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Impact of Chia (*Salvia hispanica*) Seeds Extract on Ehrlich Ascites Model Induced Kidney Toxicity in Female Mice

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ABSTRACT

Background: Ehrlich ascites carcinoma (EAC) is an undifferentiated carcinoma of the breast that was initially hyperdiploid, 100% malignantand had a short life span. It also had a high transplantability rate, no recurrence and rapid dissemination.

Methods: Therefore, our study designed to assess the potential ameliorative ability of Chia (*Salvia hispanica*) seeds extract (CSE) on Ehrlich ascites carcinoma (EAC) induced kidney toxicity and tissue damage in mice. Forty female Swiss albino mice in all were split equally into four teams (Gp1, control; Gp2, CSE; Gp3, EAC; Gp4, EAC+CSE).

Result: Current results revealed that; EAC induced elevated in the levels of serum urea, creatinine, potassium ions, chloride ions, renal tissue injury and a significant depletion in serum sodium ions, calcium ions as compared to normal control. In addition to EAC induced variable pathological changes in glomeruli and renal tubules as a marked renal damage, marked degeneration in renal tissues, moderate glomerular atrophyand marked cellular infiltration. Treatment of EAC with CSE (EAC+CSE) improved kidney functions and structure against EAC; where depletion take place in the levels of serum urea, creatinine, potassium ions, chloride ions and elevation in the levels of sodium ions, calcium ions as compared to EAC. One may draw the conclusion that CSE Having the ability to protect the kidneys from EAC cells induced kidney toxicity.

Key words: Ehrlich ascites carcinoma, Kidney functions, Mice, *Salvia hispanica*, Structure.

INTRODUCTION

According to estimates, the number of new instances of cancer will rise starting from 11.5 billion in 2007 to 16.0 billion in 2030 due in part to an ageing and growing worldwide population, making it the following largest the cause of death globally (Siegel *et al.,* 2021). Diseases like cancer are cell growth brought on by aberrant cell proliferation and it can spread to other bodily areas through the bloodstream (Bray *et al.,* 2018; Nofal *et al.,* 2023). Approximately 10 cancer was a leading cause of mortality in 2020, claiming the lives of one million individuals globally (Ferlay *et al.,* 2019). The treatment of cancer is difficult despite advances in modern medicine since treatments like surgery, radiation and/or systemic therapy get a variety of severe side effects and a low efficacy (Tousson *et al.,* 2014, 2016).

Numerous Animals used in experiments are known as *in-vivo* experimental models, including the Ehrlich tumour (Elgharabawy *et al.,* 2021; Abd Eldaim *et al.,* 2021a; Oshiba *et al.,* 2021). Ehrlich tumour is aggressive and quickly growing carcinoma (Tousson *et al.,* 2022). Ehrlich tumour can develop in either the solid and identified as Ehrlich solid tumor (EST) or ascetic form and identified as Ehrlich ascites carcinoma (EAC) depending on how it is inoculated subcutaneously or intraperitoneally, respectively (Aldubayan *et al.,* 2019; Abd Eldaim *et al.,* 2019, 2021b; El-Masry *et al.,* 2019, 2020).

EAC is cancer that has not yet undergone differentiation (Hasan *et al.,* 2023) with a hyperdiploid origin, a high transplantability, no regression, a short lifespan, quick proliferation, 100% malignancyand no tumor-specific transplantation antigen Not to mention, it is (Alotaibi *et al.,* 2021; Tousson *et al.,* 2020). Given that individuals with advanced cancer frequently have ascites with intraperitoneal spread, which indicates a bad prognosis, these characteristics of EAC cause it to mimic human cancers (Mutar *et al.,* 2020; Abd Eldaim *et al.,* 2021b).

Inside the process of discovering and developing novel medications, particularly for anticancer agents, natural compounds have become a standard (Mohamed *et al.,* 2021, 2023; Essawy *et al.,* 2022).

