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RESEARCH ARTICLE

Hepatic and Renal Protective Effects of *Annona muricata* Leaf and Fruit Extracts on Ehrlich Ascites Carcinoma in Mice

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Abstract

Cancer is a complicated disease incorporating many factors and causes which could be environmental, metabolic disorder, chemical, and genetic alteration. Many of the already used anticancer therapies are derivatives of natural sources including herbs. The current study was designed to assess the antitumor activity of Egyptian *Annona muricata* against Ehrlich ascites carcinoma (EAC) in albino mice which induced by intraperitoneal injection with EAC cells (2×10^6 cells/mouse). Eighty-eight female adult albino mice were utilized in the beginning of the current study and were separated into five groups, normal control, Ehrlich ascites carcinoma, fruit extract (200 mg/kg), leaf extract (200 mg/kg), and cisplatin (2 mg/kg) treated groups for nine successive days after 48 hours of pre-injection with EAC cells. Viability of tumor ascites cells, the volume of ascites fluid, and EAC cell count were significantly decreased after daily treatment with both extracts. Hematological parameters were enhanced, liver enzymes and creatinine regain their normal values. Oxidative stress was dimensioned via decreasing of lactate dehydrogenase (LDH) and malondialdehyde (MDA). Antioxidant activity was enhanced through the increasing of the total antioxidant capacity (TAC) and reduced glutathione (GSH) concentrations, and the activities of superoxide dismutase (SOD) and catalase (CAT). Histopathologically, residual tumor growth on the outer surface of the liver and kidney were markedly reduced without infiltration onto the tissues. Inflammation of tissues was inhibited and tissue architecture was ameliorated. In conclusion, *Annona* extracts could have anticancer activity and antioxidant properties which could be useful in dimensioning cancer progression and improves organs protection.

Keywords: Anticancer, Antioxidant, Annona extract, Ehrlich ascites carcinoma.

Introduction

Cancer is a complicated disease where cell growth is being abnormal, aggressive, invasive, and may metastasizes many times leading finally to death [1]. It is one of the top ten diseases which cause death and keep going throughout the world and expanded in the rank year after year [2]. Cancer treatment may be surgical or by radiation, chemotherapy, hormonal or biologically derived therapies. Due to the missing of potent anticancer drugs, the huge cost of chemotherapies, and the undesirable effects of most anticancer drugs, cancer is a massive cause of mortality [3]. Consequently, efforts are still being in growth to search for an impressive anticancer therapy, from natural sources, that would lessen or even impede the cancer progress [4]. It was reported that more than 50% of all cancer patients use integral and alternative anticancer medicine [5]. So, there is an increased direction in the pharmacological evaluation for the development of various natural products used in alternative or traditional medicine [6]. Components of herbal extracts like flavonoids, steroids, and terpenoids have gathered dramatic attention in the late years owing to their many impressive pharmacological effects especially antioxidant and antitumor actions [7]. In Egypt, the highly ranked cancer is liver

cancer accompanied by serious and progressive problems [8].

Liver cancer is a serious if not the most serious cancer problem in Egypt. *A. muricata* is a species of the *Annonaceae* family which has been widely studied in the last decades because of its potential therapeutic properties against many disorders such as inflammation, cancer, rheumatism and neurological disorders [9]. Cisplatin is a platinum drug approved globally and has been used as a drug for cancer chemotherapy for more than 30 years. Cisplatin is an approach to overcome drug resistance and reduce toxicity in cancer research studies [10].

EAC is an experimental cancer model which is commonly used in cancer research overall the world. In 1907, Paul Ehrlich had identified this type of tumor cells in the mammary gland of a white mouse from which the tumor was named [11]. EAC likens human tumors in their sensitivity to chemotherapy, actually, they are undifferentiated, and have rapid growth, proliferation, shorter life span, with100% malignancy [12].

Moreover, the ideal anticancer drug should be inefficient or minimally effective with normal cells, and at this point, the usage of the natural sources as an alternative way for cancer therapy is considered to have a great value for the treatment and controlling of cancer progression. To evaluate the efficacy of anticancer therapy in EAC experimental animals, the tumor fluid volume, viable and nonviable tumor cell count, hematological [11], oxidative stress, and antioxidant parameters [9] in addition to liver and kidney functions biochemical parameters [2] could be assessed. The current study aimed to estimate the potential antitumor action of the Egyptian *A. muricata* fruit and leaf extracts against EAC in adult female albino mice.

Materials and Methods

Experimental animals

Eighty-eight adult female albino mice (25-30g weight) were used in the current study, were provided from the National Research Centre, Cairo, Egypt. Animals were transferred into separate polyethylene cages at Animal House, Zoology Department, Faculty of Science, Port Said University. The animals were kept at normal conditions, room temperature of 25±5C^{o} and a natural light/dark (12 h) cycle. They were fed standard pellets and water *ad libitum*. Animal maintenance and care were in conformity with recommended International Guiding Principles for Biochemical Research Involving Animals [13].

