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Determination of antioxidant potential of *Salix aegyptiaca* L. through biochemical analysis

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Salix aegyptiaca L., commonly called musk willow, is a medicinal herb in use since ancient time. However, information on its mode of protective action is scanty. Considering its significance and wide applications, here, we explored the same using its hydroethanolic bark extract. The results of nitric oxide assay indicated the free radical scavenging ability of the bark extract. In the *ex vivo* study, the extract of bark was found to exert protective effects against protein and membrane damage caused by the Fenton's reagent in the liver homogenate of C57BL/6 mice. In *in vivo* studies, the specific activities of enzymes involved in antioxidant function and the level of reduced glutathione (GSH) enhanced in the liver of six weeks old C57BL/6 mice treated with extract of bark. On the other hand, the oxidative damage in the liver determined in terms of TBARS was decreased significantly. The concomitant increase in the free radical metabolizing enzymes and the content of GSH; and inhibition of oxidative damage is suggestive of possibility of enhanced antioxidant potential of animals. The bark extract also enhanced the specific activities of phase I and phase II enzymes, which would likely to contribute in the detoxification. In conclusion, the *S. aegyptiaca* scavenges the free radicals, elevates the endogenous antioxidant status and detoxifies the toxic agents which determine its beneficial effects.

Keywords: Antioxidants, Musk willow, Oxidative stress, Reactive oxygen species

Salix aegyptiaca, commonly known as musk willow is a member of *Salicaceae* plant family and mainly cultivated in province of Iran[1]. This plant is a traditional herb, particularly in Middle East region, used as a cardioprotectant, gastroprotector, sedative, laxative, hypnotic, nervonic, anthelmintic and vermifuge etc[2]. Anticancer activity of ethanolic bark extract of *S. aegyptiaca* against colon cancer *in vitro* and *in vivo* has been reported earlier[3,4]. Though the medicinal properties of *S. aegyptiaca* show its importance in human health, the mechanisms of its beneficial effects are not well understood. The reactive oxygen species (ROS) are known to be involved in the cause and complication of several pathophysiological conditions. The ROS produced in the animal body interact with biomolecules, and alter their structure and function. If not repaired, these changes could result in damage and death of cell. Continuation of such events leads to enhanced oxidative stress and subsequently causes diseases. The inactivation of ROS and augmentation of cells prevent the adverse effects of oxidative stress. The presence of antioxidants in *S. aegyptiaca*[5,6] may have some beneficial effect by scavenging ROS and modulating the enzyme system involved in their metabolism as well as other systems with detoxification function. Therefore, in the present study, we examined the hydroethanolic extract of bark for its hepatoprotective effects in the liver of mice.

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Materials and Methods

Chemicals

Ascorbic acid, sodium nitroprusside (SNP), guanidine hydrochloride, 1-chloro-2,4-dinitrobenzene (CDNB), 5,5'-dithiobis-2-nitrobenzoic acid (DTNB), reduced glutathione (GSH), oxidized GSH (GSSG), pyrogallol, 2,6-dichlorophenol-indophenol (DCPIP), potassium ferricyanide, triton X-100, ethylenediaminetetraacetic acid (EDTA), sodium pyruvate, thiobarbituric acid (TBA), reduced nicotinamide adenine dinucleotide (NADH) and reduced nicotinamide adenine dinucleotide phosphate (NADPH) were obtained from Sigma Chemical Co. (St. Louis, MO, USA). The rest of the chemicals used were procured from local firms (India) and were of highest purity grade.

Preparation of hydroethanolic extract of *S. aegyptiaca* bark

The bark of *S. aegyptiaca* was procured from the garden in Qazwin, Iran. The plant was identified on the morphological basis by Dr. Mohammad Irfan

Qureshi, Assistant Professor, Jamia Millia Islamia, New Delhi, India and deposited as sample no. 20150217 in the departmental herbarium. The bark was dried and powered to make a hydroethanolic extract (water:ethanol; 20:80 v/v). The powder was dissolved in solvent with ratio of 1:10 (w/v). The solution was kept on shaking at 30°C for 48 h followed by the filtration through Whatman (No. 1) filter paper. The supernatant was concentrated using rotary evaporator (Buchi, Singapore) at 45°C followed by the desiccation through lyophiliser (Labconco Corp, USA). The resulting powder was stored at −20°C till the end of experiments.

