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## Effect of treatment with essential oils on the liver toxicity induced by Ehrlich ascites carcinoma in female mouse model

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### ABSTRACT

**Background:** Essential oils (SO) are organic substances that are extracted from plant parts, which have been thought to have anti-cancer characteristics. **Aim:** To investigate the potential protective effects of SO against liver toxicity induced in EAC-bearing female mice. **Materials and Methods:** Forty female mice were equally divided into four groups (Gps). Gp1 was served as control, Gp2 was treated with SO, Gp3 was inoculated with EAC, and Gp4 were concomitantly treated with EAC + SO. **Results:** Data showed that the treatment of EAC-bearing mice with SO led to an amelioration of aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP) activities. SO treatment also induced increases in the total protein levels as compared to EAC group. There was a significant improvement in the expression of GFAP protein in brain and liver tissues in the EAC/SO treated group as compared to the EAC group. **Conclusion:** The current results indicate to the potential hepatoprotective effects of essential oils.

**Keywords:** EAC, Essential oils, Liver Enzyme, GFAP protein

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## INTRODUCTION

Cancer is a serious health problem with a high impact on the society. Cancer is considered as the second disease that causes death globally since it causes damage to almost tissues and organs of the body (Fisher et al., 2013; Chaudhuri et al., 2018). The rapid growth of abnormal cells and their spread within the body will cause them to attack other organs and tissues in the body, and this malignant tumor causes great harm and is a major cause of death throughout the world (Khan et al., 2010).

The incidence of cancer is constantly increasing despite the great development in the field of diagnosis and treatment of this disease. In addition, using essential oils has a significant impact in preventing and reducing the speed of the spread of the disease (Shynu et al., 2011; Tousson et al., 2014). This study was conducted based on many studies in which plants were

used in protection against cancer and reducing liver illness (Al-Rasheed et al., 2017).

Generally, essential oils (SOs) are a mixed group of volatile compounds consisting of hydrocarbons with advantages that they are used in foods and are used as anti-diabetic and anti-inflammatory supplements (Jing et al., 2014). Specifically, SOs are natural products that are composed of esters and alcohols, and their components extracted from plants, and they are considered anti-cancer (Oliveira et al., 2014). Experimentally, SOs have been used to induce anti-tumor effects in mice with cancer, and orange peel oils are plant extracts found in citrus peels and are considered antioxidants (Attia et al., 2005; Narciso et al., 2021). Therefore, SOs have antioxidant, antifungal and antibacterial properties and are considered an important supplement in many health foods and cosmetic industries (Wolffenbüttel et al., 2018). In addition, orange peels have a wide

range of applications in health (Attia et al., 2005), food formulations and textiles (Lawless et al., 2002). Aromatic SOs have a rich pleasant scent and are used as perfumes and disinfectants (Dosoky et al., 1966; Attia et al., 2005). These oils contain active biological substances and have been used for medicinal and cosmetic purposes in all centuries and in the field of nutrition (Burt et al., 2004). However, one of the empirical components of the orange peel SOs is limonene (Elviña et al., 2005).

In this study, we tested the SOs on the liver dysfunction induced in mice by Ehrlich ascites carcinoma (EAC) mouse model. EAC is aggressive and undifferentiated cells. Among its advantages is its ability to culture and grows rapidly (Jaganathan et al., 2010; Zeferino et al., 2018). Ehrlich's tumor is a mammary cancer that spreads in mice and mimics breast cancer in human (Mishra et al., 2018). This tumor was used for transplantation as one of the models that can grow and spread spontaneously. Ehrlich tumour is considered the most common cancer in experiments created in laboratory animals (Aldubayan et al., 2019).

## MATERIAL AND METHODS

**Essential oils from sweet orange:** The essential oils were isolated from orange peels (250g) by distillation method (Cleavenger). In this method, the plant materials were dissolved in distilled water (1.2 L) and boiled for 3h until the water was evaporated. The produced mixture of the essential oils was kept at 4°C until used according to Khalaf et al. (2021).

**Animals:** Forty adult female mice (*Swiss albino*) weighing 20-25 g and their ages were 9 to 10 weeks in the average. They were randomly housed in cages and kept at room temperature and humidity of 55% ± 5%. Mice were provided daily with water in access for consecutive fourteen days.