Plant extraction

Fresh fruits and leaves of *Annona muricata* were collected from Al-Nobaria, EL-Behera, Egypt and were identified by the Taxonomist/curator of Botany Department, Faculty of Science, Port-Said University. The plant was cleaned and washed with distilled water, dried in the oven at 60C^{o}, milled into powder, soaked in 70% ethanol for 48h and filtered through gauze then filter paper. The obtained alcoholic solution was concentrated by rotary evaporator. The obtained extract was weighed and stored at -20 C^{o} until used for treatment. Before treatment, the extracts were dissolved in 1% Tween 80 [14].

Maintenance of Ehrlich ascites cells (EAC)

EAC-bearing mouse was obtained from Tumor National Cancer Institute, Cairo, Egypt. One ml of ascites fluid which containing Ehrlich cells was aspirated and diluted with physiological saline (0.9% NaCl). Under aseptic condition, cells were counted using haemocytometer, and 0.2 ml of fresh ascites fluid providing (2×10^{6} cells) was intraperitoneally (I.P) injected into normal mice to induce peritoneal cancer.

Animal grouping

Animals were separated into five treatment groups, Group I, normal control group, healthy mice were intraperitoneal (IP) received 0.2 ml tween 80 daily for 11 successive days. Group II, a positive control group, were IP received EAC cells (2×106 cells/mouse). Group III, a leaf extract treated group, were orally received leaf extract (200 mg/kg) daily [14] after 48 h of pre-injection with EAC cells, for 9 successive days. Group IV, fruit extract treated group, were orally received fruit extract at a daily dose of (200 mg/kg) [15] after 48h of pre-injection with EAC cells for 9 successive days. Group V, cisplatin treated group, were IP treated with cisplatin at a daily dose (2 mg/kg) [16] after 48 h of pre-injection with EAC cells for 9 successive days.

Collection of samples

After 9 days of treatment, eight mice of each group were anesthetized by diethyl ether and sacrificed. Blood samples from each mouse were collected into two separate tubes;

the first was containing EDTA as an anticoagulant for assaying haematological parameters, the second was plain and centrifuged at 3000 rpm for 15 min. Obtained serum was stored at -20 Cº until used for biochemical assay. Immediately after collection of blood samples, animals were dissected; livers and kidneys were removed and washed with 0.9% NaCl. One-half of the removed liver was homogenized in ice-cold saline solution for biochemical assay while the other half in addition to kidney undergo fixation process in 10% formalin for histopathological investigation. Serum samples were used for estimation of alanine aminotransferase (ALT), aspartate transaminase (AST), lactate dehydrogenase (LDH), creatinine and Total antioxidant capacity (TAC). Liver tissue homogenates were used for estimation of superoxide dismutase (SOD), reduced glutathione (GSH), catalase (CAT), and malondialdehyde (MDA).

Determination of antitumor activity

After the dissection of mice, the peritoneal ascetic fluid was obtained from the cavity of each mouse using an 18 gauge needle and the volume of fluid was measured in a measuring tube. The number of cells present in the ascitic fluid was counted using haemocytometer to calculate the percent in the inhibition of tumor growth by comparing the number of cells in the ascitic fluid of treated mice in comparison with non treated mice group. In addition, tumor cell growth in the normal control group was taken as 100 percent cell growth. Viability test was done by staining the ascetic fluid using trypan blue dye (0.4 % in normal saline). Cells were counted using haemocytometer where the cells which cannot take up the dye were viable, with an intact membrane, and those which can take the stain were considered not viable, with a damaged membrane [16].

Hematological assay

White blood cells (WBCs), red blood cells (RBCs) count, and platelets (PT) counts in addition to hemoglobin concentration (Hb), were estimated using Abbott CELL-DYN 1800 automated hematology analyzer, USA at Animal Physiology Laboratory, Faculty of Science, Port Said University.

Determination of liver and kidney functions

Serum ALT, AST**,** and creatinine levels were estimated by a colorimetric method [17] according to the manual described by the manufacturer (Bio diagnostic, Giza, Egypt).

Determination of oxidative stress parameters and tissue damage biomarkers

MDA, TAC, SOD, GSH, and catalase were determined by the method provided by the manufacturer (Bio diagnostic, Giza, Egypt) [18-19]. LDH was determined according to the manufacturer (ELITech Clinical System) [20].

Histopathological examination

Liver and kidney tissue organs were fixed in 10% formalin for 24h, and then dehydrated, cleared, and impeded in paraffin wax. Obtained tissue blocks were sectioned by microtome at a 5µm thickness, stained with haematoxylin and eosin (H&E), examined and photographed with camera microscope system (Olympus BX53, Olympus Corporation, Tokyo, Japan) supplied with the software (Cell Sense, Version 1.4.1) for imaging. Liver and kidney tissues of the EAC groups were studied in comparison with the normal control and the other treatment groups for finding and assess the histopathological changes [21].

Statistical analysis

Values of the obtained results were expressed as means ± standard error (SE) for each animal group. Differences between groups were statistically analyzed using the Statistical Package for Social Science (SPSS), version 22 software. The significant differences were performed by One-way analysis of variance (ANOVA) test followed by Tukey's post hock test for comparison of means with control. Data were statistically significant when the P values ≤ 0.05.

