

## *Trigonella foenum graecum* and *Saliva aegyptiaca* modulates hepatic redox status in Ehrlich-ascites-carcinoma-bearing mice

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### ABSTRACT

The present study was carried out to assess the effect of plant's extract on Ehrlich ascites carcinoma (EAC) and its influence on redox status and tissue injury on liver. Among five groups of albino mice, three were treated with plant's extract, in addition, a group treated with the standard drug, 5-fluorouracil. Ascites tumor was introduced into female mice by inoculation of  $2.5 \times 10^6$  viable tumor cells/mouse. After 5 days of transplantation, the extraction of *Saliva aegyptiaca* (Egyptian sage) and *Trigonella foenum graecum* (fenugreek) were given daily for 4 days via intraperitoneal route at a dose level of 55 and 100 mg/kg body weight, respectively, to mice bearing EAC cells. The results revealed that both Egyptian sage and fenugreek normalized oxidative stress in liver of mice-bearing EAC cells evidenced by increasing the level of glutathione. On the other hand, significant decreases in the levels of malondialdehyde and nitric oxide were demonstrated in liver indicating controlled oxidative stress in these animals. As a consequence, Egyptian sage and fenugreek regulated liver enzymes namely alkaline phosphatase, aspartate aminotransferase, alanine aminotransferase, gamma-glutamyl transferase and total bilirubin. Regarding to histopathological results, treatment with Egyptian sage and fenugreek extracts diminished most of the pathological alterations induced by EAC cells in mice. In conclusion, the present data suggested that Egyptian sage and fenugreek as a potential therapeutic complement in the treatment of different pathologies that may be related to an imbalance of the cellular oxidoreductive status associated with liver injury.

### INTRODUCTION

One of the major limitations in the currently available treatment modalities for cancer is their side effects (Joensuu, 2008). Hence alternate treatment for cancer is being tested. Plant derived natural products such as flavonoids, terpenes, alkaloids and so on have received considerable attention in recent years due to their diverse pharmacological properties including cytotoxic and cancer chemo-preventive properties (Doughari *et al.*, 2009; Mann *et al.*, 2009).

*Saliva aegyptiaca* L. (English name: Egyptian sage) is a green dwarf shrub that grows in various locations in the Arabian Peninsula, Egypt, Palestine, Iran and Afghanistan (Al-Yousuf *et al.*, 2002; Rizk and El-Ghazaly, 1995). It is commonly used in local folk medicine (Rizk and El-Ghazaly, 1995) *Trigonella foenum graecum* (Fenugreek) is an annual herb belonging to

the family Leguminosea, widely grown in India, Egypt, and Middle Eastern countries (Alarcon-Aguilara *et al.*, 1998). *Trigonella foenum graecum* is one such plant whose seeds and leaves are used not only as food but also as an ingredient in traditional medicine.

Fenugreek have also been reported to exhibit pharmacological properties such as antitumor, antiviral, antimicrobial, anti-inflammatory, hypotensive and antioxidant activity (Cowan, 1999). The Ehrlich tumor was initially described as a spontaneous murine mammary adenocarcinoma. Both the solid and the ascetic forms of this transplantable tumor are frequently used (Da Silva *et al.*, 2009; Kleeb *et al.*, 1997; Salgado Oloris *et al.*, 2002).

The present study was aimed to investigate the protective effect of Egyptian sage, fenugreek and 5-fluorouracil (5-FU) on Ehrlich ascites tumor in induced oxidative stress and tissue injury on liver, because it is well established that liver enzymes are more sensitive indicator of a distant neoplasm than blood. The biochemical findings are further confirmed by the histopathological study.

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## MATERIALS AND METHODS

### Plant Material

*Salvia aegyptiaca* and *Trigonella foenum graecum* were collected from market of East Cairo, Egypt in the months of February-March, 2012. The plant materials were authenticated in Botany Department, Faculty of Science, Helwan University, Cairo-Egypt on the basis of taxonomic characters and by direct comparison with the herbarium specimens that available at the herbarium of the Botany Department.