Chia (*Salvia hispanica*) native to sections of Australia, the southwest of the United States, northwestern Argentina, Bolivia, Ecuador, Colombia, Nicaraguaand Mexico and Guatemala, is an annual herb that can reach a height of 1.75 meters. It is cultivated for its seed, which is a food source of omega-3 fatty acids (*Muńoz et al.,* 2012, 2013; Knez Hrnčič *et al.,* 2019). Chia seeds contain a high percentage of fatty acids omega-3, as well as essential

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amino acids and polyphenols and they have become important and popular in the food industry as they are nutritional supplements where it contains about protein (15-25), fats (15-35) and dietary fiber (18-35) percentage (Kulczyński *et al.,* 2019; Munir *et al.,* 2021). In addition to Chia seeds contain α-linolenic acid and because the body does not make this acid, these seeds have an essential role in the diet (Ciau-Solís *et al.,* 2014; Grancieri *et al.,* 2019; Salgado *et al.,* 2022). The polyphenols and antioxidants found in these seeds are second to none. They contain caffeic acid, rosmarinic acid, myricetin and qu (de Falco *et al.,* 2017; Al-Attar *et al.,* 2023). It can helps in preventing heart disease, nervous system disorders and diabetics. These seeds have properties for preventing heart disease, blood pressureand diabetes (Motyka *et al.,* 2023). Consequently, the purpose of this study is to examine the possibility of Chia (*Salvia hispanica*) seeds extract (CSE) in inhibition of EAC induced kidney toxicity and tissue damage.

MATERIALS AND METHODS

Transplantation Ehrlich ascites carcinoma cells in mice

Ehrlich Ascites Carcinoma (EAC) rodents that carried the disease were acquired from the National Cancer Institute of Egypt (NCI; Cairo University, Egypt). Ehrlich Ascites Carcinoma (EAC) cells, suspended in sterile isotonic saline, seven days after collection. Two and halve millions viable EAC cells were implanted intrathoracicly in every animal in order to trigger EAC.

Animals

40 female Swiss albino mice (weights 20-25 g) were obtained from EVC animal house colony. The animals were randomised and housed in a room-temperature environment of 22-25°C and relative sticky circumstances of a 12-hour light/12-hour dark cycle, a business food and water for around fourteen days , The present study was carried out at Department of Environmental Studies, Institute of Graduate Studies and Research, Alexandria University, Alexandria, Egypt.

Experimental design and animal groups

Four groups of mice were randomly assigned (Gp1-Gp4):
Gp1: Control; The mice were not treated with any medication.
Gp2:CSE (Chia seeds extract); the mice received CSE orally (200 mg/kg bw/day) intramuscularly orally or intramuscularly or both routes please explain over a period of fourteen days (Munir *et al.,* 2021).
Gp3: EAC; Mice were injected intraperitoneally once with Ehrlich cells with approximately 2,500,000 EAC/mouse (Mutar *et al.,* 2020).
Gp4: EAC+CSE; mice were inoculated once intraperitoneal with Ehrlich cells with approximately 2,500,000 EAC/mouse and at 2nd days treated with CSE (200 mg/kg bw/day) orally for 14 day, This study began in July 2020 and the practical part was completed in April 2022, The theoretical part and writing of the research beganand the research was finally completed in January 2024.

Blood and tissue sampling

Each animal was administered an intraperitoneal dose of anesthetic at the conclusion of the test of sodium pentobarbital (≥100 mg/kg). EAC fluid cells were isolated from the mice peritoneal cavity and whole blood samples were collected then centrifuged at 4000 g for 8 min. The serum was extracted and preserved at -20°C until biochemical analysis. with order to conduct histological examinations, kidney samples were extracted, rinsed with cold saline and then fixed with 10% neutral buffer formalin.

Serum kidney functions and electrolytes biomarkers

Results for serum creatinine and urea were found in the mouse sera according to Patton and Crouch (1977). The approach proposed by (Hasan *et al.,* 2022, EZZ *et al.,* 2023; Hameed *et al.,* 2023). was used to assess serum electrolyte levels (Potassium, sodium, calcium and chloride ions) using pre-made sets (Sensa core electrolyte, India).

Histological investigation

Kidney of each mouse was fixed to a formalin fixative in a neutral bufferat 10% and processed for the purpose of paraffin wax sectioningand a portion of it had stains from eosin and haematoxylin Must undergo histopathological analysis in accordance with to (Tousson *et al.,* 2016, Hasan *et al.,* 2021; Alankooshi *et al.,* 2023

Ethical approval

The housing conditions were randomly selected and included ambient temperature (22 to 25°C), relative humidity, a 12-hour light/ 12-hour dark cycleand a two-week supply of commercial food and water that was always accessible. Our Institutional Animal Care and Use Committee has given it the approval number IACUC-SCI-TU-0233.

Statistical analysis

The data was shown as the average value plus or minus the standard errorand a t-test was used for statistical analysis to see if there were any significant differences between the treatment groups. The biochemical results were deemed statistically significant in cases when the p-value was less than 0.05. The statistical software tool SPSS, version 21, was used for all statistical analyses (SPSS® Inc., USA).

