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Protective Effect of Damiana (*Turnera diffusa*) against Growth of Ehrlich Ascites Carcinoma in Female Mice

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Authors' contributions

This work was carried out in collaboration among all authors. Authors AEA and ET designed the study, performed the statistical analysis, and wrote the protocol. Author KAA wrote the first draft of the manuscript, managed the analyses of the study and the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Background and Objective: Ehrlich ascites carcinoma (EAC) is primarily derived from a murine mammary adenocarcinoma. It is a common tumor an undifferentiated carcinoma with high transplantable capability, no regression, rapid proliferation, shorter life span, 100% malignancy and also does not have tumor-specific transplantation antigen. The current study aim to study the protective role of damiana against Ehrlich ascites carcinoma (EAC) induced blood, liver and kidney damage in mice.
Materials and Methods: A total of 75 female mice were divided into 5 groups the 1st group was control group, 2nd group was damiana group, 3rd group was EAC group, 4th group was pre-treated EAC with damiana, and 5th group was co-treated EAC with damiana.
Results: Current results revealed that; EAC induce significant increase in AST, ALT, ALP, urea, creatinine, potassium ions (K+), chloride ions (Cl-), cholesterol, triglyceride and low density lipoprotein-cholesterol (LDL) while EAC induce significant decrease in total proteins, albumin, sodium ion (Na+), calcium ion (Ca2+) and high density lipoprotein-cholesterol (HDL) as compared to control group.

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Conclusion: Treatment of EAC with damiana as pre-treated or co-treated modeulates this parameters with best results for pretreatment than co-treatments. It could be concluded that; damiana has hepatic and renal protective effect against EAC induced changes in blood.

Keywords: Ehrlich ascites carcinoma; damiana; hematological studied; liver and kidney functions; lipid profiles.

1. INTRODUCTION

There are a number of in vivo experimental models based on laboratory animals including the Ehrlich solid tumor, derived from the mouse breast adenocarcinoma, which is an aggressive and fast growing carcinoma able to develop both in the ascitic and the solid form depending on whether inoculated intraperitoneal or subcutaneously, respectively [1,2,3].

Ehrlich ascites carcinoma cells (EAC), very convenient in cancer research, it is a common tumor an undifferentiated carcinoma with high transplantable capability, no regression, rapid proliferation, shorter life span, 100% malignancy and also does not have tumor-specific transplantation antigen (TSTA).

Ehrlich ascites carcinoma cells are primarily derived from a murine mammary adenocarcinoma [4,5,6]. Most approaches therapies that used in cancer treatment is chemotherapy that kill cancer cells by inducing apoptosis and causes severely affect the life of patients and represent a direct cause of death [7,8,9]. Recently, there is obvious increase in the use of complementary and alternative medicine [10,11,12,3].

Damiana (*Turnera diffusa Willd*) is used in traditional medicine, as treatment of genito-urinary diseases, and as an aphrodisiac [13]. Damiana has antioxidant properties and its leaves contain up to 1% volatile oil that is comprised of at least 20 constituents (including 1,8-cineole, pcymene, alpha- and beta-pinene, thymol, alpha-copaene, and calamene) [14]. Damiana leaves also contain tannins, flavonoids, beta-sitosterol, damianin (a brown, bitter substance), and the glycosides gonzalitosin, arbutin, and tetraphyllin B [15].

The objective of this study was to ascertain the protective role of damiana against Ehrlich ascites carcinoma (EAC) induced blood, liver and kidney damage, in mice.

2. MATERIALS AND METHODS

2.1 Transplantation of Tumor Cells and Induction of Ehrlich Ascites Carcinoma (EAC)

The Egyptian National Cancer Institute (NCI; Cairo University, Egypt) supplied the mice which had been injected with Ehrlich ascites carcinoma (EAC). These were utilized as the source of EAC cells. 0.2 ml of ascitic fluid was aspirated from each EAC bearing mice and diluted with diluted with physiological saline. Between 2.5 and 3 million EAC cells were injected intraperitoneally (IP) of each mouse.

2.2 Animals

A total of 75 female Swiss albino mice (aged between ten to twelve weeks old and weighing between 20-25 kg each) were performed for the experiments. They had been obtained from the breeding unit at the Egyptian Organization for Biological Products and Vaccines, Abbassia, Cairo. Free access to normal diet and water supplies was granted to all mice.

2.3 Experimental Design and Animal Groups

The mice were equally divided into five groups:

Group 1: Control group in which mice did not received any treatment.

Group 2: Damiana in which mice received (5g/ kgbody weight/ day) damiana for daily orally by astomachtube for two weeks.

Group 3: (EAC) involved mice that injected one time intraperitoneally with 2.5 million EAC cells to initiate carcinoma after [7].

Group 4: pre-treated EAC with damiana for two weeks.

Group 5: co-treated EAC with damiana for two weeks.