### Extraction

#### Preparation of *Salvia aegyptiaca* Extract

*Salvia aegyptiaca* was obtained from open markets and extracted according to the aqueous method described by Amin and Hamza (2005). In briefly, 10 gm of dried plants was mixed in 100 ml of boiled distilled water for 30 minutes, the extract was filtered and be ready to use.

#### Preparation of *Trigonella foenum graecum* Extract:

*Trigonella foenum graecum* seeds were obtained open markets and extracted by (100 g) were finely powdered, mixed with 80% methanol, and kept at room temperature for 5 days. After 5 days this was filtered and the solvent was evaporated by rotary evaporator (at 40-50 °C and 150 rpm) (Xia *et al.*, 1998).

### Experimental Animals

Experiments were performed on female albino mice, 6–8 weeks old, weighing 25±5 g. Mice were housed in cages (6 mice /group). The animals were obtained from research institute of ophthalmology animal house department, Al-Giza, Egypt. Animals were kept in wire bottomed cages, in a room under standard condition of illumination with a 12 hours light-dark cycle at 25±2 °C. They were provided with water and balanced diet *ad libitum*. They were acclimatized to the environment for one week prior to experimental use.

### Tumor cells

Experimental tumor cells and tumor transplantation a line of EAC was used in this study. The parent line was kindly supplied by the National Cancer Institute, Cairo University, Egypt. EAC was maintained in BALB/c mice in the ascites form by serial transplantation. Ascitic tumor cell counts were done in a Neubauer hemocytometer using the trypan blue dye exclusion method.

### Animal grouping

Mice were inoculated intraperitoneally (i.p.) on day 0 with (2.5×10<sup>6</sup>) viable tumor cells per mouse in a volume of 0.1 ml of cell suspension in physiological saline (0.9%). The groups and the design of the experiment were as follows:

Group 1: EAC control

Group 2: EAC + ESE (55 mg/ kg; i.p.)

Group 3: EAC + FE (100 mg/kg; i.p.)

Group 4: EAC + ESE+FE (half dose of each; i.p.)

Group 5: EAC + 5-FU (20 mg/kg; i.p.)

Treatment was continued for 4 days and on the day 10<sup>th</sup> after tumor transplantation, animals were sacrificed. Pieces of liver was weighed and homogenized immediately to give 50% (w/v) homogenate in ice-cold medium containing 50 mM Tris-HCl, pH, 7.4. The homogenates were centrifuged at 500 g for 10 min at 4 °C. The supernatant (10%) was used for the various biochemical determinations.

### Histopathological Estimation

A small piece of the liver was quickly removed, then fixed in neutral buffered formalin. Following fixation, specimens were dehydrated, embedded in wax, and then sectioned to 5 microns thickness. For histological examinations, sections were stained with haematoxylin and eosin (Drury and Wallington, 1981).

### Studies on biochemical parameters

#### Liver function test

Colorimetric determination of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were estimated by measuring the amount of pyruvate or oxaloacetate produced by forming 2, 4- dinitrophenylhydrazine, according to the method of Reitman and Frankel, (1957). The color of which was measured at 546 nm. Also, total bilirubin (TB) in liver homogenate, was assayed according to the method of Schmidt and Eisenburg (1975). Total protein was assayed in liver homogenate according to Lowry *et al.*, (1951).

#### Determination of malondialdehyde and nitrite/nitrate

Malondialdehyde (MDA) and nitrite/nitrate (NO) were assayed colorimetrically in liver homogenate, according to the method of Ohkawa *et al.*, (1979) and Green *et al.*, (1982), respectively. Where MDA determined by using 1 ml of trichloroacetic acid 10% and 1 ml of thiobarbituric acid 0.67% and were then heated in a boiling water bath for 30 min. Thiobarbituric acid reactive substances were determined by the absorbance at 535 nm and expressed as malondialdehyde (MDA) formed. Nitric oxide was determined in acid medium and in the presence of nitrite; the formed nitrous acid diazotise sulphanilamide is coupled with N-(1-naphthyl) ethylenediamine. The resulting azo dye has a bright reddish –purple color which can be measured at 540 nm.

