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Studies on the effect of *Salvia aegyptiaca* and *Trigonella foenum graecum* extracts on adult male mice

Amany A. Tohamy, Shaimaa R. Ibrahim and Ahmed E. Abdel Moneim

Amany A. Tohamy,
Shaimaa R. Ibrahim
Department of Zoology & Entomology, Faculty of Science, Helwan University, Cairo, Egypt.

Ahmed E. Abdel Moneim
Biomedical Research Center, University of Granada, Granada, Spain.

For Correspondence
Ahmed Esmat Abdel Moneim
Biomedical Research Center
Health Sciences Technology Park
University of Granada
Avda. del Conocimiento s/n
18100 Armilla, Granada, Spain
Tel. (+34) 602508238.

ABSTRACT

Salvia aegyptiaca (Egyptian sage) and *Trigonella foenum graecum* (fenugreek) have potential tannins, total flavonoids and total phenolics as examined *in vitro* in the present study. In addition, the antioxidant effect of Egyptian sage (ESE) and fenugreek (FE) extracts were evaluated in normal male adult mice. Also, there is no evidence about the positive and/or negative effect of those extracts on male fertility. In order to evaluate the beneficial effect of those extracts, liver and kidney functions, lipid peroxidation, nitric oxide. In addition, non-enzymatic and enzymatic antioxidant molecules as glutathione (GSH), catalase (CAT), superoxide dismutase (SOD), glutathione reductase (GR) and glutathione-S-transferase (GST) were estimated. Also, histological examination of testis was done. The results revealed that both extract of ESE and FE have potent antioxidant activity by reducing lipid peroxidation and nitric oxide formation in testis tissues of mice. Those activities were extended to non-enzymatic and enzymatic antioxidant defense components such as GSH, CAT, SOD, GR and GST. Additionally, ESE mixed to FE caused enhancement in testis structure with improved seminiferous tubules and spermatozoa. In conclusion, the results obtained showed that ESE and FE may contain some biologically active components that may be active against oxidative stress, and this may be the basis for its traditional use for environmental toxins.

Keywords: *Salvia aegyptiaca*; *Trigonella foenum graecum*; Antioxidant properties; Oxidants/antioxidants status; Mice.

INTRODUCTION

Herbs and spices have been extensively used as food additives for natural antioxidants. Spices and aromatic herbs are considered to be essential in diets or medical therapies for delaying aging and biological tissue deterioration (Bukhari *et al.,* 2008) The antioxidant property of the plant material is due to the presence of many active phytochemicals including vitamins, flavonoids, terpenoids, carotenoids, cumarins, curcumins, lignin, saponin, plant sterol and etc (Zheng and Wang, 2001).

Salvia is an impotant genus consisting of over 900 species in the family Lamiaceae (formerly Labiatae) and some species of *Salvia* have been cultivated worldwide for use in folk medicines and for culinary purposes (Gorai *et al.,* 2011; Lu and Foo, 2002).

Saliva aegyptiaca L. (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. The seeds of the plant are used as demulcent for diarrhea and for piles, and the whole plant is used in diarrhea, gonorrhea and haemorrhoids, eye diseases, and as an antiseptic, antispasmodic and stomachic (Rizk and El-Ghazaly, 1995). The plant is also used in cases of nervous disorders, dizziness and trembling and stopping perspiration (Al Yousuf *et al.*, 2002; Gorai *et al.*, 2011).

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. Its leaves are a rich source of calcium, iron, β-carotene and other vitamins (Sharma *et al.,* 1996), while the seeds contain lysine and L-tryptophan rich proteins, mucilaginous fibre and other rare chemical constituents such as saponins, coumarin, fenugreekine, nicotinic acid, sapogenins, phytic acid, scopoletin and trigonelline, which are thought to account for many of its presumed therapeutic effects (Billaud, 2001). Fenugreek has a long history of traditional use as a medicinal herb for diabetes and its antidiabetic potential has been experimentally evidenced (Kaviarasan *et al.,* 2007b). Fenugreek have also been reported to exhibit pharmacological properties such as antitumor, antiviral, antimicrobial, anti-inflamatory, hypotensive and antioxidant activity (Cowan, 1999).