Determination of antioxidant activity by nitric oxide assay

This assay was performed to investigate free radical scavenging property of *S. aegyptiaca* bark extract following the protocol of Kumaran & Karunakaran *et al*.[7]. Sodium nitroprusside (10 mM) was dissolved in phosphate buffer saline and added to different concentrations of *S. aegyptiaca* bark extract (2-20 µg/mL) dissolved in water and incubated for 150 min at 25°C. The reaction mixture without *S. aegyptiaca* bark extract served as control. After the completion of incubation, 0.5 mL of Griess reagent [1% sulfanilamide, 2% H_3PO_4 and 0.1% N-(1-naphthyl) ethylenediamine dihydrochloride] was added to each test tube and absorbance of coloured product was recorded at 546 nm in UV-1800 Spectrophotometer (Shimadzu Corp, Japan). Ascorbic acid was used as positive control.

Animals

In the present study, six weeks old male C57BL/6 mice were used for *ex vivo* and *in vivo* studies. The animals were kept and maintained in the Central Laboratory Animal Resources (Jawaharlal Nehru University, New Delhi) with a 12 h light/dark cycle and given standard food pellets and drinking water *ad libitum*. The animals were under observation throughout the experimentation, for body weight, food and water consumption, or any other sign of health toxicity. The experiments were approved by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India and Jawaharlal Nehru University Institutional Animal Ethics Committee (IAEC). The experiments were performed as per their guidelines.

***Ex vivo* studies**

The free radicals scavenging activity of was determined in an *ex vivo* system as described by Uddin *et al*.[8]. The animals were first euthanized, and their liver were excised and perfused with 0.9% saline. The livers were blot dried and 20% w/v liver tissue homogenate (LH) was prepared in phosphate buffer (25 mM, pH 7.4) using an electric homogenizer (Model no. RQ-127A, Remi, India). Free radicals were generated in homogenate using Fenton's reagent (0.5 mM $FeSO_4$: 0.5 mM H_2O_2; 1:1). Control group contained 4 mL liver homogenate (LH) + 8 mL double distilled water (DDW); positive control group, 4 mL LH + 4 mL Fenton's reagent + 4 mL DDW, while the remaining groups were mixed with *S. aegyptiaca* bark extract, giving the final reaction mixture as 4 mL LH + 4 mL Fenton's reagent + 4 mL of drug with two concentrations (400 and 800 µg/mL) each. The test tubes of reaction mixture were incubated at 37°C for 4 h. The tubes were centrifuged at 10000 rpm for 20 min (Hitachi, Japan). The resultant supernatant was then transferred into ultracentrifugation tubes and again centrifuged at 105000×g for 60 min in ultracentrifuge (Beckman Coulter, US). The cytosolic fraction supernatant was used to study protein carbonyl assay and whereas the pellet that represents microsomes were used to determine the peroxidative damage.

Estimation of protein carbonyl content

Protein carbonyl content was estimated according to method of Fagan *et al*.[9]. The protein carbonyl formation was expressed as moles of dinitrophenyl hydrazine (DNPH) incorporated/100 mg protein using a molar extinction coefficient of 21 $mM^{-1}cm^{-1}$.

Estimation of peroxidative damage

The microsomes were resuspended into homogenizing buffer and used to study the peroxidative damage by thiobarbituric acid reactive substances (TBARS) method as described by Varshney & Kale[10]. The result is expressed as nmole malondialdehyde (MDA) formed/mg protein.

***In vivo* studies**

Modulatory effect of *S. aegyptiaca* bark extract (40 and 80 mg/kg body wt.) on the reduced glutathione content, formation of malonaldehyde, specific activities of antioxidant enzymes and drug/carcinogen metabolizing enzymes of phase I and phase II systems was determined in the liver of mice. A suspension of *S. aegyptiaca* bark extract was made in 0.5% carboxymethyl cellulose (CMC, w/v) and given through oral gavage on alternate days for 14 days to C57BL/6 mice[11]. The mice were divided randomly into three experimental groups: Group I

(n=6) mice were fed with 0.5% CMC (vehicle) and served as control, Group II (n=6) and Group III (n=6) mice were fed with 40 and 80 mg/kg body wt. of *S. aegyptiaca* bark extract, respectively.

