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Role of Salvia hispanica seeds extract on Ehrlich ascites model induced liver damage in female mice.

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Abstract:

Because the Ehrlich ascites carcinoma (EAC) model can accurately predict survival time and is successful in creating neoplastic cells, it is frequently employed in experimental cancer studies. The present work is designed to investigate the antitumoral properties and therapeutic potential of Chia (Salvia hispanica) seed extract (ChSE) in inhibition of EAC-induced liver toxicity and tissue injury. All of the Forty female Swiss albino mice were equally separated into four cohorts (Gp1, control group; Gp2, CHSE group; Gp3, EAC group; Gp4, EAC+ChSE). Current results revealed that; EAC caused a notable increase in serum Aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) activities, body weight, liver weight, liver injury, and a significant depletion in albumin and total proteins as in contrast to the control Gp. Post-treatment of EAC with CHSE (EAC+ChSE) induced a significant depletion in serum ALT, AST, ALP, body weight, liver weight, liver injury, and a notable rise in albumin and total proteins as compared to EAC. One may conclude that ChSE possesses the capacity for a liver protective effect against EAC cell-induced liver injury and signposts about the potential benefits of ChSE in the treatment of liver toxicity.

Keywords: Ehrlich Ascites Carcinoma; Salvia hispanica; liver functions and structure; mice.

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Introduction

Cancer is an aberrant cell proliferation brought on by uncontrolled cell proliferation that can travel through the circulation to other parts of the body **(1).** The buildup of aberrant cells that result from cell growth and harm all organs is known as cancer **(2,3).** One of the most prevalent forms of cancer is breast cancer, which affects 99% of women and 1% of men, The term "cancer" refers to aberrant cell growth that exhibits a wide range of associated symptoms **(4,5).** Globally, cancer is a major public health issue. Every year, more than eleven million individuals are diagnosed with cancer **(6,7,1).** According to the Global Cancer Statistics 2020 study, there were 10.0 million fatalities from cancer and 19.3 million cases of cancer newly discovered patients. Approximately 10.0 million in 2020 saw many deaths from cancer, making it one of the leading causes of mortality. globally **(8)**.

Novel medications are being developed from plant secondary metabolites as an alternative to current anticancer treatments; these molecules frequently show promise in terms of reduced toxicity and/or increased efficacy over conventional chemotherapy treatments **(9,10).** These characteristics have increased our curiosity about finding anticancer drugs that come from natural sources. As previously mentioned, there is evidence that certain dietary factors, such as high fructose content and inadequate consumption of omega-3 fatty acids, lack of physical exercise, insulin resistance, excess visceral abdominal fat, and hereditary vulnerability, are significant risk factors for hepatic diseases **(11,12,6).** The highest plant source of fiber, antioxidants, and omega-3 fatty acids is chia seed **(13,14,3)**. Chia, or Salvia hispanica, is an annual herb that reaches a height of 1.75 metres. Thus, the current work is intended to look into the ameliorating potential of Chia (Salvia hispanica) seeds extract (ChSE) in inhibition of EAC-induced liver toxicity and tissue damage. Epidemics are life-threatening and pose a great risk of spreading globally**(15,1).** A transplantable tumor model called Ehrlich Ascites (EAC) has made it simple to investigate the anti-cancer properties of several chemical substances, carcinoma has a shorter life period, is fully malignant, is hyperdiploid at first, is highly transplantable, never regresses, and proliferates swiftly **(16).**

MATERIALS AND METHODS

Transplantation Ehrlich Ascites Carcinoma cells in mice

The National Cancer Institute of Egypt (NCI; Cairo University, Egypt) provided the EAC-bearing mice for purchase. EAC cells that were seven days old when extracted and kept floating in isotonic sterile saline. Two and a half million viable EAC CELLS were implanted intraperitoneally in every rodent to cause EAC **(17,16).**

Animals

40 female Swiss albino mice (weights 20 - 25g) were obtained from EVC Animal House Colony. the critters were randomized and housed in a room–temperature environment of 22 – 25 0^C and relative sticky circumstances of a 12-hour light/12-hour dark cycle, a business food, and water for around fourteen days.

Experimental design and Animal Groups

mice were split into four equal groups. (Gp1 – Gp4):

Gp1: Control. There was no treatment given to the mice.