The current study aimed to evaluate the beneficial effect of *Saliva aegyptiaca* and *Trigonella foenum graecum*, as well as antioxidant effect of those plants in adult male albino mice.

MATERIALS AND METHODS

Experimental Animals

Experiments were performed on male 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 h 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. The experiments were approved by the state authorities and followed Egyptian rules on animal protection.

Plants extract

Preparation of Salvia aegyptiaca (Egyptian sage) Extract

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

Preparation of Trigonella foenum graecum (fenugreek) Extract

Fenugreek 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).

Test for tannins

The aqueous extract (1 ml) was mixed with 10 ml of distilled water and filtered. Ferric chloride reagent (3 drops) was added to the filtrate. A blue-black or green precipitate confirmed the presence of gallic or catechol tannins, respectively.

Determination of Total Flavonoids

For the assessment of flavonoids, colorimetric method introduced by Dewanto *et al.* (2002) was modified. To determine the amount of flavonoids by the above mentioned method, 1.5 ml of the deionized water was added to 0.25 ml of the sample extract and then 90 ul of 5% Sodium nitrite ($NaNO_2$). Six min later, after addition of 180 ul of 10% $AlCl_3$, mixture was allowed to stand for another 5 min before mixing 0.6 ml of 1M NaOH. By adding deionized water and mixing well, final volume was adjusted to 3 ml. Using blank, absorbance was measured at 510 nm. Calibration curve was prepared using querestin acid as standard for total flavonoids which was measured as mg querestin equivalents (QE) per gram of the sample (mg/g).

Determination of Total Phenolics

To analyze the total phenolic content (TPC), Kim *et al.* (2003) method was followed to make the use of Folin Ciocalteu reagent. 0.4 ml of the extract (prepared in methanol with a concentration of 1.0 mg/ml), were mixed with 1.0 ml of (10%) Folin-Ciocalteu reagent and the solution was allowed to stand at 25 ºC for 5-8 min before adding 0.8 ml of 7.5% sodium carbonate solution and using deionized water, final volume was adjusted to 10.0 ml. After two hours, absorbance was measured at 765 nm. Calibration curve was prepared using gallic acid as standard for TPC which was measured as mg gallic acid equivalents (GAE) per gram of the sample (mg/g).

Experimental Design

To study the effect of some medical plants, 24 male albino mice were randomly divided into four groups, six mice of each. The first group (Con group) served as control and received saline (0.1 ml saline/ mice) by intraperitoneal injection. The second group (ESE group) injected intraperitoneal by 55 mg/ kg of Egyptian sage's extract for 7 days. The third group (FE group) injected intraperitoneal by 100 mg/kg of fenugreek's extract for 7 days. The forth group (ESE+FE group) injected intraperitoneal by mixture half dose of each Egyptian sage's and fenugreek's extract mixture for 7 days. The animals of the all groups were killed by fast decapitation and blood samples were collected. Blood was stranded for half an hour and then centrifuged at 500 g for 15 min at 4 °C to separate serum and stored at -70 °C until analysis. Pieces of liver, kidney and testis were weighed and homogenized immediately to give 50% (w/v) homogenate in ice-cold medium containing 50 mM Tris-HCl and 300 mM sucrose. The homogenate was centrifuged at 500 g for 10 min at 4 °C. The supernatant (10%) was used for the various biochemical determinations.

Histopathological Estimation

Small pieces of the liver, kidney and testis were 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).

Biochemical estimations

Liver function test

Colorimetric determination of alanine aminotransferase (ALT) or aspartate aminotransferase (AST) was 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. Alkaline phosphatase was assayed in serum, using kits provided from Biodiagnostic Co. (Giza, Egypt) according to the method that was described by Belfield and Goldberg (1971). Also, Total bilirubin (TB) in serum, was assayed according to the method of Schmidt and Eisenburg (1975). Total protein was assayed in liver, kidney and testis homogenate according to Lowry *et al.* (1951).