2.4 Sample Collection

Blood samples have been collected aseptically by venepuncture into a dry clean and sterile tube without anticoagulant substances and allow it to clot. Blood samples allowed to stand for 30 min at 4 o C for clot formation and centrifuged for 10 minutes at 3000 rpm. The collected serum was stored at -18°C until analysis for estimation of some blood parameter.

The activities of serum AST and ALP were assayed by the colorimetric method according to [16,6] respectively while alkaline phosphatase (ALP) was estimated in the rat serum according to El Moghazy et al. [17]. Serum levels of total protein were determined by using commercial kit according to Tousson et al. [18]. Serum levels of albumin were determined by using commercial kit according to Basuony et al. [19].

Serum potassium, sodium, calcium and chloride ions levels in was determined by using commercial kits (Sensa core electrolyte, India) according to Abd Eldaim et al. [20]. Last but not least, the concentration of cholesterol; triglyceride, high-density lipoprotein-cholesterol (HDL-C) and low density lipoprotein-cholesterol (LDL-C) were determined with Kits from ELLTECH according to Aldubayan et al. [21] and El Atrash et al. [22] respectively.

2.5 Statistical Analysis

Data were expressed as mean values ± SE and statistical analysis was performed using unpaired t-test to assess significant differences among treatment groups. The criterion for statistical significance was set at 0.05 for the biochemical data. All statistical analyses were performed using SPSS statistical version 21 software package (SPSS® Inc., USA).

3. RESULTS

3.1. Biochemical Investigations

Table 1 represented the Changes in liver fuctions [aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP), Total protein (TP), albumin] in different uder study groups. The results showed serum aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) levels were significantly ($p<0.05$) increased in EAC (G3) as compared with control group (G1). On the other hand; a significant ($p<0.05$) decrease in total protein and albumin in EAC group as compared to control group. In contrast; a significant ($p<0.05$) decrease in ALT, AST and ALP in pre-treated mice (G4) and in co-treated (G5) groups when compared to the EAC group. Total protein and albumin were significantly ($p<0.05$) decreased in EAC (G3) as compared with control group. However, total protein was in significantly ($p<0.05$) increased in pre-treated (G4) and significantly ($p<0.05$) increased in albumin in pre-treated (G4) and in co-treated (G5) groups when compared to the EAC group.

Tables 2 and 3 represented the changes in kidney functions and electrolytes in different groups [Urea, creatinine and electrolytes]. The results showed serum urea and creatinine levels were significantly ($p<0.05$) increased in EAC (G3) as compared with control group. While were significantly ($p<0.05$) decreased in pre-treated mice (G4) and in co-treated (G5) groups when compared to the EAC group. Sodium and calcium serum were significantly ($p<0.05$) decreased in EAC (G3) as compared with control group, but potassium and chloride were significantly ($p<0.05$) increased in EAC (G3) as compared with control group.

Table 1. Changes in serum liver enzymes (AST, ALT, ALP, total protein and albumin) in all the studied groups

Groups	Parameters			T.protein(gm/dl)	Alb (gm/dl)
	AST (U/L)	ALT (U/L)	ALP (U/L)		
Control	132.5±4.77****	44±1.472****	129±2.677****	6.04±0.0420****	4.413±0.031****
Damiana	197.5 ± 25.53*	46.75±6.549****	114.3±2.72****	6.198±0.0461****	4.905±0.040****
EAC	246.5 ± 9.314	83.75 ± 2.869	184.3± 5.072	5.245 ± 0.0606	3.42 ± 0.089
Pre-treated	136.5±4.787****	54.5 ± 3.617***	132.8±3.568****	5.245±0.0606^{NS}	4.213±0.110****
Co-treated	167.3±2.056**	67.75 ± 2.537*	166.8± 4.589*	5.625 ± 0.126**	3.9 ± 0.070**

The significance of difference was analyzed by one – way ANOVA (compare all vs. EAC group) using computer program. Values are expressed as means ± SEM. one – way ANOVA was significant at P < 0.05. where, G1, control group; G2, Damiana group; G3, EAC group; G4, Pre-treated group; G5, Co-treated groups

However, electrolytes serum were significantly ($p<0.05$) improvement in pre-treated (G4) and in co-treated (G5) groups when compared to the EAC group. Cholesterol, triglycerides and LDL levels were significantly ($p<0.05$) increased in EAC (G3) as compared with control group and damiana group. While were significantly ($p<0.05$) decreased in pre-treated mice (G4) and in co-treated (G5) groups when compared to the EAC (Table 4).