Preparation of homogenate, cytosol and microsomes

After two weeks treatment of *S. aegyptiaca* bark extract the animals were euthanized. The liver was perfused immediately with ice-cold NaCl (0.9%) and rinsed in chilled 0.15 M of Tris-KCl buffer (pH 7.4). The liver was then blotted dry, weighed quickly and homogenized in ice cold 0.15 M Tris-KCl buffer (pH 7.4) to get 10% (w/v) homogenate. An aliquot (0.5 mL) was separated to study the reduced glutathione content (GSH). The remainder homogenate was processed to prepare microsomes and cytosolic fraction as described earlier in *ex vivo* section. The specific activities of glutathione-S-transferase (GST), DT-diaphorase (DTD), and antioxidant enzymes were determined in cytosolic fraction. The specific activities of cytochrome P450 reductase, cytochrome b5 reductase and level of malonaldehyde were measured in microsomes.

Determination of reduced glutathione

Reduced glutathione (GSH) content was estimated as the total non-protein sulfhydryl using the method of Moron *et al*.[12]. Reduced glutathione (GSH) was used as a standard to calculate nmole of –SH content/g tissue.

Determination of superoxide dismutase and catalase activities

The specific activity of SOD was determined following the method of Marklund & Marklund[13] that involves the inhibition of autooxidation of pyrogallol at pH 8.0. A single unit of enzyme was defined as the quantity of superoxide dismutase required to produce 50% inhibition of autooxidation. The specific activity of SOD is expressed as µmole/mg protein. The specific activity of catalase was determined according to the method of Aebi[14], by analysing the disappearance of H_2O_2. The activity was calculated using the extinction coefficient as 40 μmol^{-1} cm^{-1}. The specific activity of catalase is expressed as moles of H_2O_2 reduced/min/mg protein.

Determination of glutathione reductase and glutathione peroxidase activities

The specific activity of GR was determined according to the method of Carlberg & Mannervik[15]. One unit of enzyme activity was defined as nmoles of NADPH consumed/min/mg protein, based on an extinction coefficient of 6.22 mM^{-1} cm^{-1}. The specific activity of GPx was determined by the method of Paglia & Valentine[16]. One unit of enzyme activity has been defined as nmoles of NADPH consumed/min/mg protein based on an extinction coefficient of 6.22 $mM^{-1}cm^{-1}$

Determination of NADPH-cytochrome P450 reductase and NADH-cytochrome b5 reductase activities

The specific activity of NADPH-cytochrome P450 reductase was determined according to Omura & Takesue[17] with some modifications, measuring the rate of oxidation of NADPH at 340 nm. The enzyme activity was calculated using extinction coefficient 6.22 $mM^{-1}cm^{-1}$. One unit of enzyme activity is defined as that causing the oxidation of 1 mol of NADPH per minute. The specific activity of NADH-cytochrome b5 reductase was measured following the method of Mihara and Sato with some modifications, measuring the rate of reduction of potassium ferricyanide at 420 nm by NADH[18]. The enzyme activity was calculated using the extinction coefficient of 1.02 mM^{-1} cm^{-1}. One unit of enzyme activity is defined as that causing the reduction of 1 mol of ferricyanide per minute.

Determination of glutathione S-transferase and DT-diaphorase activities

The specific activity of cytosolic GST was determined according to the method of Habig *et al*.[19]. The specific activity was expressed as micromoles of GSH–CDNB conjugate formed/min/mg protein using the extinction coefficient 9.6 mM^{-1} cm^{-1}. The specific activity of DTD was determined carring out the method of Ernster *et al*.[20]. The activity was calculated using extinction coefficient 21 mM^{-1} cm^{-1}. One unit of enzyme activity is defined as amount of enzyme required to reduce one micromole of DCPIP per minute.

Estimation of peroxidative damage and Protein

Lipid peroxidation was estimated in microsomes, following the method of Varshney & Kale[10] which has been described in the *ex vivo* section. Protein content of the samples was determined using Brafordford's reagent with BSA as standard at 595 nm.

