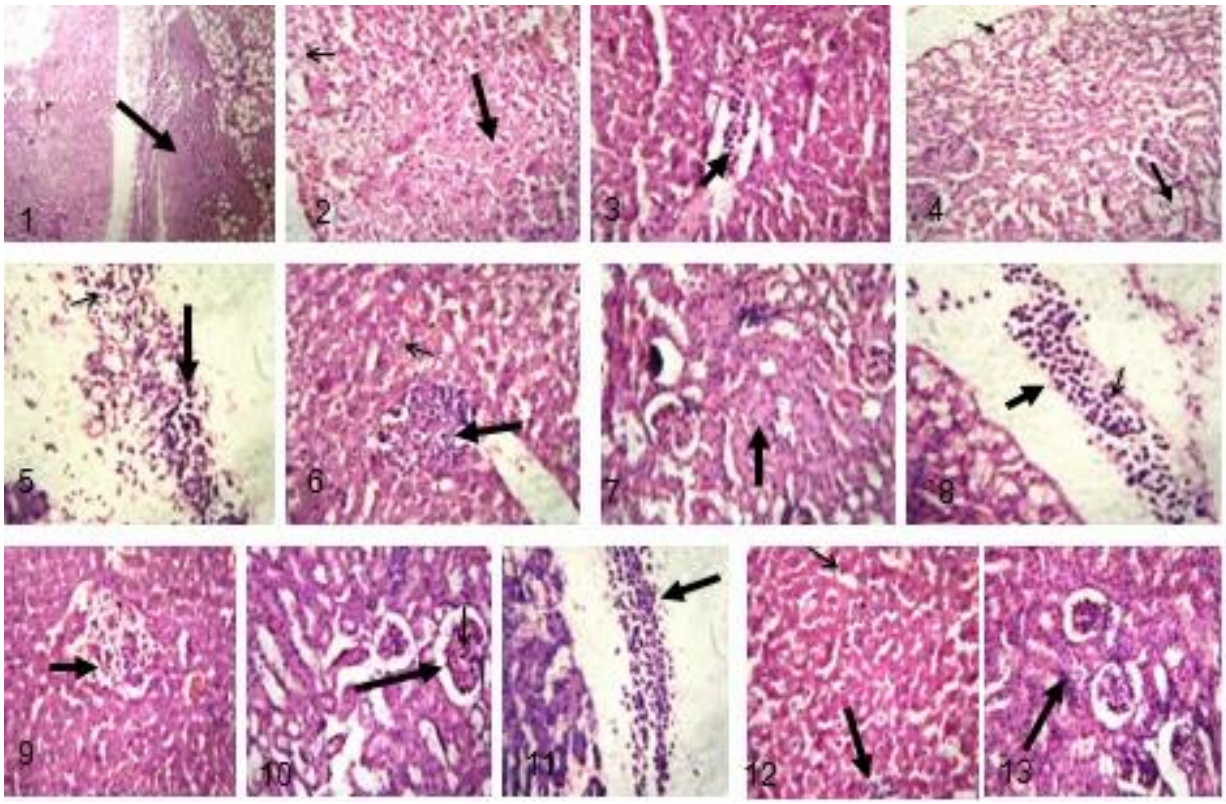

Figure 2: 1. Peritoneum (group 2)A large tumour mass within the peritoneum (arrow) that replaced the omental fat (H&E x 300). 2: Liver (group 2) necrosis (long arrow) and hydropic degeneration (short arrow head) of hepatic cells (H&E x 1200). 3: Liver (group 2) tumour emboli within portal vien (H&E x 1200). 4: Kidney (group 2) nephrosis (arrow) of renal tubules(H&E x 1200). 5: Peritoneum (group 3) little neoplastic cells (long arrow) and numerous leukocytes (short arrow) replacing the omental fat (H&E x 1200). 6: Liver(group3) portal mononuclear cells (arrow) and degenerative changes(short arrow) in the hepatic cells (H&E x 1200). 7: Kidney (group 3) mild degenerative changes in renal tubules (arrow) (H&E x 1200). 8: Peritoneum (group4) small tumour mass with apoptotic changes(long arrow)and invaded by leukocytes (short arrow) (H&E x 1200). 9: Liver (group 4) focal replacement of hepatic parenchyma with erythrocytes and leukocytes(arrow) (H&E x 1200). 10: Kidney (group 4) dilated glomerular space(long arrow) and contracted tufts(short arrow) (H&E x 120). 11:Peritoneum (group5) small tumour mass invaded by leukocytes (arrow) (H&E x 1200). 12: Liver (group4) focal interstial leukocytic infiltration with dilated sinusoids (arrow) and hyperplastic kupffer′s cells(short arrow) (H&E x 1200). 13: Kidney (group5) interstial lymphocytic aggregation(long arrow with normal renal parenchyma(H&E x 120).