Results

Antitumor activity

The viability percent of tumor ascites cells in leaf and fruit extracts and cisplatin treated groups (44.69±1.69, 61.58±1.05, and 0.010±0.001) were significantly decreased ($P<0.001$) in comparison with cancer group (95.18±0.69). The ascites fluid volume showed a significant decrease ($P<0.01$) in leaf and fruit extracts and cisplatin treated groups (1.04±0.09, 1.9±0.09, and 0.006±0.001) in comparison to the cancer group (3.84±0.14). The EAC cell counting in the leaf and fruit extracts and cisplatin treated groups (41.2±2.20, 77.0±1.76 and 0.120±0.007) were significantly decreased ($P<0.001$) in

comparison to cancer group (246.0±2.91) as revealed in Table (1).

Table (1): Effect of *Annona muricata* leaf (200 mg/kg) and fruit (200mg/kg) extracts in comparison to cisplatin (2mg/kg) for treatment of tumor EAC cell count (x10^6/ml), in albino mice

Parameters	Groups			
	Cancer	Leaf	Fruit	Cisplatin
Viability percent	95.18 ± 0.69 [a]	44.69 ±1.69 [c]	61.58 ± 1.05 [b]	0.01 ±0.001 [d]
Ascitic fluid volume	3.84±0.14 [a]	1.04±0.09 [c]	1.92± 0.09 [b]	0.006±0.001[d]
EAC count (x10^6/ml)	246±2.91[a]	41.2±2.20[c]	77.0±1.76[b]	0.12 ±0.007[d]

All values were expressed as means ± Standard error (SE) where n=8. Superscripted litters (a-d) referring to the significant differences between groups using one way ANOVA statistical analysis test. Values shared the same letters are none significantly differ.

Hematological assay

Data in table (2) illustrated that RBCs count and Hb concentration were significantly decreased (P<0.001) in cancer non treated control group (2.7±0.07 and 8.3±0.23) when compared with normal control group (5.17±0.12 and 11.9±0.11), significantly increased (P<0.001) in leaf (4.50±0.12 and 10.90±0.15) and fruit (3.88±0.09 and 9.72±0.25) extracts and cisplatin (4.0±0.14 and 10.6±0.18) treated group compared with cancer control group. WBCs count was significantly increased (P<0.001) in the cancer control group (9.4±0.26) in comparison to the control group (6.21±0.23), significantly decreased in leaf, fruit and cisplatin treated groups (6.74±0.7, 7.66±0.20 and 7.50±0.10) when compared with the non treated cancer group. Platelets count showed a significant decrease (P<0.001) in the cancer group (150.8±13) in comparison to the control group (304.4±5.27), while leaf and fruit extract and cisplatin treated groups (285.2±4.15, 273.2±4.02, and 279.6±6.76) revealed a significant increase (P<0.001) in comparison to control cancer group, nevertheless, non statistically significant differences between leaf, fruit extracts and cisplatin treated groups in hematological parameters were observed.

Table (2): Effect of treatment with *Annona muricata* leaf (200mg/kg) and fruit (200mg/kg) extracts or cisplatin (2mg/kg) on hematological parameters of EAC-bearing albino mice.

Parameters	Control	EAC	Groups	Fruit	Cisplatin
WBCs x(10^3/ml)	6.21±0.23 [b]	9.4±0.26[a]	6.74±0.71 [b]	7.66±0.20 [b]	7.5±0.1 [b]
Platelets x(10^3/ml)	304.4±5.27[a]	150±13.1[b]	285.2±4.15[a]	273.20±4[a]	279.6±6.7[a]
RBCs x(10^6/ml)	5.17±0.12 [a]	2.72±0.07 [c]	4.5±0.12[a]	3.88±0.09 [b]	4.0±0.14[a]
Hb (g/dl)	11.98±0.11[a]	8.32±0.23[d]	10.9±0.15[b]	9.72±0.25[c]	10.6±0.18 [b]

WBCs: White blood cells, RBCs: Red blood cells and Hb: hemoglobin. All values were expressed as means ± Standard error (SE) where n=8. Superscripted litters (a-d) referring to the significant differences between groups using one way ANOVA statistical analysis test. Values shared the same letters are none significantly differ.

Biochemical study

Effect of A. muricata on serum transaminases AST and ALT

Regarding serum AST and ALT activities, data in table (3) showed a significant increase (P<0.001) in cancer group (102.36±1.56 and 93.10±2.36) in comparison to the control group 49.64±0.72 and 38.80±1.01). Treatment with leaf (60.24±1.29 and 48.67±2.08) and fruit (71.68±2.34 and 68.00±1.14) extracts revealed a significant decrease when compared with the cancer group. On another hand, there were a significant increase (P<0.001) in ALT and AST levels in cisplatin treated group (82.4 ±0.92 and 78.8± 0.86) versus normal control in addition to leaf and fruit extracts treated groups.

Effect of A. muricata on serum creatinine concentration

Serum creatinine concentration presented in table (3) showed a significant increase (P<0.001) in the non treated cancer group (1.83 ± 0.03) in comparison to the control group (0.79 ±0.032). Where in both leaf and fruit extracts and cisplatin treated groups (0.86±0.08, 0.86 ±0.112, and 1.38 ±0.139) it was decreased (P<0.001, P<0.001, and P=0.017) significantly in comparison to the cancer group respectively. On the other hand, creatine concentration showed a significant increase in the cisplatin group compared to the control group, leaf and fruit treated groups (P<0.005, P<0.005 and P<0.002).