Statistical analysis

The values were presented as mean ± SEM. The mean and significance of the differences between the data pairs was calculated by t-test (SigmaPlot 8.0). A value of P <0.05 was considered significant.

Results

Nitric oxide scavenging activity

Salix aegyptiaca bark extract exhibited nitric oxide ($NO^{\cdot}$) scavenging property. These $NO^{\cdot}$ free radicals

were generated by the addition of sodium nitroprusside, dissolved in PBS at physiological pH. The addition of *S. aegyptiaca* bark extract inhibited the production of nitrite ions which were estimated by adding Griess reagent (Fig. 1). The IC_{50} value of the extract was found to be 17.71±0.34 µg/mL. Ascorbic acid was used as positive control for comparison.

Protein carbonyl estimation

In the *ex vivo* studies, the treatment of *S. aegyptiaca* bark extract showed a significant protection by 12.77% (P <0.05) at concentration of 800 µg/mL against protein carbonyl damage caused by ˙OH free radicals, generated by the Fenton's reagent (Fig. 2).

Peroxidative damage

Inhibition of peroxidative damage by *Salix aegyptiaca*, in the *ex vivo* studies, was investigated in the microsomes. The peroxidation was initiated by addition of the Fenton's reagent and determined in terms of TBARS formation. The *S. aegyptiaca* bark extract @800 µg/mL of showed a significant reduction in the formation TBARS by 21.34% (P <0.05) (Fig. 3).

Reduced glutathione

Reduced glutathione content was found to be significantly elevated by 1.19 fold (P <0.05) and 1.31 fold (P <0.01), respectively, when the animal treated with 40 and 80 mg/kg body wt. of *S. aegyptiaca* bark extract (Fig. 4A).

Superoxide dismutase and catalase

The animals treated with 40 and 80 mg/kg body wt. of *S. aegyptiaca* bark extract exhibited significant increase in the specific activity of SOD by 1.41 fold (P <0.05) and 1.58 fold (P <0.005), respectively (Fig. 4B). Similarly, specific activity of catalase was also found significantly elevated in animals treated with 40 and 80 mg/kg body wt. of the extract by 1.26 fold (P <0.05) and 1.32 fold (P <0.05), respectively (Fig. 4C).

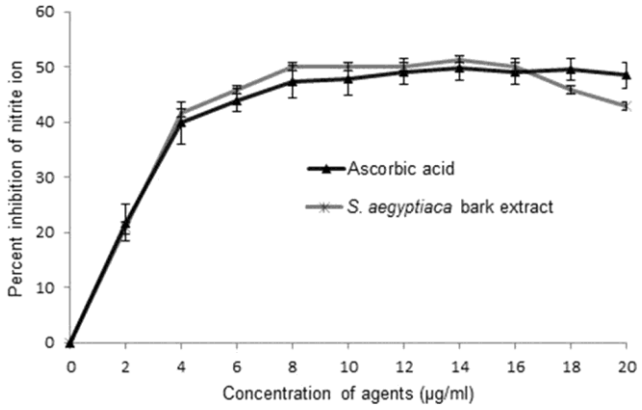

Fig. 1 — Nitric oxide assay; percent inhibition of nitrite ion by *Salix aegyptiaca* bark extract. [Sodium nitroprusside was added to different concentrations of *S. aegyptiaca* bark extract (2-20 µg/mL) and incubated for 150 min at room temperature. After the completion of incubation, Griess reagent was added to each test tube and absorbance of coloured product was recorded at 546 nm. Ascorbic acid (2-20 µg/mL) was used as positive control. Values are represented as mean ± SEM of three samples]