Gp2: ChSE (Chia seeds extract); The mice were given ChSE verbally (200mg/ kg/bw /day) through a stomach tube for 14 days **(18).**

Gp3: EAC: Mice were inoculated once intraperitoneal with Ehrlich cells with about 2,500,000 EAC/mouse **(19,17,16).**

Gp4: EAC+ChSE; mice were inoculated once intraperitoneal with Ehrlich with about 2,500,000 EAC/mouse and at 2^{nd} days treated with ChSE (200mg/kg bw/day) orally for 14 days.

Blood and tissue Sampling

At the end of the experiments, all animals were given an intraperitoneal dose of sodium pentobarbital to induce anesthesia (≥ 100 mg/kg). EAC fluid cells were isolated from the mice's peritoneal cavity and whole blood specimens were collected and then centrifuged at 4000 g for 8 min. Serums were separated and stored at– 20^oC up to a biochemical analysis. For histological analysis, kidney samples

were extracted and stored in 10% neutral buffer formalin after being cleaned in cold saline.

Cytological of Ehrlich ascites carcinoma cells

Following the anesthesia of the mice, the peritoneal cavity was cautiously opened, all ascites fluid was collected, and any smeared cells were checked for. After the group animals were infected with EAC cells extracted by centrifugation from the ascitic fluid, the smears were put on glass slides, let to dry naturally, and then fixed in 70% ethanol before being stained with 0.1% Giemsa solution and analyzed under an electric microscope for cytological investigations.

Liver enzymes and functions

Alanine transaminase (ALT), and serum aspartate transaminase (AST) activities were assessed based on Reitman and Frankel (1957) and alkaline phosphate (ALP) was assessed according to **(20).** Albumin and total protein levels were measured in serum according to **(15,21,22,23).**

Histological investigation

The liver lobe 10% neutral buffer was used to correct each mouse formalin restorative and processed about paraffin was sectioning, and a portion of it had stains from eosin and hematoxylin for histopathological examination according to **(24,10,25,26,27).**

Statistical Analysis

To determine if there were any statistically significant differences between the treatment groups, the data were reported as mean values ± SE and subjected to an unpaired t-test statistical analysis. The statistical significance criterion of $p<0.05$ was selected for the biochemical data. All statistical analyses were performed using the SPSS statistical version 21 software package (SPSS® Inc., USA).

Results:

Effects of EAC and/or ChSE on mice body and liver weights

Table 1 shows the alterations in the weights of the bodies and livers of the various experimental groups. When compared to the control group, the body and liver weights of mice injected with EAC were considerably higher. However, handling of EAC With ChSE revealed a significant decrease in mice Weights of the body and liver compared to EAC-bearing mice.

Effect of ChSE on cytological examinations of EAC

The EAC group's ascites fluid cells revealed increased peritoneal fluid volume, a high quantity of mitotic cells, and many tumor cells that have nuclear enlargement; Moreover, there was architectural disarray in the cells, a notable level of anisocytosis, pleomorphism, and cellular anaplasia, as well as Hyperchromasia and nuclear vascularity (Figure 1B). In contrast to the EAC Gp, the post-treatment EAC with ChSE displayed a lower rate of mortality, mitotic cells, and cellular alterations in addition to a higher quantity of apoptotic cells (Figure 1C).

Effect of ChSE on liver functions

Table (2) showed that; EAC induced a notable increase in the amounts of ALT, AST, ALP, and GGT and a significant depletion in albumin and total proteins when compared to the control group. Contrariwise, treatments of EAC with CHSE (EAC+ChSE) induced a significant depletion in ALT, AST, ALP, and GGT and a significant elevation in albumin and total proteins when compared to EAC (Table 2).

Liver histopathology

Figure 1 and Table 3 show the alterations in liver tissues' morphometry and histology in different groups. The hepatocytes in the control and ChSE-treated mice liver section revealed a normal hepatocyte framework, with polygonal liver cells featuring large round nuclei and cytoplasm that is eosinophilic, and sparsely between the hepatic cords are separated hepatic sinusoids with finely arranged Kupffer cells (Figure 1A&1B). Liver sections in mice inoculated with Ehrlich cells (EAC Gp) exhibited severely injured hepatocytes, as marked degeneration, marked focal necrosis, marked cellular infiltration, and congested blood sinusoids were also observed (Figure 1C). In contrast, liver sections in treated EAC with ChSE revealed a small level of hepatocyte improvement with slightly degenerated hepatocytes, mild vacuolated hepatocytes, and mild focal necrosis (Figure 1D).

Table 1: Effects of EAC and/or CHSE on body and liver weights.