**Transplantation of EAC cells:** Ehrlich Ascites Carcinoma (EAC) cells were obtained from Egyptian National Cancer Institute, Cairo University, Egypt. A 7-day-old EAC cells were collected from EAC bearing mice, and then mixed with sterile saline solution,  $2.5 \times 10^6$  cells/20 g body weight were transplanted into all mice. The growth of EAC cells was observed

in all mice that were received these cells on the 7<sup>th</sup> – 14<sup>th</sup> days after imaging the mice according to Mutar et al. (2020).

**Experimental design:** Mice were equally distributed into four equal groups in a randomly. Gp1 included mice with no treatment and used as a control. Gp2 includes mice that were received oral injection of essential oils (25 mg/Kg body weight/day) by stomach tube for 14 consecutive days according to Parmar et al. (2008). Gp3 included mice received intraperitoneal (IP) injection of EAC cells (250,000 EAC/mouse) according to Alankooshi et al. (2023). Gp4 included mice that were IP injected with EAC cells and then treated the next day with daily oral treatments with SO for consecutive 14 days.

**Blood and Tissue samples:** After the end of the experimental period, all mice were anesthetized using a sedative sodium pentobarbital ( $\geq 100$  mg/kg). They were then dissected, and the EAC cells were isolated from the peritoneal cavity. Blood samples were withdrawn through the inferior *vena cava* into tubes containing heparin and mixed well to avoid clots formation in the samples. Thereafter, the sera were separated by a centrifugation at 3000 g for 20 min then kept in clean stopper vials at -20°C until biochemical analysis. Moreover, the liver and brain tissues were dissected out, cleaned with saline solution, and preserved in plastic tubes containing 10% formalin fixative to conduct immunohistochemical preparation and examination.

**Biochemical Assay:** The activity of aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) and serum total protein concentration has been identified by spectrophotometry using commercial kits (Hasan et al. 2021).

**Immunohistochemical investigation:** The liver and brain tissues of all groups were excised, fixed in 10% formalin for a period between (24-48 hours), dehydrated in ascending alcohol concentrations, paraffin wax infiltrated and then were cut at 5 m sections to be mounted on glass slides. Then, the slide tissue sections were deparaffinized and hydrated again then stained with hematoxylin and eosin (H&E) for

histological examination by light microscopy according to Hameed et al. (2022) and Fatoh et al. (2023).

### Statistical analysis

The Statistical Package for the Social Sciences (SPSS software version 16) was used to analyze the findings. The data were displayed as mean  $\pm$  standard error of mean (SEM) and subjected to one-way analysis of variance (ANOVA) and Dunnett test statistical analysis. Comparisons using the Dunnett test were used to determine how significant the differences between the groups were. To compare the significant difference between groups, an unpaired T-test was used.  $P < 0.05$  was established as the threshold for statistical significance.

## RESULTS

### Effect of Essential oils on the tumor volume

As shown in Figure 1, when mice were injected only with EAC cells, they showed high tumor growth rate as indicated by the swollen abdomen as compared to mice treated with essential oils of orange peels. On the contrary, mice injected with tumor cells and co-treated with oral SOs had positive effects by reducing the size of the Ehrlich tumor and improvement of the animal performance.

### Effect of Essential oils (sweet orange) on serum liver function tests

Table 1 and Figure 2 show the toxic effect of EAC induction and development on the liver function tests and the protective role of the SOs on the hepatic toxicity. EAC tumor significantly increased the serum activity of transaminases (AST & ALT) and alkaline phosphatase (ALP) as compared to the corresponding control values. Treatment of EAC mice with daily oral doses of SOs for 14 consecutive days reduced the serum activity of AST, ALT, and ALP close to normal values. In the contrary, EAC tumor reduced total protein level in the serum as compared to the control group and the SOs treatment restored the protein level around normal value in the control group.