Table (3): Effect of treatment with *Annona muricata* leaf and fruit extracts or cisplatin on biochemical, oxidative stress markers and antioxidant parameters of EAC-bearing albino mice.

Parameter	Groups				
	Control	EAC	Leaf	Fruit	Cisplatin
ALT (U/L)	38.8±1.01^{e}	93.1±2.36^{a}	48.67±2.08^{d}	68±1.14^{c}	78.8± 0.86^{b}
AST (U/L)	49.64±0.72^{e}	102.36±1.5^{a}	60.24±1.29^{d}	71.68±2.3^{c}	82.4 ±0.92^{b}
Creatinine (mg/dl)	0.79 ±0.03^{e}	1.83 ± 0.03^{a}	0.86±0.08^{d}	0.86 ±0.1^{c}	1.38 ±0.139^{b}
LDH (U/L)	755 ±2.87^{e}	1700±34^{a}	799.8±6.9^{d}	1140 ±2^{b}	907±8.25^{c}
MDA (nmol/g tissue)	31.6±1.2^{bc}	51.3±1.4^{a}	27.1±0.67^{c}	34.7±1.4^{b}	29.7±0.81^{b}
TAC (mM/L)	2.55±0.01^{a}	1.3±0.02^{b}	2.4±0.06^{a}	2.4±0.04^{a}	2.37±0.11^{a}
GSH (mg/g tissue)	21.9±0.56^{a}	14 ±0.3^{c}	19.4±0.38^{b}	18.5±0.5^{b}	18.68±0.47^{b}
Catalase (U/ g tissue)	6.6±0.04^{a}	4.7±0.06^{c}	6.36±0.08^{b}	6 ±0.09^{b}	6.14±0.16^{b}
SOD (U/g tissue)	5.58±0.12^{a}	3.20±0.03^{d}	5.08±0.05^{b}	4.48±0.1^{c}	4.9±0.13^{c}

ALT: alanine aminotransferase, AST: aspartate transaminase, LDH: lactate dehydrogenase, malondialdehyde (MDA), TAC: Total antioxidant capacity, GSH: reduced glutathione and SOD: superoxide dismutase. All values were expressed as means ± Standard error (SE) where n=8. Superscripted litters (a-e) referring to the significant differences between groups using one way ANOVA statistical analysis test. Values shared the same letters are none significantly differ.

Tissue damage and oxidative stress assay

Effect of A. muricata on serum LDH activity and MDA content

Serum LDH activity and liver MDA content were significantly increased (P<0.001) in the cancer group (1700.2±34.7 and 51.34±1.4) in comparison to the control group (755.20±2.8 and 31.60±1.2). Where leaf (799.80±6.9, 27.18±0.67) and fruit (1140±2.6, 34.74±1.4) extracts and cisplatin (907±8.25, 29.7±0.81) treated groups showed a significant decrease (P<0.001) in comparison to the cancer group. Non statistically significant differences (P=0.08, P=0.77, P=0.33) between leaf, fruit and cisplatin treated groups in the LDH activity and MDA content were observed in comparison to the normal control group (Table 3).

Effect of A. muricata on serum TAC

Serum TAC content was significantly decreased (P<0.001) in the cancer group (1.3±0.02) in comparison to the control group (2.55±0.01). Treatment with both leaf and fruit extracts and cisplatin (2.44±0.0065, 2.41±0.04 and 2.37±0.11) exhibited a significant increase (P<0.001) in TAC compared to the cancer group. There were non statistically significant differences between leaf, fruit and cisplatin treated groups (P=0.57, P=0.37, and P=0.136) in the TAC content in comparison to normal control group respectively, Table (3).

Effect of A. muricata on liver GSH content, SOD and CAT activities

Liver GSH content, CAT and SOD activities presented in Table (3) were significantly decreased (P<0.001) in the cancer non treated group (14.02±0.31, 4.78±0.06 and 3.2±0.03) in comparison to the control group (21.98±0.5, 6.62±0.04 and 5.58±0.12). In leaf (19.40±0.38, and 6.36±0.08) and fruit (18.52±0.5, 6.02±0.09, and 4.48±0.14) extracts treated groups in addition to cisplatin treated group GSH, CAT, and SOD were significantly increased (P<0.001) in comparison to the cancer non treated group. Non significant differences were observed between leaf and fruit extracts treated groups in comparison to cisplatin treated group.

Liver examination

Histopathological examination of the liver was illustrated in figure (1), the normal control group showed normal hepatic architecture. Hepatocytes have abundant cytoplasm and small nuclei, they are arranged in lobules which are separated by blood sinusoids with thin walls. Ascites tumor non treated group revealed massive growth of malignant cells arranged in multiple layers on the outer liver surface. Hepatocytes were severely degenerated, enlarged and infiltrated by hyperchromatic tumor cells with a massive inflammatory response. The liver of leaf extract treated group has very minimized tumor cell growth on the liver surface without infiltration into hepatic tissue. Moderate necrosis and few apoptotic bodies could be seen. Hepatocytes showed moderate hydropic degeneration with restored normal lobular architecture. Fruit extract treated group revealed a minimal presence of tumor growth on the liver surface without being infiltrated into hepatic tissue, moderate hydropic degeneration of hepatocytes, and moderate congestion with mild inflammatory infiltrate. Cisplatin treated group has no evidence of tumor cell growth on the liver surface. Liver tissue has marked hydropic hepatocytes degeneration, moderate congestion with severe inflammatory infiltrate which could reveal hepatotoxicity.