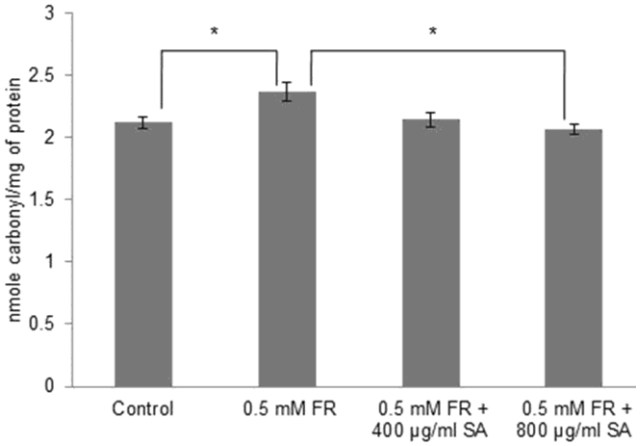

Fig. 2 — Protein carbonyl content; formation of protein carbonyls against Fenton reagent. [*Salix aegyptiaca* bark extract was added at concentration of 400-800 µg/mL. The test tubes were incubated for four hours at 37°C followed by two consecutive centrifugations. The supernatant was used for protein carbonyl assay. Abbreviations: FR, Fenton's reagent; SA, *S. aegyptiaca* bark extract. Values are represented as mean ± SEM of three samples. *(P <0.05) represents significant changes relative to control]

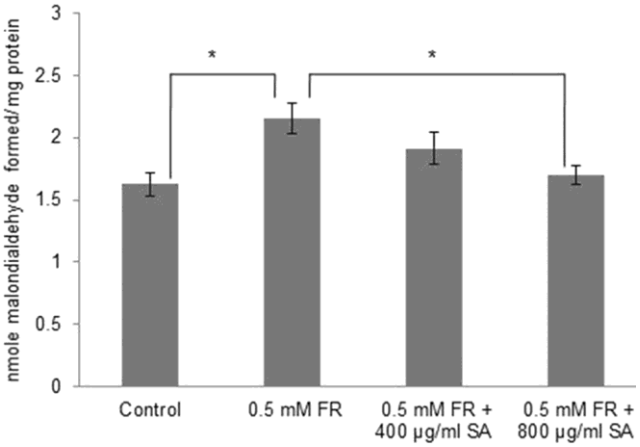

Fig. 3 — Peroxidative damage; formation of MDA against Fenton's reagent. [*Salix aegyptiaca* bark extract was added at concentration of 400-800 µg/mL. The test tubes were incubated for four hours at 37°C followed by two consecutive centrifugations. The resulting microsome was used for peroxidative damage estimation. Abbreviations: FR, Fenton reagent; SA, *S. aegyptiaca* bark extract. Values are represented as mean ± SEM of three samples. *(P <0.05) represents significant changes relative to control]

Glutathione reductase and glutathione peroxidase

The specific activity of GR was found to be significantly increased by 1.22 fold (P <0.05) and 1.38 fold (P <0.05), respectively, in the animal treated with 40 and 80 mg/kg body wt. of *S. aegyptiaca* bark extract (Fig. 4D). The specific activity of GPx was also found elevated by 1.29 fold (P <0.01) in the animals treated with 80 mg/kg body wt. of *S. aegyptiaca* bark extract (Fig. 4E).

Cytochrome P450 reductase and cytochrome b5 reductase

Treatment of *S. aegyptiaca* bark extract resulted in a significant increase in the specific activity of cytochrome P450 reductase by 1.25 fold (P <0.05) and 1.27 fold (P <0.005), respectively, when animals were treated with 40 and 80 mg/kg body wt. (Fig. 5A). The specific activity of cytochrome b5 reductase showed significant increase by 1.09 fold (P <0.01) in the animals treated with 80 mg/kg body wt. of *S. aegyptiaca* bark extract (Fig. 5B).

Glutathione S-transferase and DT-diaphorase

The significant enhancement in the specific activity of glutathione S-transferase (GST) was found by 1.43 fold (P <0.05) and 1.91 fold (P <0.01) in the animals treated with 40 and 80 mg/kg body weight of *S. aegyptiaca* bark extract, respectively (Fig. 5C). The specific activity of DT-diaphorase (DTD) was also found to be significantly elevated by 1.52 fold (P <0.05) and 1.63 fold (P <0.05), respectively, in the group treated with 40 and 80 mg/kg body wt. of *S. aegyptiaca* bark extract (Fig. 5D).