4. DISCUSSION

The present study aimed to distinguish the hepatic ameliorative role of damiana against Ehrlich ascites carcinoma induced liver toxicity in female mice. Our results revealed that; EAC induced elevations in ALT, AST and ALP, and depletions in albumin and total proteins. These results are in harmony with the results of Haldar et al. [23]. The elevation of liver enzymes is an index of deterioration hepatic functions due to cancer proliferation as observed in the EAC group. damiana had hepatic ameliorative effects against EAC, which was confirmed by depletion of serum AST, ALT and ALP, and elevations of albumin and total protein levels. These findings confirmed those of Bezerra et al. [24] and Marques et al. [25] who reported that damiana improved liver

Table 2. Changes in serum kidney functions (urea and creatinine) in all the studied groups

Groups	Parameters	
	Creatinine (mg/dL)	Urea (mg/dL)
Control	0.55 ± 0.0178****	27.13 ± 0.966****
Damiana	0.49 ± 0.0274****	24.88 ± 1.76****
EAC	0.895 ± 0.0665	54.13 ± 3.003
Pre-treated	0.545 ± 0.0126****	32.25 ± 1.797****
Co-treated	0.7 ± 0.0372**	43.63 ± 1.375**

The significance of difference was analyzed by one – way ANOVA (compare all vs. EAC group) using computer program. Values are expressed as means ± SEM. one – way ANOVA was significant at P < 0.05. where, G1, control group; G2, Damiana group; G3, EAC group; G4, Pre-treated group; G5, Co-treated groups

Table 3. Changes in serum electrolytes [chloride ions (Cl-), calcium ions (Ca2+), potassium ions (K+), and sodium ions (Na+)] in all the studied groups

Groups	Parameters			
	Na^+ mmol/l	K^+ mmol/l	Ca^{++} mmol/l	Cl^- mmol/l
Control	136.5±0.533****	4.313 ± 0.033****	1.118±0.003****	100.9± 0.8495****
Damiana	135.3±0.323****	4.375 ± 0.043****	1.212±0.006****	100.1±0.512****
EAC	120 ±1.646	5.718 ± 0.127	0.912 ± 0.0126	116.1 ± 1.405
Pre-treated	134.4±1.026****	4.943±0.083****	1.079± 0.029****	103.2 ± 0.704****
Co-treated	129.5±0.979****	5.235±0.085**	1.012 ± 0.014**	108.1 ± 1.212***

The significance of difference was analyzed by one – way ANOVA (compare all vs. EAC group) using computer program. Values are expressed as means ± SEM. one – way ANOVA was significant at P < 0.05. where, G1, control group; G2, Damiana group; G3, EAC group; G4, Pre-treated group; G5, Co-treated groups

Table 4. Changes in serum lipid profiles (cholesterol, Triglyceride, HDL and LDL) in all the studied groups

Groups	Parameters			
	Chlost. mg/dL	Tg mg/dL	HDL mg/dl	LDL mg/dl
Control	102.3±2.333****	175.3± 3.844****	36.97±1.633**	30.3 ± 1.212****
Damiana	127 ± 4.163****	228.3±5.364****	38.17±1.59***	43.17 ±2.168****
EAC	237.3 ± 7.126	406 ± 16.04	26.83 ±0.601	129.3 ± 3.879
Pre-treated	130 ± 2.082****	247 ± 9.165****	35.00 ± 0.764**	45.6 ± 3.57****
Co-treated	122 ± 1.528****	202.3 ± 3.18****	38.00 ± 1***	43.43 ± 1.09****

The significance of difference was analyzed by one – way ANOVA (compare all vs. EAC group) using computer program. Values are expressed as means ± SEM. one – way ANOVA was significant at P < 0.05. where, G1, control group; G2, Damiana group; G3, EAC group; G4, Pre-treated group; G5, Co-treated groups

enzymes, total protein and albumin levels in mice.

Ehrlich tumor causes alterations in the biochemical parameters electrolytes sodium (Na+) and potassium (K+) ions in the mice according with Abd Eldaim et al. [20] who reported that Ehrlich tumor has led to significant changes in the renal function in mice through an increase in the levels of potassium, and chloride ions and decreased sodium ions.

Also, these results appear that the development of EAC was accompanied by changes biochemical parameters observed in EAC untreated mice group according to Habib et al. [26] and Khanam et al. [27]. However, the presence of damiana in treated groups leads to ameliorative the levels of potassium ions, and sodium ions in the mice, our results correspond with Guo et al. [28]. Current results showed that; chlosterol, triglycerides and HDl were significantly increase in EAC as compared to control, this results agree with Alotaibi et al. [29] who reported that; EAC induced elevation in lipid profiles.

5. CONCLUSION

Damiana has an enhanced role against EAC-induced changes in liver and kidney functions, electrolytes and lipid profiles in female mice. Hence, these results suggest that damiana could be a reliable treatment for Ehrlich's ascites cancer.

CONSET

It is not applicable.

ETHICAL APPROVAL

The experiments were conducted according to guidelines issued by the Ethical Committee of Faculty of Science at Tanta University and subject to approval by the Institutional Animal Care and Use Committee (IACUC-SCI-TU-0156).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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