#### Estimation of glutathione

The hepatic glutathione (GSH) levels was determined by the method of Ellman (1959). The method is based on the reduction of Elman's reagent (5, 5' dithiobis (2-nitrobenzoic acid) "DTNB") with GSH to produce a yellow compound. The reduced chromogen is directly proportional to GSH concentration and its absorbance can be measured at 405 nm.

### Statistical analysis

Results were expressed as the mean ± standard error of the mean (SEM). Data for multiple variable comparisons were analyzed by one-way analysis of variance (ANOVA). For the

comparison of significance between groups, Duncan's test was used as post hoc test according to the statistical program statistical package program (SPSS version 17.0).

## RESULTS

After 10 days of EAC inoculation, there were abnormal decreases of hepatic ALT, AST and total bilirubin levels in the liver of the EAC-bearing mice in comparison to the control values. ESE, FE and ESE + FE treatments significantly suppressed ( $p \leq 0.05$ ) the decrease in hepatic parameters levels in the liver of ESE, FE and ESE + FE + EAC-treated groups compared with EAC implanted mice (Table 1). Nevertheless, liver function markers were still lower than the normal control levels. Total protein level in EAC implanted mice was showed significantly decrease ( $p \leq 0.05$ ) in comparison to the control values. ESE+FE and 5-FU+EAC-treated groups were showed significantly increased in total protein level as compared with EAC implanted mice (Fig. 1).

Fig. 2 (a) showed hepatic histological features of the control group. EAC bearing mice showed hepatocytes had vacuolated cytoplasm, loss of cord arrangement of hepatocytes and sinusoidal spaces with degenerated plasma membranes and nuclei. Severe pyknotic nuclei, necrosis, inflammation and congested portal vein were also seen (Fig. 5b). All the EAC induced alterations in the hepatic architecture were significantly modulated by ESE, FE and ESE+FE treatments.

EAC implantation resulted in significant ( $P < 0.05$ ) elevation in MDA and NO contents in the liver homogenates compared to the control group (Fig. 3 and 4). Treatments with ESE and FE caused significant reductions in NO level when compared to the EAC bearing mice, while 5-FU was showing evaluation in NO level when compared to the EAC bearing mice.

Changes in liver non-enzymatic antioxidant molecules after 10 days of EAC implantation were shown in Fig. 5. EAC-bearing mice demonstrated significant decreases in the concentration of GSH in comparison to the control mice. Daily intraperitoneal supplementations with ESE, FE and ESE+FE of EAC-bearing mice significantly maintained the hepatic GSH concentrations near the normal values.

## DISCUSSION

Liver enzymes such as ALT and AST are marker enzymes for liver function and integrity. These enzymes are usually raised in serum and decreased in liver homogenate in acute hepatotoxicity or mild hepatocellular injury (Abdel Moneim *et al.*, 2011), but tend to decrease with prolonged intoxication due to damage to the liver. Increases in both transaminases are found in liver diseases, with ALT much higher than AST. Very high values are usually obtained in toxic hepatitis (Sunil *et al.*, 2013). Biochemical measurements of these parameters in EAC mice and treated EAC mice liver tissue showed significantly decreased in

hepatic ALT in treated groups except in ESE group, while showed significantly increased in AST in treated groups except in ESE group. Bilirubin is also indicative of hepatotoxicity. The results in the present study showed that all treated groups caused significant increased in total bilirubin against EAC control group.