	Control	ChSE	EAC	EAC+ChSE
Body weight (gm)	$21.95^{\#} \pm 1.60$	$22.40^{\#} \pm 1.35$	$32.15^{*} \pm 1.88$	$27.05^{\#*} \pm 1.39$
Liver weight(gm)	$1.21^{\#} \pm 0.08$	$1.14^{\#} \pm 0.04$	$1.88^{*} \pm 0.09$	$1.54^{\#*} \pm 0.08$

Ten observations are presented as mean ± S.E. When compared to the control and EAC groups, respectively, (*) and (#) are significant at 0.05.

Table 2: Changes in serum liver enzymes (ALT, AST, ALP, Albumin, total protein) levels in experimental groups.

	ALT (U/L)	AST (U/L)	ALP (U/L)	Albumin (gm/dL)	TP (gm/dL)
Control	$33.6^{\#} \pm 2.19$	$98.0^{\#} \pm 5.13$	$109.5^{\#} \pm 7.05$	$4.12^{\#} \pm 0.33$	$7.01^{\#} \pm 0.49$
ChSE	$31.0^{\#} \pm 2.01$	$73.7^{\#} \pm 3.85$	$96.0^{\#} \pm 6.29$	$4.22^{\#} \pm 0.35$	$6.85^{\#} \pm 0.45$
EAC	$63.2^{*} \pm 3.75$	$180.5^{*} \pm 7.48$	$170.2^{*} \pm 7.88$	$3.29^{*} \pm 0.27$	$5.30^{*} \pm 0.35$
EAC+ChSE	$41.0^{\#} \pm 2.36$	$122.5^{\#*} \pm 6.20$	$117.9^{\#*} \pm 8.45$	$3.90^{\#*} \pm 0.25$	$6.26^{\#*} \pm 0.41$

We provide the data as mean ± S.E. of 10 observations. (*) & (#) significant 0.05 concerning the EAC group and the control group, respectively.

Table 3: Effect of EAC and/or ChSE on liver tissue structure of mice.

Histological changes	Control	ChSE	EAC	EAC+ChSE
Atrophy	-	-	+	-
degenerated hepatocytes	-	-	+++	+
Focal necrosis	-	-	+++	+
Cellular infiltration	-	-	+++	+
Vacuolation	-	-	+	-

The values (n = 6) are presented as mean ± SE.
Grade: Marked or severe (+++); Slight or moderate (+); Negative or normal (-); Moderate (++).