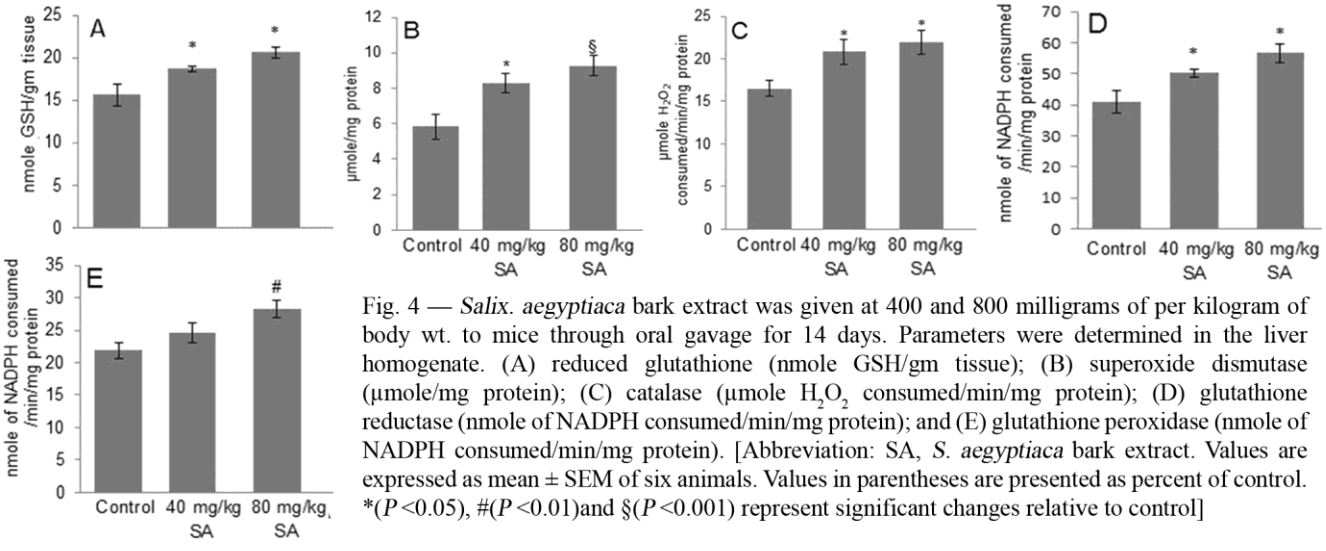

Fig. 4 — *Salix. aegyptiaca* bark extract was given at 400 and 800 milligrams of per kilogram of body wt. to mice through oral gavage for 14 days. Parameters were determined in the liver homogenate. (A) reduced glutathione (nmole GSH/gm tissue); (B) superoxide dismutase (µmole/mg protein); (C) catalase (µmole H_2O_2 consumed/min/mg protein); (D) glutathione reductase (nmole of NADPH consumed/min/mg protein); and (E) glutathione peroxidase (nmole of NADPH consumed/min/mg protein). [Abbreviation: SA, *S. aegyptiaca* bark extract. Values are expressed as mean ± SEM of six animals. Values in parentheses are presented as percent of control. *(P <0.05), #(P <0.01)and §(P <0.001) represent significant changes relative to control]