Kidney function test

Uric acid (UA), blood urea nitrogen (BUN) and serum creatinine (Cr) were assayed in serum, using kits provided from Biodiagnostic Co. (Giza, Egypt) according to the methods that were described by Fossati *et al.* (1980), Fawcett and Scott (1960) and Szasz *et al*. (1979), respectively.

Determination of malondialdehyde and nitrite/nitrate

Malondialdehyde (MDA) and nitrite/nitrate (NO) were assayed colorimetrically in liver, kidney and testis homogenate, according to the method of Ohkawa *et al.* (1979) and Berkels *et al.* (2004), 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 and anti-oxidant enzymes

The hepatic, renal and testicular glutathione (GSH) levels were determined by the methods 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. In addition, the activity of hepatic, renal and testicular antioxidant as catalase (CAT) was determined catalase reacts with a known quantity of H_2O_2, according to the method of Aebi (1984). The reaction is stopped after exactly 1 min with catalase inhibitor. In the presence of peroxidase (HRP), the remaining H_2O_2 reacts with 3,5-dichloro-2-hydroxybenzene sulfonic acid (DHBS) and 4-aminophenazone (AAP) to form a chromophore with a color intensity inversely proportional to the activity of catalase in the original sample. Superoxide dismutase (SOD) activity was assayed by the method of Nishikimi *et al.* (1972). This assay relies on the ability of the enzyme to inhibit the phenazine methosulphate-mediated reduction of nitroblue tetrazolium dye. Also, the activity of glutathione-S-transferase (GST) and glutathione reductase (GR) activities were determined by the methods of Habig *et al.* (1974) and Factor *et al*. (1998), respectively.

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

The Egyptian sage extract (ESE) and fenugreek extract (FE) gave positive tests for gallic tannins while the extracts gave negative result for catechol tannins as shown in Table 1. The total phenolic and flavonoids contents in ESE and FE are shown in Table 2. It is well-known that plant phenolics and flavonoids are highly effective free radical scavengers and antioxidants. *Salvia aegyptiaca* extract contained phenolic and flavonoids compounds at 0.411 mg GAE/g and 1.63618 mg QE/g, respectively. *Trigonella foenum graecum* extract contained phenolic and flavonoids compounds at 0.44702 mg GAE/g and 0.95064 mg QE/g, respectively. ESE contained high amounts of flavonoids compounds compared to FE. These results indicated that the potent antioxidant activity of *Salvia aegyptiaca* and *Trigonella foenum graecum* may be related to the phenolic and flavonoids compounds in these extracts.

Table. 1: Gallic tannins and catechol tannins contents in Egyptian sage and fenugreek extracts.

Plants / Parameter	ESE	FE
Gallic tannins	+ve	+ve
Catechol tannins	-ve	-ve

The results of our study shown that ESE administration caused significant reduction in ALP and total bilirubin comparing to control group (-31.16% and -70.47% at *p<0.05* respectively), while AST, ALT and total protein were non-significantly changed indicating that ESE administration support the function of liver (Table 3 and Fig. 1). The results of FE administration caused significant reduction in ALP and total bilirubin and total protein of testis according to control group (-29.61%, -65.95% and -42.23% respectively), while AST and ALT and total protein of liver and kidney were non-significantly changed indicating that FE administration support the function of liver (Table 3 and Fig. 1). Moreover, The results of ESE+FE administration caused significant reduction in AST and total bilirubin and total protein of testis comparing to the control group (-12.47%, -71.66%, and -42.43% at *p<0.05* respectively), while shown non-significantly changed in ALP and ALT, also total protein of liver and kidney (Table 3 and Fig. 1). In addition, ESE+FE had shown significant increase in ALP comparing to ESE group and FE group.

Table. 2: Total phenolic and flavoniod contents in Egyptian sage and fenugreek extracts.

Plants / Parameter	ESE	FE
Total phenolic (mg GAE/g sample)	0.411± 0.0084	0.447± 0.0083
Flavoniod (mg QE/ g sample)	1.636± 0.0156	0.950± 0.0026

Table. 3: Effect of plants' extract on liver function of mice.