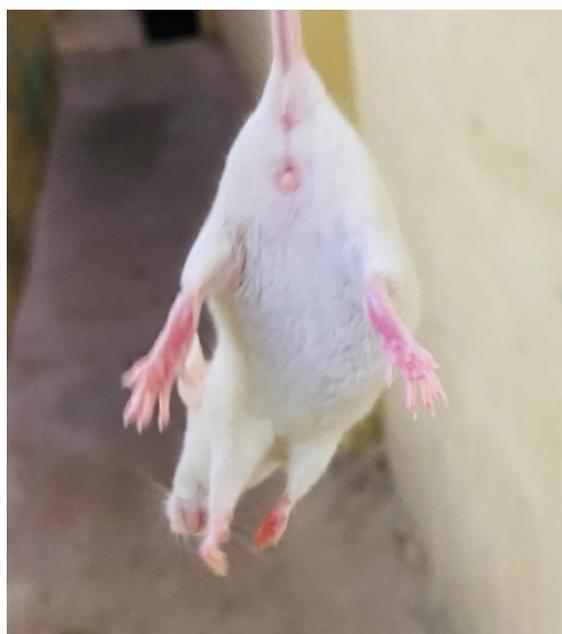
### Effect of Essential oils on the hepatic GFAP protein expression

Regarding the level of protein gene expression in the liver cells (Figure 3), it was observed that

there was no GFAP expression for protein in the liver tissues of control, SOs and EAC + SOs groups indicating negative expression of GFAP. In the contrary, EAC group have a positive GFAP expression where the liver sections show GFAP immunopositively staining in hepatocytes compared to other groups under investigation.



Gp3: EAC cells



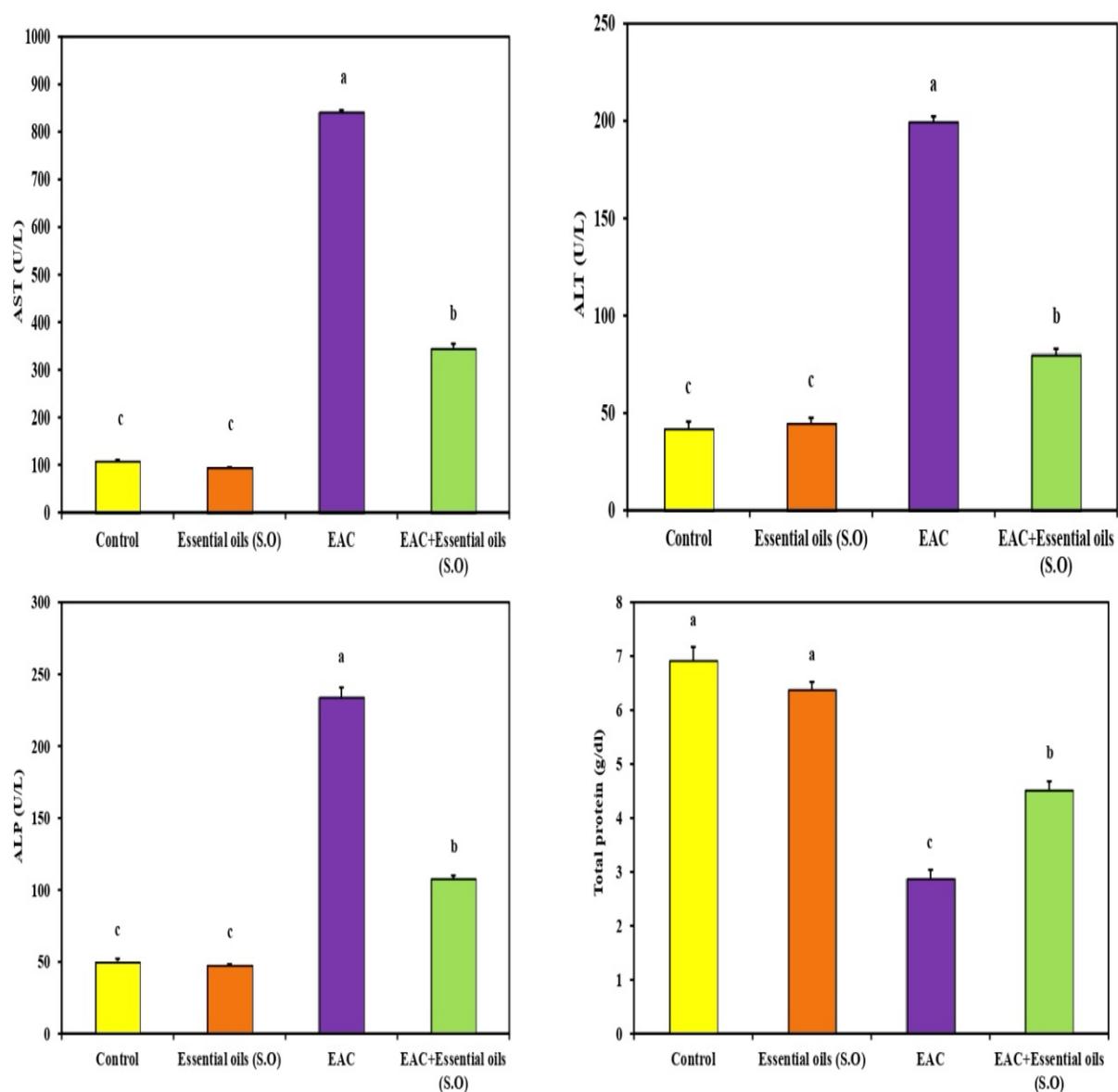
Gp4: EAC + Essential oils (S.O)