RESULTS AND DISCUSSION

Effects of EAC and/or CSE on mice body and kidney weights

Table (1) showed the changes in experimental groups of mice with varying amounts of fat around their kidneys and bodies. The weights of the mice's bodies and kidneys were considerably grew following EAC injection as in contrast to the control group. Nevertheless, therapy of EAC with CSE

Table 1: Effects of EAC and/or CSE on mice body and kidney weights.

	Control	CSE	EAC	EAC+CSE
Body weight (gm)	23.55$^{\#}$±1.37	23.26$^{\#}$±1.05	32.72*±1.60	26.11$^{\#*}$±1.39
Kidney weight (gm)	0.33±0.04	0.33±0.03	0.34±0.03	0.34±0.02

Data are expressed as mean ± S.E of 10 observations. (*) and (#) significant 0.05 compared to control and to EAC groups respectively.

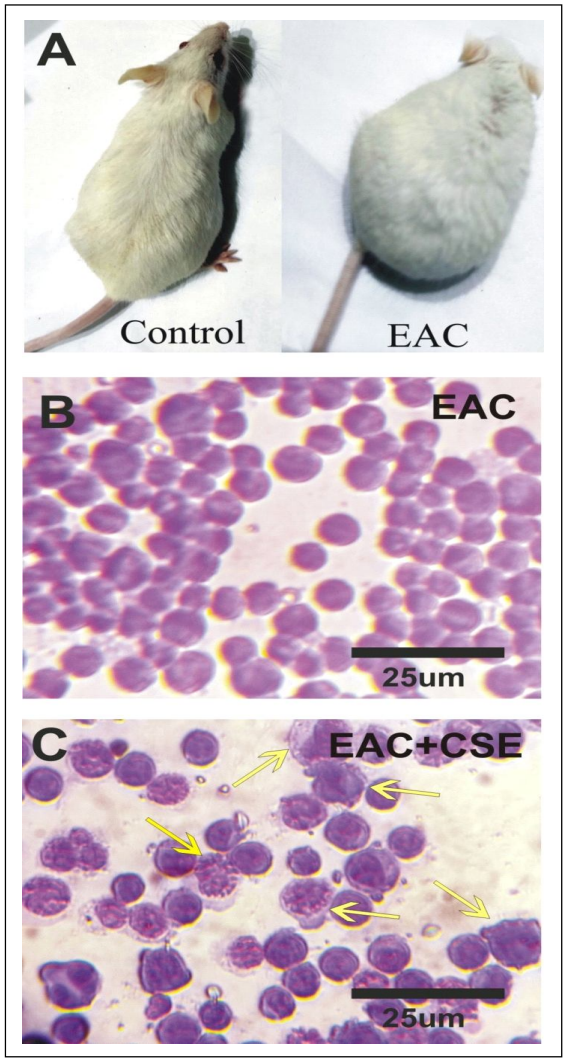


Fig 1: A: Control and EAC bearing mice. **B and C:** Ascites fluid smear stained with Giemsa stain in EAC and in EAC+CSE groups. B: Ehrlich ascites cells cellular anaplasia, mitosis, nuclear expansionand many tumor cells are seen in the EAC group's smear. C: Ascites fluid in EAC+CSE showed reduction at mitotic cell density and increased in apoptotic cells.

revealed a significant decrease in mice body and kidneys weights as in comparison to to EAC bearing mice.

Cytological examinations of EAC

Fluid with ascites in EAC mice group revealed the presence of many mitochondrial cells (Fig 1A), a profusion of tumor cells with nuclear enlargement; pleomorphism, anisocytosis, cellular anaplasiaand nuclear vascularity and hyperchromasia (Fig 1B). However, ascites fluid in EAC+CSE showed reduction in the numbers of mitotic cells and presence of many relative to apoptotic cells, to the EAC group (Fig 1C).

Effect of CSE on kidney functions and electrolytes level

Table (2) showed that; EAC induced a significant elevation in the levels of urea, creatinine, potassium, chloride ions and a significant depletion in sodium and calcium ions when compared to control group. Conversely; treatments of EAC with CSE (EAC+CSE) induced a significant depletion in urea, creatinine, potassium, chloride ions levels and a significant elevation in sodium and calcium ions when compared to EAC (Table 2).