Groups / Parameter	Control	ESE	FE	ESE + FE
AST (U/L)	4.49± 0.080	4.31± 0.112	4.33± 0.056	3.93± 0.078[a]
ALT (U/L)	9.35 ± 0.098	9.01± 0.399	9.28± 0.172	9.10± 0.180
ALP (U/L)	69.97± 3.308	48.17± 2.989[a]	49.25± 3.553[a]	74.15± 4.496[bc]
Total Bilirubin (mg/dL)	1.18± 0.014	0.35± 0.021[a]	0.40± 0.031[a]	0.33± 0.010[ac]

Values are means±SE (n=5). a: significant change at $p < 0.05$ with respect to control group. b Significant change at $p < 0.05$ with respect to ESE group. c Significant change at $p < 0.05$ with respect to FE group.

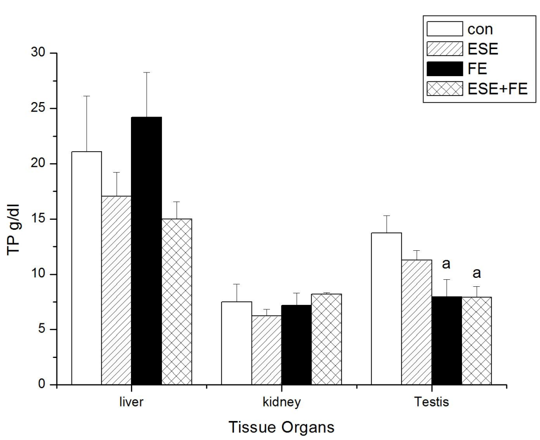

Fig. 1: Effect of plants' extract on total protein of liver kidney and testes of mice.
Values are means±SE (n=5). a: significant change at $p < 0.05$ with respect to control group.

The results of kidney function tests in Egyptian sage group, shown that ESE administration caused significant decrease in urea (-38.07%, *p<0.05*) comparing to control group, while creatinine and uric acid were non-significantly changed indicating that ESE administration support the function of kidney (Table 4). In FE shown non-significantly changed in creatinine, urea and uric acid were indicating that FE administration support the function of kidney. The results of ESE+FE administration caused significant reduction in urea (-39.63%) comparing to control group, while creatinine and uric acid were shown non-significantly changed indicating that the ESE+FE administration support the function of kidney (Table 4). In addition, FE administration caused significant increase in urea comparing to ESE group. Also, ESE+FE administration caused significant reduction in urea comparing to FE group.

Administration of ESE caused significant reduction in MDA of liver (-11.82% at *p<0.05*) comparing to control group, while shown non-significantly changed in nitrite/nitrate, indicating that ESE has antioxidant properties (Table 5). FE administration induced significant reduction in MDA of kidney and testis (-19.86% and -17.21%, respectively) comparing to control group. FE induced significant reduction in nitrite/nitrate of kidney (-19.80%), while shown significant increase in nitrite/nitrate of testis (28.39%) comparing to control group. There was non-significantly changed in nitrite/nitrate of liver after FE administration (Table 5). The results of ESE+FE administration shown non-significant in MDA, but induced significant reduction in nitrite/nitrate of kidney (-28.60%, *p<0.05*) comparing to control group. Those results indicating that mixture Egyptian sage and fenugreek extract administration has antioxidant properties (Table 5). Moreover, FE administration induced significant reduction in MDA of kidney and testis, while shown significant increase in MDA of liver comparing to ESE group. FE treatment induced significant reduction in nitrite/nitrate of kidney, while shown significant increase in nitrite/nitrate of testis comparing to ESE group. In addition, The results of ESE+FE administration caused a significant increase in MDA of kidney and testis comparing to FE group, and induced significant reduction in nitrite/nitrate of liver and kidney comparing to ESE group, while shown significant reduction in nitrite/nitrate of liver and testis comparing to FE group (Table 5).