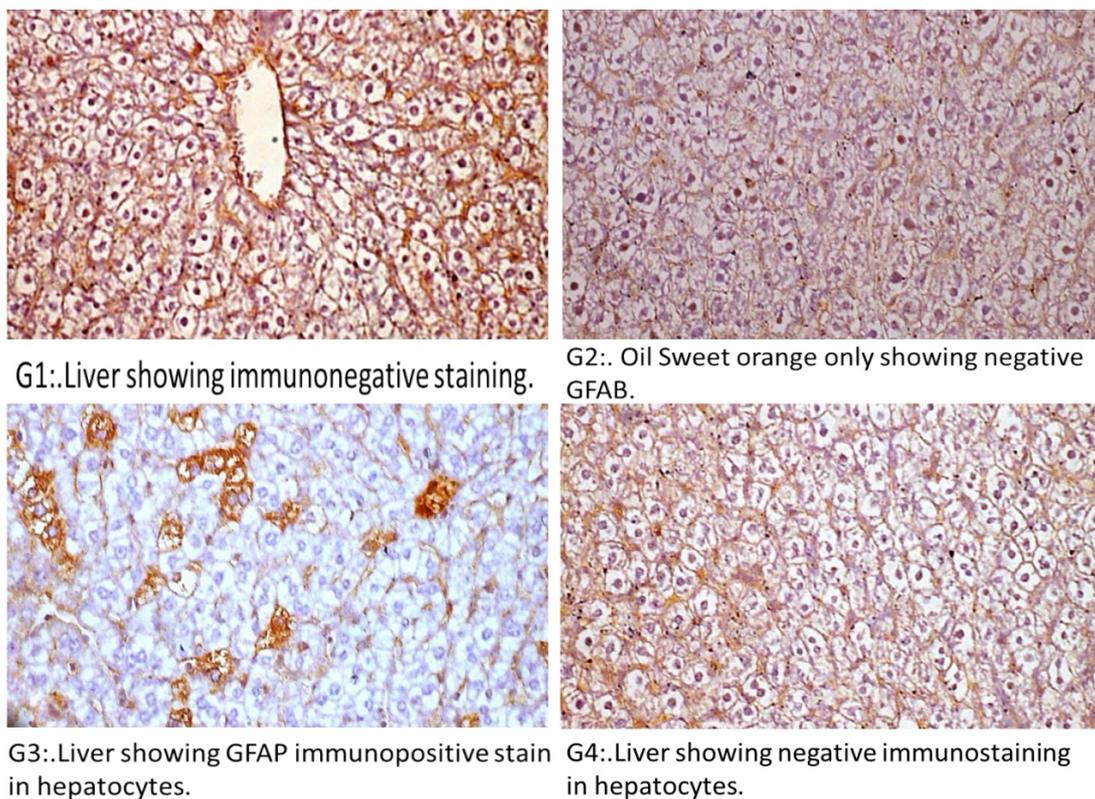
**Figure 1.** Mice Bearing EAC were treated with essential oils (orange peels) in the fourteenth day before anatomy (before and after treatment).