Cancer chemotherapy using antioxidant formulations is an exciting pharmaceutical research involving the use of either natural or synthetic components to delay inhibit or reverse the development of cancer in normal or preneoplastic conditions (Balasubashini *et al.*, 2006). Antioxidant can inhibit proliferation of cancer cells (Rebecca *et al.*, 1998). Among possible mechanisms of protection against chemical carcinogenesis could be anti-oxidant dependent induction of detoxifying enzymes (Iqbal and Okada, 2003). Oxidative stress is potentially harmful to cells and reactive oxygen species (ROS) are implicated in the etiology and progression of many diseases including cancer (Cerutti 1994; Sur and Ganguly, 1994 and Sardar *et al.*, 2000).

Under conditions of excessive oxidative stress, however antioxidants are depleted and ROS can damage cellular components and interfere with critical cellular activities (Sardar *et al.*, 2000). Cancer cells have highly elevated protective mechanism to prevent lipid peroxidation. Several studies have demonstrated that lipid peroxidation is significantly decreased in tumor cells and tissues compared with that of corresponding normal cells (Gauchez *et al.*, 1995).

Excessive production of free radicals resulted in oxidative stress, which leads to damage of macromolecules such as lipids can induce lipid peroxidation in vivo (Fenninger and Milder, 1954). Malondialdehyde (MDA) the end product of lipid peroxidation, are seen to be higher in cancer tissues than in non-diseased organ (Meister, 1988). GSH, an important non-protein thiol, plays a significant role in protecting cells from neoplastic process. In addition, GSH plays a role as an endogenous antioxidant molecule that is found particularly in high concentration in liver and is known to have key function in the protective process (Sinclair *et al.*, 1990).

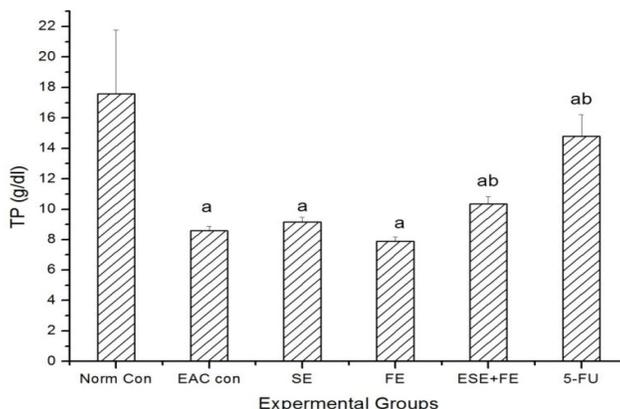
The present study showed that, administration of ESE, FE and ESE+FE caused a non-significant decreased in MDA level and caused a significant decrease in nitric oxide level. The administration of ESE and FE showed significantly increase in GSH level. Agrawal *et al.*, (2011) reported that the inhibition of MDA, nitric oxide level and increased of GSH activities as a result of tumor growth was also reported with the administration of Boswellic acid. At the same manner, Thirunavukkarasu *et al.*, (2003) reported that fenugreek seed administration reduced the increase in the levels of biomarkers of oxidative stress in the liver of rats dosed with ethanol.

In conclusion, the present data suggested that *Salvia aegyptiaca* and *Trigonella foenum graecum* as a potential therapeutic complement in the treatment of different pathologies that may be related to an imbalance of the cellular oxidoreductive status associated with liver injury.