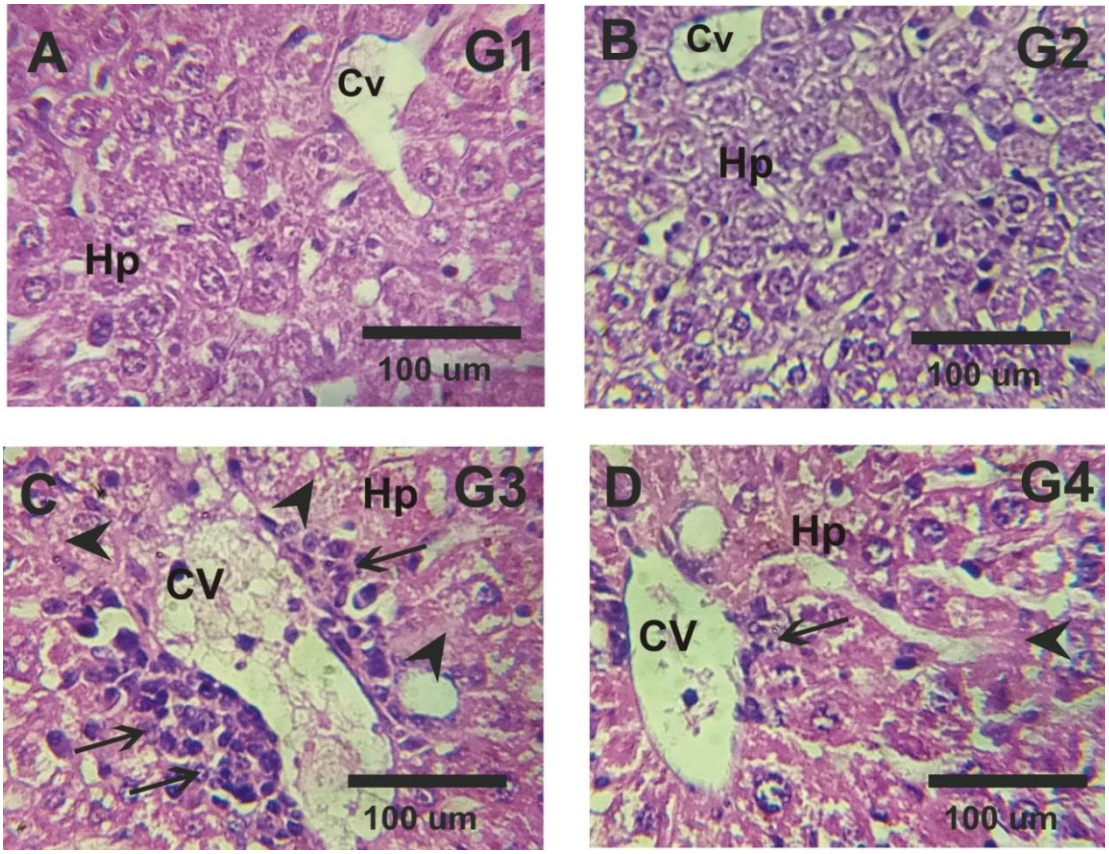

Figure 1: Liver sections photomicrographs in all groups stained with Hematoxylin & Eosin. A&B: Normal structure of hepatocytes (Hp) and central (CV) and portal veins (PV) in the liver in control and ChSE groups. C: Liver sections in EAC-bearing mice showed marked degeneration, marked inflammatory cells (arrows), and marked diffuse necrosis of hepatic tissue (arrowheads). D: Liver sections in treated EAC with ChSE exhibited a moderate degree of improvement with slightly degenerated hepatocytes, mild inflammatory cells (arrows), and mild focal necrosis.

Discussion

The current study is designed to investigate the antineoplastic activity and curative role of Chia seed extract (ChSE) in inhibition of EAC-induced liver toxicity. Since ascetic fluid satisfies the nutritional needs of carcinoma cells, it is the direct source of sustenance for the growth of carcinomas. Current cytological analyses of ascites fluid smears showed an increase in ascites fluid volume, cellular alterations, an abundance of EAC cells, contains a significant quantity of mitotic cells; however, ascites fluid smears in EAC+ChSE showed an abundance of apoptotic bodies and a low number of EAC cells. These results were consistent with those of **(28,23).** who observed that EAC-bearing mice had significantly higher ascitic fluid volume, viable cancer cell count, final body weight, and belly circumference. Our results concur with that of **(29).** who reported that: salba – chia (Salvia hispanica L.) in the management of patients with diabetes type 2 who are overweight or obese. Our results are consistent with that of **(30).** who reported that: in mice given a high-fat diet, the digested chia seed protein reduces obesity and the corresponding inflammation of adipose tissue. our results do not agree with **(31).** who reported that: Chia seeds don't help overweight adults lose weight or change their risk factors for disease.

The current study indicated that: EAC induced hepatic dysfunction represented by an increase in the activities of serum ALT, AST, and ALP and a decrease in serum levels of total protein and

albumin. The finding of this study indicated that: EAC hepatic dysfunction. Our findings agree with that of **(32).** and Tousson et al. (2020) indicated that: EAC elevated serum ALT, AST, and ALP activities. Also, treatments of EAC with ChSE induced a decrease in the activities of serum ALT, AST, and ALP increase in serum levels of total protein and albumin as compared to control. Our results matched that of **(33,11,34).** who reported that; ChSE induced improvements in liver functions.

The reality is that histological Hepatotoxicity was found in the liver tissues of EAC-bearing mice, and this was connected with **(17,19).** whose research implies that these elevations in liver enzyme activity might be the consequence of liver tissue damage. Their findings also show that EAC produced liver damage and the death of hepatocytes, which released the plasma to release their enzymes. elevated serum concentrations of ALT, AST, and ALP may demonstrate how these compounds can stabilize hepatic cellular membrane damage and shield hepatocytes against membrane fragility, potentially lowering the amount of enzyme leakage into the bloodstream. These results concurred with those of **(35,26,19).** who reported that: EAC induced liver injury in female mice.

However, the treatments of EAC with ChSE showed improved liver structure and functions. These results were in line with the findings of **(36).** who found that; In an experimental metabolic syndrome model, chia seeds reduce endothelial dysfunction and liver inflammation. These outcomes were consistent with those of **(37).** who find that; chia Rats given a high-fat, high-fructose diet show improvements in their liver's metabolic abnormalities when they are fed wheat or oil.

Conclusion:

EAC induced liver dysfunction and tissue damage and ChSE can enhance liver structure and functioning. We can conclude that: ChSE might be applied as a supplemental treatment for ascites in EAC, Hepatitis, and Schistosoma.

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Compliance with ethical standards.

Conflict of interest: Each author stated that they had no competing interests with the manuscript's publication.

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