**Table 1.** Comparison between the different studied groups according to liver enzymes

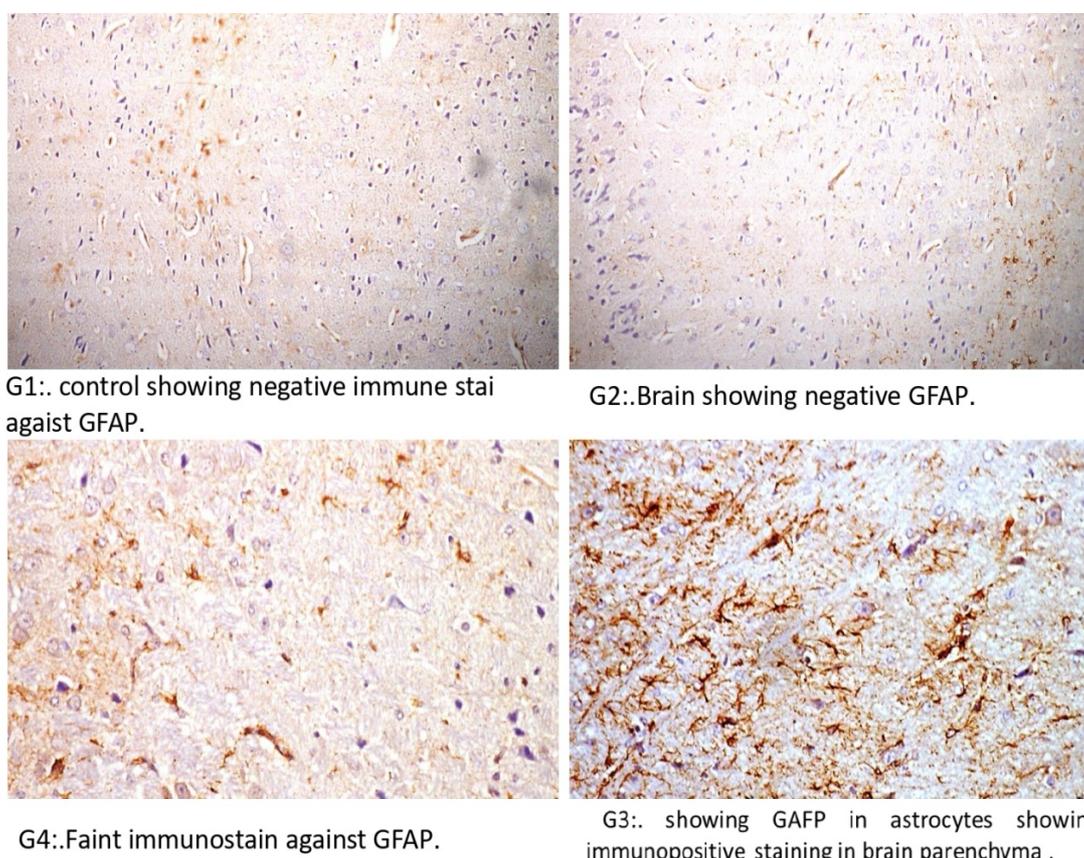
Parameters	Control	Essential oils (S.O)	EAC	EAC + Essential oils (S.O)
AST (U/L)	107.7 <sup>c</sup> ± 2.88	93.87 <sup>c</sup> ± 0.87	840.7 <sup>a</sup> ± 5.03	344.7 <sup>b</sup> ± 10.19
ALT (U/L)	42.0 <sup>c</sup> ± 3.63	45.03 <sup>c</sup> ± 2.57	199.5 <sup>a</sup> ± 2.90	79.83 <sup>b</sup> ± 3.20
ALP (U/L)	50.32 <sup>c</sup> ± 1.84	47.32 <sup>c</sup> ± 0.99	233.6 <sup>a</sup> ± 7.21	107.6 <sup>b</sup> ± 2.46
Total protein (g/dl)	6.92 <sup>a</sup> ± 0.25	6.38 <sup>a</sup> ± 0.14	2.87 <sup>c</sup> ± 0.17	4.52 <sup>b</sup> ± 0.16