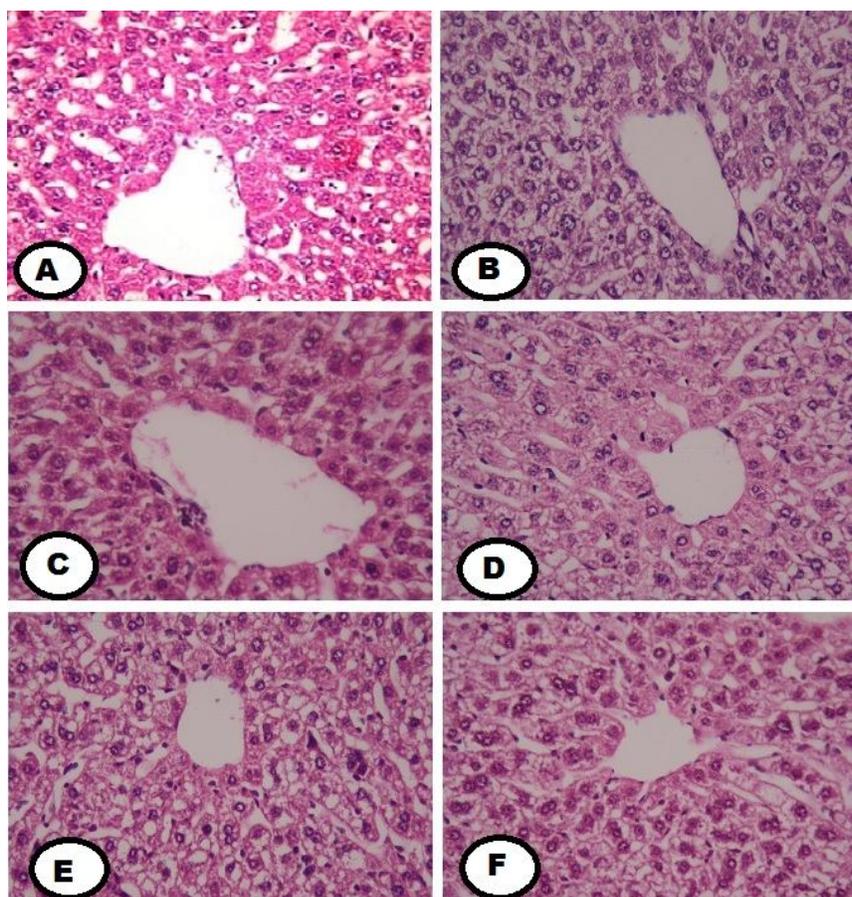
**Table 1:** Activities of ALT (alanine aminotransferase), AST (aspartate aminotransferase) and total bilirubin in hepatic homogenates of different animal groups.

Group	AST (U/mg tissue)	ALT (U/mg tissue)	Total Bilirubin (mg/mg tissue)
Control	72.3±2.5	86.4±3.8	2.98±0.2
EAC	48.5±1.7 <sup>a</sup>	63.8±1.1 <sup>a</sup>	0.6±0.2 <sup>a</sup>
ESE + EAC	53.463±0.9 <sup>ab</sup>	61.472 ±0.5 <sup>a</sup>	0.933±0.2 <sup>ab</sup>
FE + EAC	54.696 ±0.7 <sup>ab</sup>	40.914±0.8 <sup>ab</sup>	1.375±0.1 <sup>ab</sup>
ESE + FE + EAC	54.439±1.0 <sup>ab</sup>	53.911±1.7 <sup>ab</sup>	2.090±0.1 <sup>ab</sup>
5-FU + EAC	57.698 ±1.1 <sup>ab</sup>	56.012±1.2 <sup>ab</sup>	2.475±0.1 <sup>ab</sup>

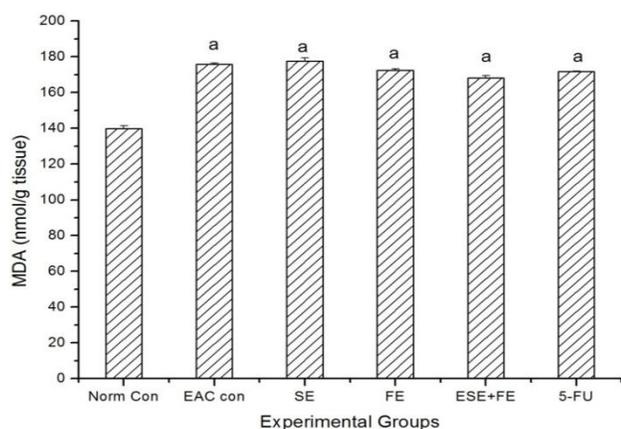
Values are means ± SE (n=6). a: Significant change at  $p < 0.05$  with respect to Control group; b: Significant change at  $p < 0.05$  with respect to EAC group.