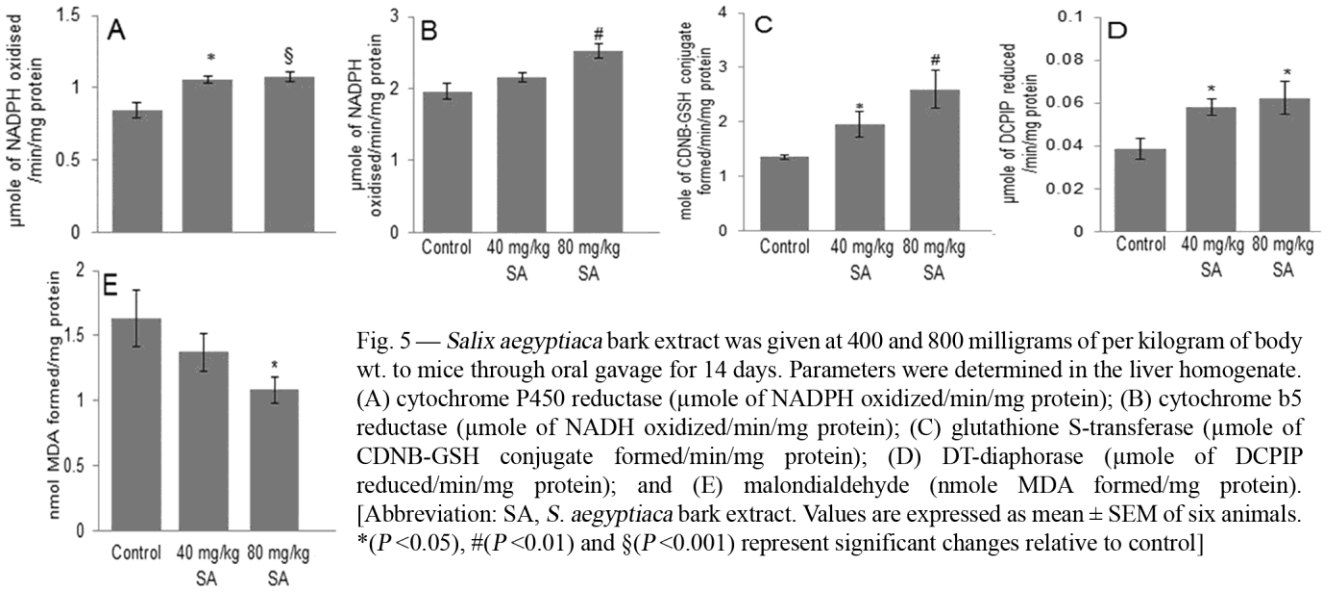

Fig. 5 — *Salix aegyptiaca* bark extract was given at 400 and 800 milligrams of per kilogram of body wt. to mice through oral gavage for 14 days. Parameters were determined in the liver homogenate. (A) cytochrome P450 reductase (µmole of NADPH oxidized/min/mg protein); (B) cytochrome b5 reductase (µmole of NADH oxidized/min/mg protein); (C) glutathione S-transferase (µmole of CDNB-GSH conjugate formed/min/mg protein); (D) DT-diaphorase (µmole of DCPIP reduced/min/mg protein); and (E) malondialdehyde (nmole MDA formed/mg protein). [Abbreviation: SA, *S. aegyptiaca* bark extract. Values are expressed as mean ± SEM of six animals. *(P <0.05), #(P <0.01) and §(P <0.001) represent significant changes relative to control]

Peroxidative damage

The peroxidative damage was determined in the microsomes prepared from the liver of mice, where a significant decrease in peroxidative damage was seen by 33.73% (P <0.05) with the treatment of 80 mg/kg body wt. of *S. aegyptiaca* bark extract (Fig. 5E).

Discussion

Reactive oxygen species, such as $O_2^{\cdot-}$ (superoxide), $^{\cdot}OH$ (hydroxyl), $HO_2^{\cdot}$ (per hydroxyl), $^1O_2$ (singlet oxygen), $NO^{\cdot}$ (nitric oxide), $ROO^{\cdot}$ (peroxyl), $RO^{\cdot}$ (alkoxyl) and H_2O_2 (hydrogen peroxide) generated in the animal body cause oxidative stress leading to cellular damage, which if not repaired, may induce several pathological conditions and their complications[21]. Since, *Salix aegyptiaca* has medicinal properties and antioxidant activity, it is expected to scavenge the free radicals and exhibit the protective role in biological systems. This possibility was examined in *ex vivo* studies, using the peroxidative damage and formation of protein carbonyl in microsomal and cytosolic fractions, respectively prepared from the liver homogenate and incubated in presence of Fenton's reagent to generate the free radicals as well as using nitroprusside to get nitric oxide free radicals in chemical system.

The peroxidation, a free radical chain reaction initiated by $^{\cdot}OH$ and, propagated by $ROO^{\cdot}$ and $RO^{\cdot}$, is a determinant to membrane damage[22]. In case of proteins, the interaction with free radicals, particularly $^{\cdot}OH$ could lead to the formation of carbonyl which is used as a measure of protein damage[23]. The nitric oxide assay is widely used to ascertain the antioxidant activity of a given agent[24]. In the present study, the bark extract of *S. aegyptiaca* significantly inhibited the peroxidation, protein carbonyl formation as well as nitric oxide generation. These findings are suggestive of the free radical scavenging ability of the extract of bark. It would be pertinent to mention that the several primary and secondary free radicals are generated in the cells. However, among those $^{\cdot}OH$ free radicals mainly contribute to the detrimental effects, being most reactive (for example the rate of its addition to carbon double bond is $k = 4.8\times10^9$ dm^3 $mol^{-1\text{-}s}$)[25] and also evident from its shortest biological half-life (1×10^{-9} s)[26]. It can be inferred from the above findings that *S. aegyptiaca* scavenges $^{\cdot}OH$ and other free radicals quite effectively and can be attributed to its medicinal properties.