Discussion

Ascitic fluid is acting as a direct source for nutrition of tumor cells [31]. The increased volume of ascitic fluid during the growth of tumor acts as a mediator for nutritional requirement for tumor cells [32]. The obtained results supported this hypothesis as intraperitoneal inoculation of EAC cells into mice caused a significant increase in the mice body weight. Such increase may be due to the increased number of life EAC cells and subsequant accumulation of ascitic fluid in the peritoneal cavity. The presence of large tumour mass within the peritoneum that extended to replace the omental fat confirmed the results.The administration of curcumin, and / or chlorambucil to EAC bearing mice (Groups 3-5) significantly decreased the body weight, ascitic fluid volume, total tumor cell count and number and percent of life EAC cells. Moreover, they significantly increased the number and percent of dead cells .This indicates the effectiveness of both curcumin and chlorambucil on EAC cells. . similarly ,Badr et al [3] reported that, The increasing of life span of animals provide a good criteria for judgment on the value of any anticancer agents . It could be explained by cytotoxic effect of Chlorambucil on tumour cells [8] since it has the ability to make covalent conjugation of DNA in tumour cells [7].besid

the antitumour effect of diferuloylmethane , an active principle present in curcumin[11]. The decrease in ascitic fluid volume, total tumor cell count and the number and percent of life tumor cells as mentioned above reduced the tumor burden and enhanced the survival parameters of EAC bearing mice (MST, ILS% and T/C %).The decrease of the tumour mass that present within the peritoneum in groups (3-5) compared with EAC bearing mice confirmed the results.

Several investigators reported that EAC cells caused liver damage and disturbances in hepatic cell metabolism [34,35]. In this study, the intraperitoneal inoculation of EAC cells was associated with a significant increase in serum activities of aminotransferases (AST and ALT), ALP and LDHwhich may attributed to hepato cellular damage induced by EAC. The presence of necrosis and hydropic degeneration in hepatic cells, beside tumour emboli within portal vien proved this concept and the obtained data are in conformity with the earlier observations [10,36,37]. The hypoproteinemia, hypoalbuminemia and decreased albumin / globulin ratio in group (2) may be due to the decreased feed intake, loss through intestine and disturbed metabolism in the liver, in addition to the effect EAC on the kidneys which leads to albuminuria. The treatment with curcumine, and / or chlorambucil significantly decreased the elevated serum enzymes and significantly improved the proteins profile compared with EAC bearing mice, indicating the effectiveness of these treatments against tumor cells.

The present work showed that EAC damaged the renal tissue as clarified by both the clinico–and histopathological means was reflected by a significant increase in serum creatinine and blood urea nitrogen. Such biochemical changes, are the outcome of nephropathy which is manifested by nephrotic changes in renal tubules. Nearly similar findings were reported by Hassanet al, and Habib et al., [31,38].

The antioxidant defense systems play an important role in protecting living tissues from the deleterious effects of reactive oxygen metabolites [39]. Among these protective systems, superoxide dismutase and catalase enzymes play an important role in free radical scavenging system. The main function of the two enzymes is to provide a defence against the superoxide anions and hydrogen peroxide [10]. In our study oxidative stress following the intraperitoneal inoculation of EAC cells into mice caused increased use and weakened protective effect of the antioxidant enzymatic system and was manifested by increase of the lipid peroxidation and the MDA formation. Similar results of oxidant damage of the liver have been reported by Samudrala et al., [10] who recorded a significant decrease in endogenous antioxidant enzymes (SOD & CAT) with enhanced free radical generation and MDA formation in EAC bearing mice. The results revealed that the chlorambucil enhanced the antioxidant defense system compared with EAC bearing mice. This improvement may be due to the effect of chlorambucil on EAC cells and subsequantly decreased the ROS released during their metabolism. Although the antioxidant status improved , it did not return to the normal level as chlorambucil itself increased the ROS [40] . The current study also revealed that the adminestration of curcumin reduced the elevated levels of MDA and increased CAT and SOD activities in EAC bearing mice, which indicate the possible antioxidant and free radical scavenging property of curcumin. The ability of curcumin to decrease the cellular levels of reactive oxygen species has been recognized and has been discussed in several reports. Basically, the antioxidant activity of curcumin seems to be mediated by its ability to both scavenges reactive oxygen species (ROS) [41,42] and activates endogenous antioxidant mechanisms that reduce the cellular levels of ROS [43,44]. The using of curcumin and chorambucil in combination was synergistcally better than the treatment with either agent alone.