ESE administration caused significant decrease in glutathione content of testis by (-35.10% at *p<0.05*) and ESE had shown significant increase in CAT of kidney and testis (12.06 % in both organs) comparing to control group, while SOD shown non-significantly changed (Table 6). FE administration caused non-significantly changed in GSH, while shown significant increase in CAT of kidney (30.58%) and shown significant increase in SOD of kidney and testis (19.88% and 23.83%, respectively) comparing to control group (Table 6). The results of ESE+FE administration caused non-significantly changed in GSH and SOD, while shown significant increase in CAT of liver (11.13 % at *p<0.05*) comparing to control group. In addition, FE administration caused a significant increase in GSH content of kidney and testis and shown significant increase in CAT of kidney and testis, while shown significant increase in SOD of testis comparing to ESE group (Table 6).

Table. 4: Effect of plants' extract on kidney function of mice.

Groups / Parameter	Control	ESE	FE	ESE + FE
Uric acid (mg/dL)	8.52±0.26	8.13±0.437	8.99±0.502	8.36±0.241
Urea (mg/dL)	51.74±2.86	32.04±0.909[a]	45.36±4.103[b]	31.23±2.247[ac]
Creatinine (mg/%)	0.68±0.01	0.63±0.032	0.62± 0.023	0.66±0.016

Values are means±SE (n=5). a: significant change at $p < 0.05$ with respect to control group. b Significant change at $p < 0.05$ with respect to ESE group. c Significant change at $p < 0.05$ with respect to FE group.

Table.5: Effect of plants' extract on malondialdehyde and nitric oxide content of liver, kidney and testes of mice

Groups / Parameter	Control	ESE	FE	ESE + FE
Hepatic MDA (nmol/g tissue)	17.03±0.780	15.02± 0.447[a]	17.61±0.528[b]	15.74±0.713
Renal MDA (nmol/g tissue)	7.05±0.075	6.71±0.232	5.65±0.097[ab]	6.59±0.248[c]
Testicular MDA (nmol/g tissue)	2.96±0.162	3.172±0.061	2.45±0.067[ab]	3.13±0.109[c]
Hepatic NO (µmol/ g tissue)	97.39± 6.465	107.81±9.789	116.10±11.509	76.58± 5.776[bc]
Renal NO (µmol/ g tissue)	43.65±1.827	42.35± 2.915	35.00±1.373[ab]	31.16±1.214[ab]
Testicular NO (µmol/ g tissue)	32.03± 3.768	28.58±2.525	41.129± 2.25[ab]	26.61± 0.904[c]

Values are means±SE (n=5). a: significant change at $p < 0.05$ with respect to control group. b Significant change at $p < 0.05$ with respect to ESE group. c Significant change at $p < 0.05$ with respect to FE group.

Table. 6: Effect of plants' extracts on reduced glutathione, catalase, and superoxide dismutase levels of liver, kidney and testes of mice .

Groups / Parameter	Control	ESE	FE	ESE + FE
Hepatic GSH (mg/g tissue)	20.34±0.797	25.26±1.717	19.705± 1.8264	26.25±2.870[c]
Renal GSH (mg/g tissue)	20.59±1.894	15.38±1.519	24.909± 1.7890 [b]	19.07±2.176
Testicular GSH (mg/g tissue)	31.47±2.669	20.42±1.714[a]	32.19±1.292[b]	26.66±2.504
Hepatic CAT (U/g tissue)	0.92± 0.026	1.01±0.005	0.95±0.009	1.03± 0.017[a]
Renal CAT (U/g tissue)	1.94± 0.079	2.17±0.012[a]	2.53±0.056[ab]	2.13± 0.023[c]
Testicular CAT (U/g tissue)	2.35± 0.116	2.04±0.091[a]	2.48±0.112[b]	2.15±0.042[c]
Hepatic SOD (U/g tissue)	1000.00±14.999	948.43±15.930	906.59±36.531	1010.46±13.230[c]
Renal SOD (U/g tissue)	1978.70±29.029	1949.33±11.456	2372.08±12.122[a]	1988.98±15.275[c]
Testicular SOD (U/g tissue)	4056.85±62.004	3822.77±31.080	5023.81±46.277[ab]	3867.75±16.811[c]

Values are means±SE (n=5). a: significant change at $p < 0.05$ with respect to control group. b Significant change at $p < 0.05$ with respect to ESE group. c Significant change at $p < 0.05$ with respect to FE group.