Values are expressed mean ± SE; n = 4 for each treatment group. Mean values within a row not sharing common superscript letters were significant different, p<0.05. F: F for One way ANOVA test, pairwise comparison bet. each 2 groups were done using Post Hoc Test (Tukey)

**Figure 2.** Comparison between the different studied groups according to ALT , AST , ALP , Total protein in serum.



**Figure 3.** Immunohistochemical variation on the liver in all studied groups stained with GFAB immunoreactivity.



**Figure 4.** Immunohistochemical variation on the Brain in all studied groups stained with GFAB immunoreactivity.

### Effect of Essential oils on the brain's GFAP protein expression

As shown above in liver cells reactivity with FGAP, the level of protein gene expression in the brain tissue showed negative GFAP reactivity in mice of the control and SOs groups (Figure 4). In the contrary, both EAC-bearing mice and EAC-mice treated with SOs in groups 3 and 4 had a positive GFAP expression where the brain sections showed faint to dense GSAP immunopositively staining in astrocytes, respectively compared to the control groups.

### DISCUSSION

EAC cell line is considered a transplantable undifferentiated malignant mammary carcinoma model in the experimental female mouse (Segura et al., 2000). The current study aims to investigate the preventive role of essential oils (orange peels) against EAC that causes liver damage and to study its effect on liver enzymes and glial fibrillary acidic protein in liver and brain tissues. Our results demonstrated that EAC caused an increase in serum ALT, AST, and ALP and a decrease in total proteins of the liver. These results are in consistent with Haldar et al. (2010). The sera increase in liver enzymes of EAC group is evidence of the rapid damage of the liver cells, which leak out these enzymes in the surrounding blood. This was evident in EAC mice dosed with SOs, which prevent hepatocytes damage leading to a significant effect in improving sera liver enzymes through a decrease in AST, ALT, and ALP, and an increase in total proteins in the serum compared to the EAC group. These outcomes interpretate the decrease in liver enzymes and proves the protective role of orange peels against cancer in mice. These results agree with those of Firdaws et al. (2018). In addition, our results demonstrated that EAC causes a decrease in the levels of total proteins in female mice compared to the control group, which are consistent with Badr et al. (2011) and Donia et al. (2018) who demonstrated that essential oils have an essential role in preventing cancer in mice, and are also consistent with Chu et al. (2017) who showed the effectiveness of these peels against liver infections in mice.

Glial fibrillary acidic protein (GFAP) is a member of the type 111 subclass of intermediate filament (IF) proteins, which includes astrocyte specific IF proteins. Among type III IF proteins, GFAP has the smallest head domain with a molecular mass of ~50 KDa (Inagaki et al., 1994). GFAP is an intermediates filament that was initially found and described in astrocyte cells (Pekny et al., 2005). HSCs and central nervous system astrocytes are similar in several ways, including their proximity to capillaries, stellate-like appearance, and ability to respond to tissue damage (Blomhoff et al., 1991). It is still unknown what function of GFAP expression serves in liver and brain tissue. Prior research on rats demonstrated that HSCs have an exceptionally wide range of intermediate filament proteins (Unitarian et al., 1996).

In mice that following CCl<sub>4</sub> injury, HSCs near the edges of fibrotic septa and most HSCs in a normal liver are identified by GFAP (Neubauer et al., 1996). However, GFAP expression dropped with activation of rodent HSCs (Niki et al., 1996). Reduced expression of GFAP at a more advanced stage of fibrosis raised the possibility that GFAP is a signal for quiescent cells in rodents. On the other hand, the development of GFAP/desmin-positive HSCs in the early stages of fibrosis (Erts et al., 1991). GFAP was found in localized periseptal regions of cirrhotic liver but not in normal liver HSCs. Quantifying the hepatic expression of GFAP at various phases of human chronic hepatitis has not been the subject of many investigations (Martinelli et al., 2004). our current results are consistent with Hashemi et al. (2014) that FGAP was more expressed in the hepatocytes and astrocytes in the liver and brain of EAC mice group and that dosed with SOs.

The present results conclude that SOs from orange peels have a protective role in improving serum liver enzymes and total proteins against injury induced by EAC in female mice. SOs can also improve GFAP expression in both liver and brain tissues compared to EAC bearing mice.

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