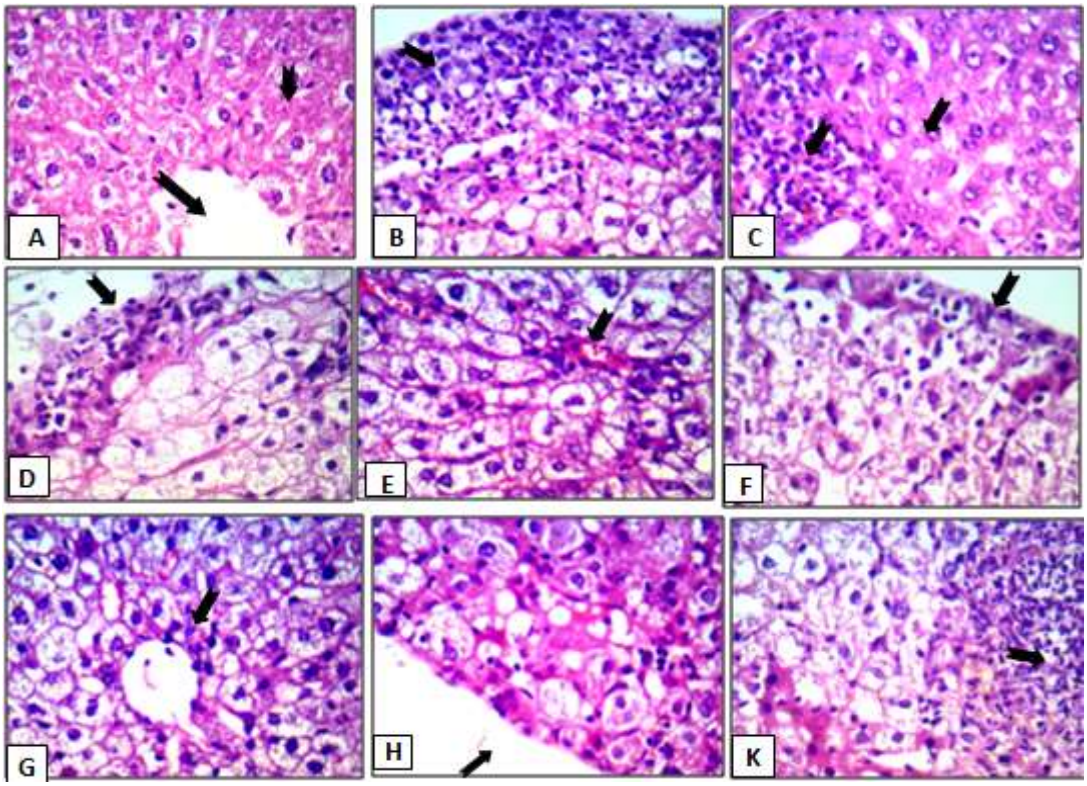

Figure (1): Hematoxylin and Eosin (H&E) stained liver sections of albino mice 9 days post treatment. A: negative control rats with normal liver architecture, hepatocytes are normal and arranged as cords in hepatic lobules. Thin walled blood sinusoids (short arrow) and central vein are seen (long arrow), cells have acidophilic cytoplasm with basophilic regions. B and C, liver of EAC bearing mouse showing massive growth of malignant Ehrlich carcinoma cells (arrow) on the outer surface of the liver. Hepatocytes have hydropic and vacuolar degeneration (B). C: marked infiltration by tumor cells into liver tissue with inflammatory response and marked degeneration of hepatocytes. D and E, liver of EAC bearing mice after treatment with *A. muricata* fruit extract illustrating mild tumor growth on the surface of liver tissue (arrow) (D). E: moderate hydropic degeneration of hepatocytes and moderate congestion with mild inflammatory infiltrate and improvement of liver tissue architecture with mild hemorrhage. F and G, liver of EAC bearing mice after treatment with *A. muricata* leave extract showing mild tumor growth on the surface of liver tissue (arrow) (F). G: mild hydropic degeneration of hepatocytes and moderate congestion with no inflammatory infiltrate. Central vein is seen with normal appearance. H and K, liver of EAC bearing mice after treatment with cisplatin showing no evidence of tumor growth on the surface of liver tissue (arrow), marked hydropic and vacuolar degeneration of hepatocytes (H). K: no evidence of tumor cells infiltration into liver tissue, moderate congestion with severe inflammatory infiltrates. The magnification power is 400 x.