Table. 7: Effect of plants' extracts on glutathione reductase, and glutathione-S-transferase levels of liver, kidney and testes of mice.

Groups / Parameter	Control	ESE	FE	ESE + FE
Hepatic GR (µmol/ g tissue)	494.34±45.788	666.35±34.652[a]	418.78±48.568[b]	311.87±26.127[ab]
Renal GR (µmol/ g tissue)	352.06±17.729	373.77±34.526	440.48±37.327	500.77±43.743[ab]
Testicular GR (µmol/ g tissue)	110.12±9.477	65.91±2.049[a]	64.30± 3.595[a]	86.81±13.724
Hepatic GST (µmol/ h/ g tissue)	5.07±0.119	5.76±0.137[a]	6.00±0.229[a]	5.31±0.246[c]
Renal GST (µmol/ h/ g tissue)	10.35± 0.743	9.79±0.404	12.31±0.203[ab]	12.34±0.559[ab]
Testicular GST (µmol/ h/ g tissue)	17.17±0.916	22.81±1.394[a]	31.60±1.427[ab]	19.29±0.581[bc]

Values are means±SE (n=5). a: significant change at $p < 0.05$ with respect to control group. b Significant change at $p < 0.05$ with respect to ESE group. c Significant change at $p < 0.05$ with respect to FE group.

The results of ESE+FE administration caused increase in GSH content of liver and shown decrease in SOD content of kidney and testis, while an increase in SOD content of liver comparing to FE group was noticed. In addition, ESE+FE administration showed significant decrease in catalase of kidney and testis comparing to FE group (Table 6).

ESE caused significant increase in GR content of liver by 34.79% at $p<0.05$), while shown significant decrease in GR content of testis (-40.14%) comparing to control group. In addition, ESE had shown significant increase in GST of liver and testis (13.60% and 32.86%, respectively) comparing to control group (Table 7). FE caused significant decrease in GR content of testis by -41.60% at $p<0.05$ and shown significant increase in GST of liver, kidney and testis (18.47%, 18.99% and 84.07%, respectively) comparing to control group (Table 7). The results of ESE+FE administration caused significant decrease in GR content of liver by -36.91%, while shown significant increase in GR content of kidney by 42.23% comparing to control group. In addition, ESE+FE administration showed significant increase in GST of kidney (19.29%) comparing to control group. In addition, FE caused significant decrease in GR content of liver and shown significant increase in GST of kidney and testis comparing to ESE group. The results of ESE+FE administration showed significant decrease in GR content of liver, while shown significant increase in GR content of kidney comparing to ESE group. In addition, ESE+FE administration showed significant increase in GST of kidney comparing to ESE group, while shown significant decrease in GST of liver and testis comparing to FE group, and showed significant decrease in GST of testis comparing to ESE group. Histopathology of liver and kidney in all groups displayed nearly normal architecture like control (data not shown). However, histopathology of testis in ESE and FE groups displayed nearly normal architecture like control (there was no change) as shown in Fig. 2 (B and C). Moreover, Fig. 2 (D) of mice treated with mixed Egyptian sage and fenugreek showed improved structure with well developed seminiferous tubules and spermatozoa.