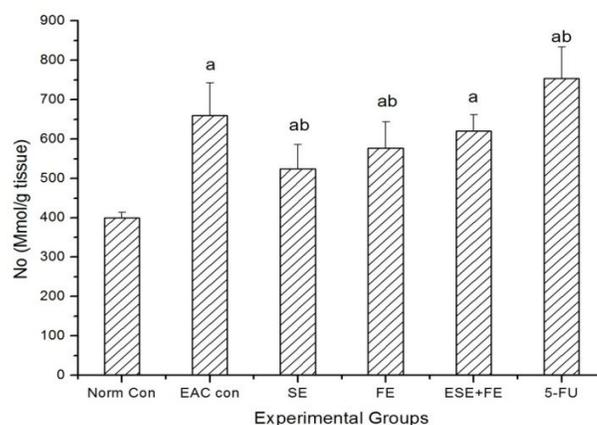
**Fig. 1.** Effect of plants' extract on total protein level on liver mice. Values are means ± SE (n=6). a: Significant change at  $p < 0.05$  with respect to Control group; b: Significant change at  $p < 0.05$  with respect to EAC group.



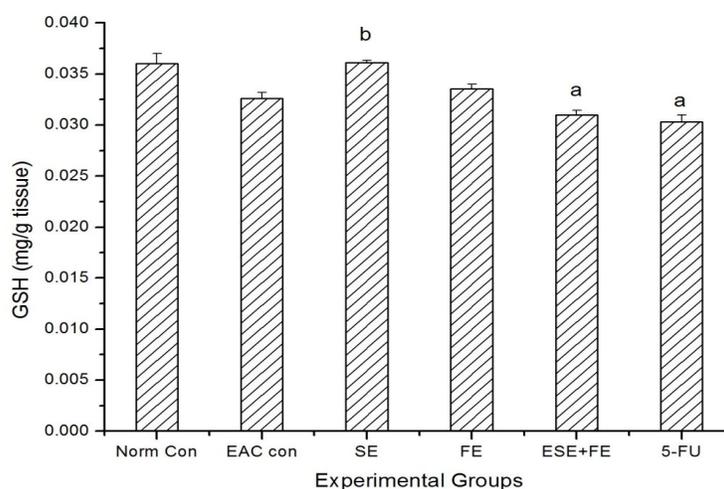
**Fig. 2:** (A) Normal histological features of liver of control group with well formed central vein (CV). (B) Section of EAC-bearing mice liver with several irregularities, irregular CV, loss of cord arrangement of hepatocytes and loss of sinusoidal spaces, vacuolated hepatocytes and lymphocyte infiltration. (C) Section of EAC-bearing mice liver after *Salvia aegyptiaca* treatment with preserved histo-architecture. (D) Section of liver after treatment of *Trigonella foenum graecum* treatment liver with well formed CV, hepatocytes arranged in cord like fashion with well arrayed sinusoidal spaces around the CV. (E) Section of EAC-bearing mice liver after *Salvia aegyptiaca* plus *Trigonella foenum graecum* treatment with preserved histo-architecture. (F) Section of EAC-bearing mice liver after treatment of 5-flourouracil treatment liver with well formed CV, hepatocytes arranged in cord like fashion with well arrayed sinusoidal spaces around the CV (400X).



**Fig. 3:** Effect of plants' extract on MDA level on liver mice. Values are means  $\pm$  SE (n=6). a: Significant change at  $p < 0.05$  with respect to Control group.



**Fig. 4:** Effect of plants' extract on NO level on liver mice. Values are means  $\pm$  SE (n=6). a: Significant change at  $p < 0.05$  with respect to Control group; b: Significant change at  $p < 0.05$  with respect to EAC group.



**Fig. 5:** Effect of plants' extract on GSH level on liver mice.

Values are means  $\pm$  SE (n=6). a: Significant change at  $p < 0.05$  with respect to Control group; b: Significant change at  $p < 0.05$  with respect to EAC group.

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