Kidney histopathology

Fig 2 and Table 3 showed the histological and morphometric alterations in the kidney tissues of the control group and every treatment group. The control group's kidney portions showed normal histological structure; Both the renal cortex and the medulla, the two primary components of the kidney, exhibit typical histological characteristics. Each of the several renal corpuscles encases the renal cortex with its own set of glomeruli and Bowman's capsule. To facilitate renal filtration, a typical normal gap is present between the glomeruli and Bowman's capsule. A network of convoluted tubules encircles the renal corpuscles (Fig 2A). Kidney sections in treated mice with CSE revealed normal structure of glomeruli and renal tubules as in control (Fig 2A and 2B). Kidney samples from Ehrlich cell-inoculated mice (EAC group) revealed variable pathological changes in glomeruli and renal tubules as a marked renal damage, marked degeneration in renal tissues, moderate The malpighian corpuscles, which had lost their distinctive shape due to glomerular atrophy, were noticed and marked cellular infiltration (Fig 2C). Kidney sections in treated with EAC with The histological structure of the kidneys showed moderate improvement and organization when compared using CSE to EAC group, where it demonstrated a notable improvement in the glomruli with moderate degeneration in renal tubules atrophy (Fig 2D).

Although chemotherapy treatments are successful against many types of cancer, they are constrained by a variety of negative side effects and problemsand cancer is thought to be the second biggest cause of death globally (Tousson *et al.,* 2018). Ehrlich ascites carcinoma first manifested as a breast cancer that developed on its own, mimicking the symptoms of the more common breast cancer

Table 2: Changes in kidney functions and Electrolytes level in different groups.

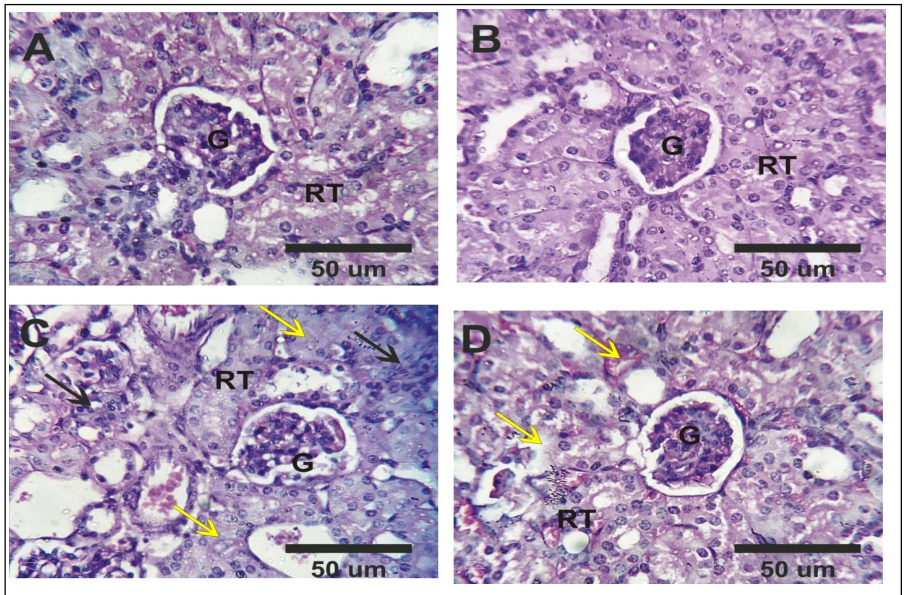
	Control	CSE	EAC	EAC+CSE
Creatinine (mg/dl)	0.64$^{\#}$±0.04	0.55$^{\#}$±0.03	1.06*±0.05	0.71$^{\#}$*0.04
Urea (mg/dl)	34.2$^{\#}$±1.75	32.5$^{\#}$±2.08	50.6*±2.54	34.8$^{\#}$±2.33
Na^{+} (mmol/l)	135.5$^{\#}$±6.2	135.1$^{\#}$±7.5	127.4*±6.0	133.0$^{\#}$±5.9
K^{+} (mmol/l)	3.61$^{\#}$±0.25	3.85$^{\#}$±0.41	4.80*±0.29	4.06$^{\#}$*±0.30
Ca^{++} (mmol/l)	1.24$^{\#}$±0.11	1.25$^{\#}$±0.09	0.91*±0.05	1.08$^{\#}$*±0.13
Cl^{-} (mmol/l)	101.5$^{\#}$±8.0	100.5$^{\#}$±7.3	114.0*±9.8	107.1$^{\#}$*±6.4

Data are expressed as mean ± S.E of 10 observations. (*) and ($^{\#}$) significant 0.05 compared to control and to EAC groups respectively.

Table 3: Effect of Ehrlish ascites carcinoma (EAC) and/or 9-Diaminoacridine (9-DAAD) on kidney tissues structure of mice.