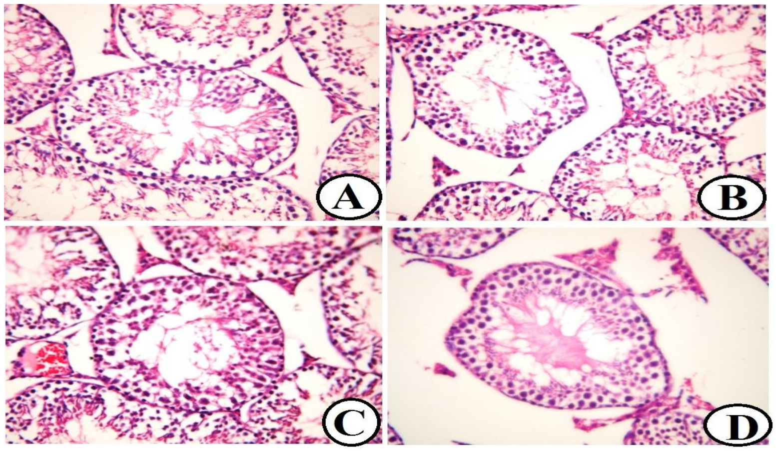

Fig. 2: (A) Testis of mice in control group, showing the normal histological structure of the seminiferous tubules with different series of spermtogenic layers and spermatozoa. **(B and C)** Testis of normal mice treated with Egyptian sage extract (ESE) and fenugreek extract (FE), respectively, for one week showing the normal histological structure of the seminiferous tubules was control-like. **(D)** Testis of normal mice treated with Egyptian sage and fenugreek extract (ESE+FE) for one week showing the normal histological structure of the seminiferous tubules was control-like with different series of improved spermtogenic layers and spermatozoa. Sections were stained with hematoxylin and eosin.

DISCUSSION

Herbal medicine has been used for more than 5000 years. The interest in polyphenols has grown considerably because of their high capacity to trap free radicals associated with different diseases. Phenols and flavonoids are very impotant plant constituents because of their antioxidant activity (Abdel Moneim *et al*., 2011; Annegowda *et al.,* 2010). The plant phenolics are commonly present in fruits, vegetables, leaves, nuts, seeds, barks, roots and in other plant parts (Kaviarasan *et al.,* 2007a). The antioxidant activity of phenolic compounds is mainly due to their redox properties which play an important role as free radical scavengers, reducing agents, quenchers of singlet oxygen and complexes of pro-oxidant metals (Mustafa *et al.,* 2010).

The total phenolic compounds may contribute directly to the antioxidant action therefore, it is necessary to investigate total phenolic content. Bukhari *et al.* (2008) showed TPC was in the range of 1.35-6.85 mg/g of the fenugreek extract in different solvents, and the total flavonoids are in the range from 208-653 µg/g of quercetin equivalent. Fenugreek seeds are rich in flavonoids. Flavonoids of fenugreek extract have been observed to possess antioxidant activity (Moskaug *et al.,* 2005; Myhrstad *et al.,* 2002; Ozcan *et al.,* 2005). Phytochemically, the Egyptian sage contains flavoniods, tannins, sterols/triterpenes and coumarins. Several flavonoids have been isolated from the plant, namely, apigenin-7-glucoside, Iuteolin 7-glucoside, chrysoeriol-7-glucoside, 6,8-di-C-β-glucosyl Iuteolin and chrysoeriol-7-glucoside (Rizk and El-Ghazaly,1995). The results indicate that the extract of fenugreek seeds and Egyptian sage contains antioxidants and protects cellular structures from oxidative damage.

Fenugreek administration showed non-significant change in liver enzymes as well as kidney function parameters, indicates its protective role against liver and kidney damage. Khalil (2002) showed that fenugreek extract treated rats showed non-significant change in serum AST, ALT, serum total protein and ALP. Also, Bin-Hafeez *et al.,* (2003) observed that, no elevation in liver function enzymes by fenugreek treatment. Fenugreek treated rats, showing normal appearance in kidney and Liver displayed nearly normal architecture (Khalil, 2002). Similarly, fenugreek powder did not alter AST, ALT and alkaline phosphates levels either in serum or liver in rats (Toppo *et al*., 2009).

Egyptian sage treatment showed non-significant change in liver and kidney parameters except for urea that significantly decreased after 7 days of sage administration, so these results indicated the protective role of Egyptian sage against liver and kidney damage. Despite the extensive phytochemical work on *Salvia aegyptiaca*, there are only few reports on the pharmacological properties of the plant, and no toxicological studies (Al-Yousuf *et al.,* 2002).