Kidney examination

In figure (2), the normal control group has normal kidney structure composed of glomeruli and tubules. Glomeruli showed capillary tuft with thin Bowman's space. Proximal tubules lined by columnar cells with abundant eosinophilic cytoplasm. Distal tubules lined with low cubical cells and separated by interstitium showed thin blood vessels. Cancer non treated group has extensive growth of multilayers of Ehrlich cells on the outer kidney surface without tissue infiltration, cells were enlarged with hyper chromatic nuclei and nuclear pleomorphism, no infiltration with tumor cells marked hydropic degeneration of tubular epithelium with marked swelling of cytoplasm and marked congestion of vessels with areas of hemorrhage and focal cystic changes. Leaf treated group demonstrated mild tumor residual growth on the kidney surface without being infiltrated and mild necrosis with few apoptotic bodies. Minimal hydropic degeneration of epithelium tubules which mildly congested was observed. Fruit treated group has no evidence of tumor growth in kidney tissue and only showed mild hydropic degeneration of tubules. Evidence of marked necrosis was shown, moderate hydropic degeneration of tubules with moderate congestion. Cisplatin treated group showed no evidence of tumor cell growth. Kidney tissue revealed marked hydropic degeneration of tubules with marked congestion of vessels. Glomeruli showed focal increased cellularity of mesangial cells, focal shrinkage, and hyalinosis. Marked secondary changes on kidney due to toxicity are observed.

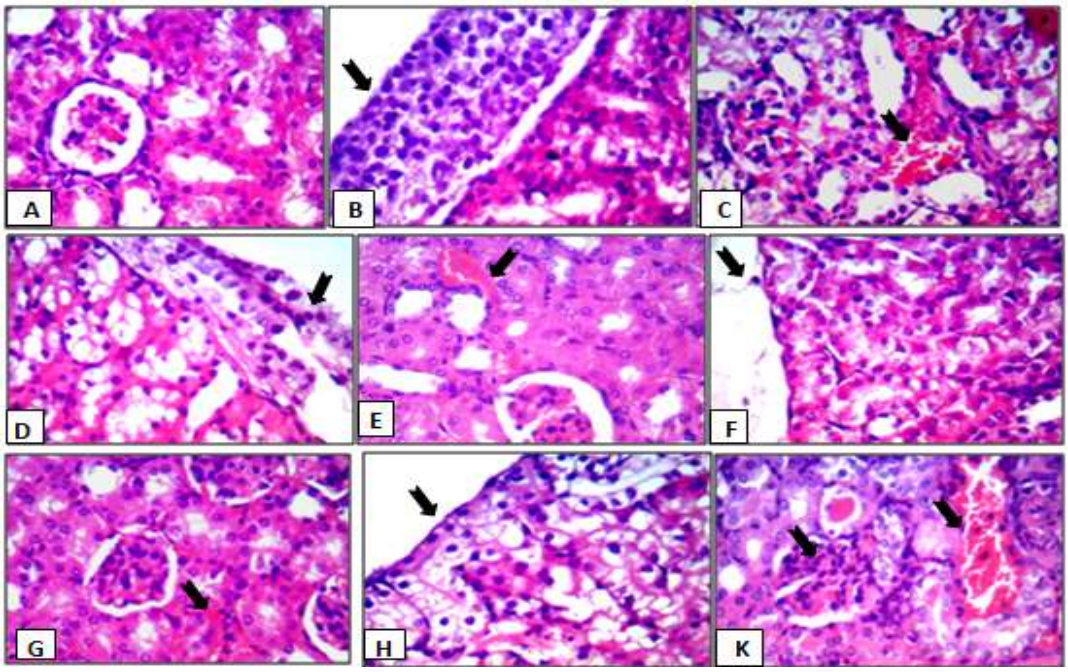

Figure 2: Hematoxylin and Eosin (H&E) stained transverse sections in kidneys of albino mice 9 days post treatment. A: normal control mouse showing normal glomeruli and tubules. Glomeruli have capillary tuft, thin Bowman's capsule space, and mesangial cells. Most of the tubules are lined by normal cuboidal cells with abundant eosinophilic cytoplasm and separated by interstitium. B and C, kidney of EAC bearing mouse, B: showing massive growth of malignant cells in multilayers on the outer surface of the kidney, C: showing marked hydropic degeneration of tubular epithelium and marked congestion of vessels with areas of hemorrhage and focal cystic changes. D and E, kidney of EAC bearing mouse after treatment with *A. muricata* fruit extract, D: showing residuals growth of malignant cells on the outer surface of the kidney, E: marked improvement of kidney tissues architecture with few areas of hemorrhage and mild congestion. F and G, kidney of EAC bearing mouse after treatment with *A. muricata* leaves extract, F: very few residuals of malignant cells on the outer surface of the kidney, G: revealing a marked improvement of kidney tissues architecture with few areas of hemorrhage and no signs of inflammation could be seen. H and K kidney of EAC bearing mouse after treatment with cisplatin, H: no evidence of malignant cells growth on the outer surface of the kidney, K: wide areas of hemorrhage and marked signs of inflammation could be seen with hydropic changes in kidney cells and moderate congestion. The magnification power is 400 x.

Discussion

Cancer is a sophisticated disease originating from the multiple effects of mutant gene products, proto-oncogenes, tumor suppressor genes, and DNA repair genes, promoting the non-controlled growth and proliferation of cancer cells [22]. Humans are exposed to a diversity of cancer-inducing agents which may act as initiators for the tumor formation. In fact, the initiation of carcinogenesis may occur many years before it is being diagnosed [23]. However, treatment with chemotherapies leads to various kinds of toxicities and marked problems in the treatment of cancer. Many cancer therapies already in use are plant-derived products [24, 25]. Meanwhile, annonacin was reported to reduce tumor size comparable to the chemical traded drug cisplatin *in vivo* [26] and inhibited prostate cancer proliferation [27].