Histological changes	Control	EAC+CSE	EAC	CSE
Glomerular atrophy	-	-	++	+
Degeneration in renal tubules	-	-	+++	+
Focal necrosis	-	-	+++	-
Interstitial infiltrations	-	-	++	+
Vacuolation	-	-	++	+
Apoptosis	+	+	++	+

The mean is used to express the values ± SE (n = 4). Results: normal or negative (-); Slight or mild (+); Moderate (++); Marked or severe (+++).



A and B: Normal histological features in the glomruli (G) and tubules (RT) in kidney sections of control and CSE groups'. C: Marked degeneration (Black arrows) in renal tissues, moderate glomerular atrophy (G) and marked cellular infiltration (yellow arrows) in EAC. D: Mild glomruli atrophy (G) with mild degeneration in the renal tubules (yellow arrows) in EAC+CSE.

Fig 2: Hematoxylin and Eosin-stained photomicrographs of kidney sections from the various experimental groups.

(Alotaibi *et al.,* 2021). There is evidence that when EAC cells invade internal organs, it causes inflammatory cell aggregations and mitochondrial degradation (Abd El-Wahab and Fouda, 2009; Hasan *et al.,* 2023). Ehrlich ascites carcinoma is comparable to tumors found in humans in that it lacks differentiation, grows quicklyand responds well to treatment (Kabel *et al.* 2013). Ascetic fluid is the direct nutritional source for carcinoma advancement as it satisfies the dietary needs of cancer cells. Current cytological examinations in ascites fluid Tests showed that the ascites fluid volume was rising, cellular changes, large number of EAC cells and mitotic cells number, but ascites fluid smears in EAC+CSE revealed many of apoptotic bodiesand small number of EAC cells. These results agreed with those of

Hashem *et al.* (2020) who reported that; the ascitic fluid volume, viable tumour cell count, ultimate body weight and belly circumference were all noticeably increased in EAC-bearing mice. These findings were in line with that of Brissette *et al.* (2013) They found that those who were overweight or obese and had type 2 diabetes mellitus lost weight after eating seeds from the *Salvia hispanica* L. plant.

The present investigation indicated that; EAC induced renal dysfunction represented by the elevation in the levels of urea, creatinine, potassium ions (K^+), chloride ions (Cl^-) and by the depletion in the levels of sodium ions (Na^+) and calcium ions (Ca^{++}), this variation in kidney functions and electrolytes levels could be associated with damage to renal tissue caused by by EAC. These results corroborated those of Abd Eldaim *et al.* (2021b) and Hasan *et al.* (2023) who reported that; increased in the levels of creatinine, urea and potassium after EAC induction in mice. Also; current results were consistent with that of Hashem *et al.* (2020) and Saleh *et al.* (2022) who reported that; EAC induced renal damage after elevation in kidney functions. Current results revealed that; treatments of EAC with CSE (EAC+CSE) induced improvements in tested renal functions and electrolytes levels as compared with EAC. These results were consistent with that of Mohamed *et al.* (2023) who reported that; black chia (Salvia hispanica) seeds improved kidney functions on Nå-CML development in rats administered streptozotocin.

Kidney in microscopic structure is an apparatus like tubular gland that consist of intrarenal efferent urinary tract and nephron. Current histopathological The results showed that EAC caused kidney damage, which manifested as significant glomeruli and tubular cell atrophy and tubular cell degeneration. Symptoms include kidney damage and abnormalities in the urine tubules and glomeruli in kidney sections based on the presence of renal damage in EAC bearing mice group. These results concurred with those of Abd Eldaim *et al.* (2021b) and Hasan *et al.* (2023) who reported that; EAC induced kidney injury on urinary tract and nephron in female mice. These results corroborated those of earlier research conducted by Medhat *et al.* (2017) who demonstrated that; The production of reactive oxygen species (ROS) by EAC may be the cause of the changes in kidney functions and architecture. ROS are critical in oxidative stress, which disrupts cellular equilibrium and causes tissue damage. However, the treatments of EAC with CSE showed improved glomeruli and renal tubules in kidney structure and glomerular basement membrane thickening and There are several hemodynamic and metabolic variables that can affect the proximal tubule and the enlarged glomeruli that fill the whole capsule.

CONCLUSION

EAC induced renal tissue injury and it elevated the activities of serum urea, creatinine, potassium ions (K^+), chloride ions (Cl^-) and by the reduction in the levels of sodium ions (Na+) and calcium ions (Ca^{++}). However, treatment of EAC with CSE ameliorated the effects of EAC on electrolytes, kidney functions indicating that CSE had a potent renal protective potential.

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Data availability

Data supporting our study results are accessible from the relevant author whenever needed.

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Conflict of interest

The authors declare no conflict of interest.

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