Fenugreek mixed with Egyptian sage showed decrease in some parameters of liver. In kidney function, the administration of mixed extracts showed decrease in urea, while non-significantly changed in uric acid and creatinine, indicates that it improve the liver and kidney function.

When diabetic rats were treated with aqueous extract of fenugreek seeds, marked recovery of testis and well-developed spermatogenic activity and Leydig cells were seen (Khalil, 2002). Hamden *et al.* (2010) reported that oral treatment of diabetic rats with fenugreek steroids improved the histological appearance of testis and epididymis with significant decrease in sperm shape abnormalities.

Peroxidation is important in food deterioration and in the oxidative modification of biological molecules particularly lipids. Inhibition of lipid peroxidation by any external agent is often used to evaluate its antioxidant capacity (Kaviarasan *et al.,* 2007a). Nitric oxide (NO) is a free radical involved in numerous and diverse cellular pathways in mammals (Torreilles, 2001).

The antioxidant property of fenugreek, inhibit lipid peroxidation of the erythrocytes (Thirunavukkarasu *et al.,* 2003). Several studies were found an improvement in organs functions after treatment with fenugreek (Devasena and Menon, 2002;

Thirunavukkarasu *et al.,* 2003). Fenugreek seeds have also been reported to raise the antioxidant levels and lower the lipid peroxidation in liver of ethanol intoxicated (Thirunavukkarasu *et al.*, 2003) and diabetic rats (Anuradha and Ravikumar, 2001). The antioxidant property of fenugreek has been studied *in vivo* and *in vitro* in ethanol-induced toxic rats which reduced lipid peroxidation and prevented enzyme leakage (Thirunavukkarasu *et al.,* 2003). Because of flavonoids in fenugreek, these act as antioxidant and potential inhibitors of nitric oxide synthase (Rao *et al.,* 2006; Sharififar *et al.*, 2009).

In this study, Fenugreek showed decrease in lipid peroxidation content of kidney and testis and decrease in nitric oxide content of kidney, but showed increase in nitric oxide content of testis. Egyptian sage showed decrease in lipid peroxidation content of liver, but showed non-significantly changed in levels of lipid peroxidation content of kidney and testis and nitric oxide. In the fenugreek, Egyptian sage mixture showed decrease in nitric oxide content of kidney and showed non-significantly changed in levels of nitric oxide content of liver and testis and lipid peroxidation.

The antioxidant enzymes SOD, CAT, GR are some of the biological antioxidant enzymes that directly scavenge free radicals or prevent their conversion to toxic products (Abdel Moneim, 2011; Freeman and Crapo, 1982). Superoxide dismutase enzyme is an important cellular antioxidant enzyme, which converts superoxide radical into H_2O_2 and O_2 (Kaviarasan *et al.*, 2007a). Catalase converts H_2O_2 to water and molecular oxygen, thus preventing the formation of extremely dangerous hydroxyl radical from H_2O_2 via the Fenton reaction (Bagnyukova *et al.,* 2005; Kehrer, 2000).

Fenugreek extract exhibited antioxidant property (Thirunavukkarasu *et al.,* 2003), which protects the functional organs and increase body weight (Khalil, 2002). Fenugreek administration to diabetic animals showed a reversal of the disturbed antioxidant levels of enzymes such as catalase, superoxide dismutase (Genet *et al.,* 2002). Choudhary *et al.* (2001) showed the diet containing fenugreek seeds probably enhances the antioxidant potential of animals through increased levels of GSH and specific activity of GST. In our study, fenugreek showed non-significantly changed in levels of GSH, but showed increase in catalase content of kidney, SOD content of kidney and testis and GST content of liver and kidney, while showed decrease in GR level on testis. Egyptian sage showed non-significantly changed in level of SOD, while showed significantly decrease in levels of GSH and GR in testis but showed increase in levels of catalase in kidney and testis, GR in liver and GST in liver and testis. In the mixture of two extracts showed non-significantly changed in levels of GSH and SOD, while showed increase in catalase content of liver, GST content of kidney and GR content of kidney, but showed decrease in GR level in liver.

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