The ascitic fluid is believed to be a nutritional requirement for cancer cells growth, and the rapid increase in the ascitic fluid accompanied with growth of tumor could be meet the nutritional components needed for tumor cells [28]. The reduction in the viability percent and tumor cell count in treated animals may be due to the selective inhibition of cancer cells via the epidermal growth factor receptor (EGFR) down-regulation and inhibition [29,30]. *In vitro* studies suggested that *A. muricata* leaves extract is selectively toxic against cancerous cells without harmful effects on the healthy cells [31]. Moreover, the stimulation of macrophages might promote the release of different types of cytokines in the peritoneal cavity that could have a role in the killing of tumor cells [32]. Cisplatin has a cytotoxic effect on EAC tumor cells which contribute the major cellular component of ascites fluid volume [33, 34].

White blood cells (WBCs) well known to play a major role in the immune responses and body protection against disease-causing agents or abnormal cells [35]. The significant increase in WBCs count in the cancer group may be due to the activation of bone marrow [33]. Anemia, reduction in RBC and/or hemoglobin, may result either due to insufficient iron content or due to hemolytic or myelopathic cases [36]. The administration of both leaf and fruit extracts almost restored Hb concentration and ameliorate RBCs and WBCs counts is in agreement with Usunobun and Okolie, 2016 [37]. They proved the improvement of immunological function and decreased inflammation as protective actions of *A. muricata* on the hemopoietic system. Platelets play a major role in clot formation during tissue damage and the regeneration of new cells and tissues [38]. Reduction in platelets count in experimental animals indicated an adverse effect on the blood oxygen-carrying capacity as well as thrombopoietin [39]. Platelets count was increased in both extracts treated groups due to platelets activation in order to the high contents of alkaloids, flavonoids, and phenols with potent abilities to act as antioxidant agents according to [40].

The most common liver enzymes AST and ALT are favorable biomarkers of liver injury [39]. When the liver is injured for any reason, these enzymes are released into the blood stream [41]. The significant increase in AST and ALT activities in the cancer group could be due to hepatocellular damage caused by inoculation of EACs**.** The presence of tumors is obvious to alter many activities of the body vital organs, mostly the liver, regardless of the tumor site [42]. AST and ALT were dimensioned in both leaf and fruit extracts treated groups perhaps due to the hepatoprotective role of *A. muricata* [43] in which the extracts are rich in antioxidants [44] that have a role in the plasma membrane stabilization in addition to repairing of the hepatic damaged tissues [45]. AST and ALT activities in cisplatin treated group significantly increased in comparison to the normal control group. Liver damage induced during that treatment with cisplatin had been reported [46].

Anticancer chemical therapies induce nephrotoxicity due to the consequences accumulation of certain metabolites in the kidneys [47]. The significant increase in serum creatinine in the cancer group may be due to the nephrotoxicity induced by EAC [48]. Creatinine was significantly decreased in both extracts groups which may be due to the nephroprotective role which attributed to their antioxidant effect [49]. Creatinine was

increased in cisplatin treated group in which cisplatin can induce renal damage and nephrotoxicity [50].

LDH is a metabolic enzyme commonly expressed in various tissues and can be detected in serum. The increased LDH activity in the cancer group may be due to membrane damage of cells [51]. LDH is increased in the cases of tissue injury and could be a marker of tumor burden in various types of cancer [52]. The decrease in LDH activity in both extracts treatment groups may be due to the protective effects of *A. muricata* against loss of membrane integrity in which the extracts are rich with flavonoids, saponins, alkaloids, tannins, and ascorbic acid [37].

Many researchers suggested that ROS and oxidative stress play a crucial role in the pathogenesis of cancer [53]. Oxidative damage leads to alteration in both membrane lipid bilayer fluidity and permeability properties that can induce lipid peroxidation [54]. MDA is the end product of lipid peroxidation was increased in the cancer group which may be due to the increase of lipid peroxidation induced by EAC. MDA was markedly decreased in both extracts treated groups due to the antioxidant activities of *A. muricata* [55].

TAC has been developed to quantify the intake of all known and unknown antioxidants [56]. TAC revealed a significant decrease in cancer group and almost restored to normal levels after treatment with both extracts. This could be attributed to the presence of glycosides, proteins, saponins, tannins, different phenolic compounds, alkaloids, flavonoids, steroids and vitamin C [57].

The antioxidant glutathione, a powerful inhibitor of the neoplastic operation, is mainly found in a high concentration in the liver [35]. A relationship was defined between glutathione content and catalase activity, that any decrease in glutathione content causes a decrease in catalase activity [58]. Lobo *et al*., [59] had reported that the decrease of GSH content may be a result of the proliferating rate of EAC cells. GSH can act either to detoxify activate oxygen species such as H_2O_2 or reduce lipid peroxides themselves [60]. The decrease in hepatic GSH in cancer group could be due to inhibition in synthesis, or increased consumption of GSH by oxidative stress [61]. While the increased GSH content in treated groups may be due to repair of hepatic tissue damage in which the liver is the organ of GSH synthesis [45]. It was reported that *A. muricata* extract enhances SOD and catalase activities, increases GSH content and reduces MDA [55].