In the animal body, free radicals are formed due to exposure to agents such as ionizing radiations and toxic chemicals as well as during the disturbed and normal metabolic processes like the release of electrons from the mitochondrial electron transport chain, conversion of molecular oxygen into superoxide by xanthine oxidase, β-oxidation in peroxisomes and others including inflammation, phagocytosis and arachidonate pathway and in turn cause an increase in oxidative stress and its burden[27]. In response to such situation, the defense systems both enzymatic and non-enzymatic, present in the body metabolize the free radicals and their molecular products and prevent the oxidative burden and its consequent complications. Therefore, it was interesting to see whether *S. aegyptiaca* modulates the enzymatic and non-enzymatic defense system.

Mice were treated with the bark extract for 14 days and the specific activities of enzymes involved in the free radical metabolism, and the level of glutathione (GSH) as a representative of the non-enzymatic system were determined in the liver. The extract enhanced the specific activities of superoxide dismutase (SOD), catalase, glutathione peroxidase (GPx) and glutathione reductase (GR) and the level of GSH as well. As a consequence, SOD is likely to dismutate $O_2^{\cdot-}$ to H_2O_2 and subsequently H_2O_2 into water and molecular oxygen by catalase and GPx. GR reduces the glutathione disulphides (GSSG) to GSH and helps to recycle and make it available for the antioxidant function of GPx[28]. As GSH is known to be free radical scavenger[29], its increased level would also contribute and enhance the free radical scavenging potential of *S. aegyptiaca.* Apart from the scavenging of free radicals, the extract of *S. aegyptiaca* augmented the metabolism of free radicals and restored the antioxidant potential of animals. It was supported by the lowered levels of peroxidative damage in the liver, under similar circumstances.

Since, the cytochrome P450 system, consisting of the phase I and phase II enzyme systems, metabolizes the toxic chemicals including carcinogens and prevents from their detrimental effects[30], it was sought to determine the possible link with medicinal properties of *S. aegyptiaca.* In the liver–of mice, fed with its bark extract for 14 days, the specific activities of cytochrome P450 reductase and cytochrome b5 reductase, important members of phase I enzymes system as well as of glutathione-S-transferase and DT-diphorase, members of the phase II enzymes system, elevated significantly. Under such conditions, the phase I enzymes metabolise the toxic agents and

convert them into hydrophilic metabolites, to serve as substrate for phase II enzymes system which subsequently increase their polarity and hydrophilicity, and facilitate their rapid elimination from the body[31]. DT-diaphorase which is member of phase II system, also functions as an antioxidant enzyme. Its enhanced specific activity in the liver of the mice treated with bark extract, is likely to accelerate the two-electron reduction of the xenobiotics, and reduce the quinones to prevent their conversion into semi quinones and provide protection from their reactive intermediate metabolites[32] and in turn maintains the antioxidant status of the animals. The modulation of cytochrome P450 system by *S. aegyptiaca* is suggestive of its chemopreventive efficacy against carcinogenesis and other toxic agents.

Conclusion

In this work we report that the extract of bark of *Salix aegyptiaca* has an antioxidant activity and able to enhance the specific activities of superoxide dismutase (SOD), catalase, glutathione reductase (GR) and glutathione peroxidase (GPx) which metabolize the free radicals generated in the cells through the modulation of cytochrome P450 system. The extract was also found to exert protective action against the membrane, protein and DNA damage and support its ability to scavenge the free radicals. The enhanced specific activities of phase I and II enzymes due to the extract treatment indicate its potential to augment the chemopreventive efficacy of *Salix aegyptiaca.*

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Conflict of interest

The authors declare no conflict of interests.

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