The results of molecular analysis revealed a significant decrease in the transcriptional levels of P53 and Bax genes expression, beside a significant decrease in the Bcl-2 gene expression in EAC bearing mice. The P53

prevents cancer formation [45]. As it has been described as the guardian of the genome because of its role in conserving the stability by preventing genome mutation [46]. Hence P^{53} is classified as a tumor suppressor gene [47]. The expression of Bax is upregulated by P53 and Bax has been shown to be involved in P53-mediated apoptosis [48]. Bcl-2 is considered as an important anti-apoptotic protein and classified as an oncogene [49].The obtained results supports the previously obtained by Hassan and Abdel-Gawad [31] who reported a significant decrease in the P53 and Bax genes expression, with a significant decrease in the Bcl-2 gene expression in EAC bearing mice. Our results revealed a significant increase in the transcriptional levels of proapoptotic gene P53 and Bax gene with a significant decrease in e Bcl-2 gene expression in chlorambucil and / or curcumin treated groups compared with the 2nd group. Nearly similar results were recorded in breast cancer treated with curcumin [50,51]. Also similar findings were recorded by Bosanquet *et al.* [52].

Conclusion

The obtained data demonstrated the suppressive effect of curcumin and chlorambucil on EAC cells in mice . It could be cocluded that although using of curcumin as a single agent in the treatment of EAC has shown promising results, there is a significant interest for using the compound as an adjuvant agent in combination with standard chemotherapy such as chlorambucil.

Conflict of interest

The authors declare no conflict of interest.

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الملخص العربى

كفاءة الكركمين و / أو الكلورامبيوسيل على سرطان إرليخ في الفئران

نصر عبد الوهاب محمد نصر الدين[١]-هيثم عبدالله على[٢]
[١]قسم الباثولوجيا الإكلينيكية – كلية الطب البيطرى – جامعة الزقازيق
[٢] قسم الكيمياء الحيوية– كلية الطب البيطرى – جامعة الزقازيق

السرطانات هي السبب الأكثر شيوعا لموت الإنسان بعد اضطرابات القلب والأوعية الدموية. وقد أجريت هذه الدراسة للتحقق من فعالية الكركمين و / أو الكلورامبيوسيل على السرطان الناجم عن خلايا إرليخ في الفئران. ولهذا الغرض تم استخدام عدد ١٢٥ من إناث الفئران قسمت بالتساوى إلى خمس مجموعات. المجموعة الاولي استخدمت كمجموعة ضابطة سالبة. أما الاربع مجموعات الاخري(٢-٥) فقد تم حقنهم بخلايا سرطان ارليخ(١.٤ × ١٠^{٦}) داخل الغشاء البريتونى . المجموعة الثانية تركت بدون علاج كمجموعة ضابطة موجبه اما المجموعة الثالثة و الربعة و الخامسة فقد تم علاجهم بالكركيمين (٢٠ مجم / كجم من وزن الجسم) الكلورامبيوسيل (٢و٠ مجم / كجم من وزن الجسم) و بالكركيمين (٢٠ مجم / كجم من وزن الجسم) مع الكلورامبيوسيل (٢و٠ مجم / كجم من وزن الجسم) على التوالى عن طريق الفم بعد ٤٨ ساعة من زرع خلاي ارليخ . بعد ١٠ أيام من بداية التجربة تم تجميع عينات الدم لفصل السيرم مع أخذ عينات من الغشاء البريتوني والكبد والكلى من١٥ فار من كل مجموعة في نفس التوقيت لقياس الإجهاد التأكسدي للكبد، التحليل الجزيئي وفحص الأنسجة. تركت الفئران المتبقية (١٠) في كل مجموعة لتحليل البقاء على قيد الحياة. وقد أظهرت النتائج عن وجود انخفاض ملحوظ في وزن الجسم، الاستسقاء، العدد الكلي لخلايا إرليخ، وعدد ونسبة الخلايا الحية، ومستويات الاسبرتيت أمينوترانسفيريز ، الألانين أمينوترانسفيريز ، الفوسفاتيز القلوي، اللكتيت ديهيدروجينيز، الكرياتينين، اليوريا نيتروجين فى الدم ،المالوندالديهيد الكبدى ومعدل التعبير الجينى للبى٥٣ و الباكس فى الكبد في جميع المجموعات المعالجة بالمقارنة مع الفئران التي تعانى من سرطان إرليخ ولم تعالج. وعلاوة على ذلك زاد متوسط البقاء على قيد الحياة ، العمر الافتراضي ، عدد الخلايا الميتة ، مستويات السيرم من البروتين الكلي ، الزلال ، نسبة الزلال الى الجلوبيولين ، أنشطة المواد المضادة للاكسدة بالكبد و التعبير الجيني

للبي سي إل ٢ الكبدي في الفئران المعالجة بالكركم و / أو الكلوراميوسيل (مجموعات ٣-٥) مقارنة بالمجموعة الثانية . أظهر فحص الأنسجة أن أقل درجة من التغييرات فى الغشاء البريتوني والكبد و الكلى كانت في المجموعة الخامسة .ونستخلص من ذلك ان استخدامه الكركم كعلاج مكمل مع العلاج الكيماوي التقليدي مثل الكلورامبيوسيل له تأثير اكثر ايجابية.