Histopathological examination of the liver revealed extensive growth of malignant EAC cells in cancer group on the outer surface of the liver accompanied with marked inflammation. Migration of EAC tumor cells into the liver parenchyma through ascites fluid can lead to liver carcinoma and cellular degeneration [62]. Leaf and fruit extracts treated groups represented just residual tumor growth without being infiltrated inside the tissue, mild inflammation, and restored normal lobular architecture which suggested to be due to the hepatoprotective role of *A. muricata.* These findings were in agreement with Samin *et al*., [63]. Anyhow, histological results were in parallel with the biochemical results which showed enhancement in the levels of liver enzymes ALT and AST. Cisplatin treated group marked hydropic degeneration of hepatocytes and massive inflammatory infiltrate that indicate hepatotoxicity in agreement with Yu *et al*., [46].

Regarding histopathological examination of the kidney, In leaf and fruit extracts treated groups, kidney showed mild residual tumor growth without tissue infiltration, This could be suggested due to *A. muricata* anti-tumorigenic and anti-metastatic activities onto internal tumors [64]. Current data indicates that *A. muricata* extract has a protective effect against kidney damage due to the presence of saponins, flavonoids, tannins, and glycosides that contribute to the treatment of various diseases, including treatment for kidney impairment [43]. Cisplatin treated group showed marked regression of tumor and good response, but with massive secondary changes on the kidney. It was reported that cisplatin inhibited proliferation and increased the percentage of dead cells [65]. On the other hand, cisplatin is a strong cellular toxin and its nephrotoxicity is one of the most common complications in clinical and experimental trials [66].

In conclusion, it could be suggested that the leaf and fruit extracts of *A. muricata* have beneficial anti-inflammatory effects and anticancer activity. Both extractions have the ability to ameliorate liver and kidney functions resulting from oxidative stress occurred during carcinogenesis. Biochemical and histopathological results were in harmony and supported this suggestion.

Conflict of interest

The authors declare that they have no competing interests.

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الملخص العربي

التأثيرات الوقائية الكبدية والكلوية لمستخلصي الفاكهة والأوراق لنبات الفشطة المصري *Annona muricata*) على سرطان إرليخ الاستسقائي في الفئران

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يعتبر السرطان من الأمراض المعقدة متعددة المراحل. قد يتسبب في حدوثه العديد من العوامل الكيميائية والوراثية والفيزيائية والبيئية بالاضافة الى مشاكل التمثيل الغذائي. يوجد العديد من العلاجات المضادة للسرطان المستخدمة حاليا و معظمها مشتقة بطريقة أو بأخرى من مصادر طبيعية بما في ذلك بعض النباتات. وقد أجريت هذه الدراسة لتقييم النشاط المضاد للسرطان لمستخلصي الفاكهة والأوراق لنبات القشطة المصري *Annona muricata* . تم استحداث الورم الاستسقائي في الفئران البيضاء عن طريق حقنها في التجويف البريتوني بخلايا إرليخ (Ehrlich). تم استخدام ثمانية وثمانون من إناث الفئران البالغين وتم تقسيمها إلى خمس مجموعات ، الضابطة الطبيعية ، والمريضة بسرطان إرليخ الاستسقائي الغير معالجة ، والمريضة المعالجة بمستخلص الفاكهة (٢٠٠ ملجم / كجم) ، والمريضة المعالجة بمستخلص الأوراق (٢٠٠ ملجم / كجم) ، والمريضة المعالجة بعقار سيسبلاتين (٢ ملجم / كجم) لمدة تسعة أيام متتالية بعد ٤٨ ساعة من الحقن المسبق بخلايا إرليخ (Ehrlich). وقد أظهرت النتائج انخفاض معنوي في حجم السائل الاستسقائي ، وعدد خلايا إرليخ بشكل ملحوظ بعد العلاج بكل من المستخلصين. تحسن في المعلمات الدموية واستعادة إنزيمات الكبد والكرياتينين لقيمها الطبيعية. تحسن الإجهاد التأكسدي عن طريق انخفاض قيم انزيم (LDH) و المالونداي الدهيد (MDA). تم تحسن نشاط مضادات الأكسدة من خلال زيادة تركيز مضادات الأكسدة الكلية (TAC) ، ونشاط انزيم سوبر أوكسيد ديسموتاز (SOD) ، و الجلوتاثيون (GSH) وأنشطة الكاتاليز (CAT). كما أظهر الفحص الميكروسكوبي لأنسجة الكبد والكلى انخفاض ملحوظ لنمو الخلايا السرطانية على السطح الخارجي للكبد والكلى دون تخلل تلك الخلايا إلى داخل الأنسجة كما هو الحال مع المجموعة المريضة الغير معالجة. وأيضا تم تثبيط الالتهاب الملحوظ في الأنسجة و تحسين بنية الأنسجة التركيبية واستعادة الانسجة الى نسبة كبيرة لتركيبها الطبيعي. وقد خلصت الدراسة الى أن مستخلص الفاكهة والأوراق لنبات الفشطة المصري *Annona muricata* له نشاط مضاد للسرطان وخصائص مضادة للأكسدة يمكن أن تكون مفيدة في تحديد أبعاد تطور السرطان وتحسين حماية الأعضاء.