Antioxidant Properties of Aqueous Extract of *Salvadora persica* in Rats Subjected to Forced Swimming Test

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Abstract: *Salvadora persica* (SP) has been used as a medicinal purpose among global Muslim community. The present study was carried out to evaluate the antioxidant activity of aqueous extract of SP in male albino rats subjected to forced swimming test (FST) and related biochemical parameters. These activities were tested at dose of 900 mg/kg extracts of SP administered orally for 28 successive days. Rats were divided into two groups (12 animals/group): control group (distilled water) and extract group (SP aqueous extract at 900 mg/kg for two weeks. On day 14, animals will be further classified into four equal groups (six animals each): Undepressed control, depressed rats, extract plus undepressed rats and extract plus depressed for next two weeks. The weight of the animals will be measured every week. HPLC analysis revealed the profile of SP extract. It was found that extract significantly (p<0.001) increases mobility time in rats. It also showed significant (p<0.001) decreased in blood glucose and DNA fragmentation as compared to depressed group. Additionally, there was an imbalance of oxidants/antioxidants level in brain of depressed rats and a significant inhibition on serum butyrylcholinesterase (BuChE) activity in rats exposed to FST. The obtained results revealed that SP extract has got significant antioxidant activity. Hence, aqueous extract of SP may be explored further for the management of mental depression.

Keywords: *Salvadora persica*, Depression, Force Swimming Test, DNA Fragmentation, Oxidative Stress

1. Introduction

Depression can be defined as a high common disorder with increasing lifetime rates [1]. World health organization reported that about 450 million people suffer from behavioral disorder [2] and this percentage represent 12.3% of the global load of disease, and prophesied to rise up to 15% by 2020 [3]. Depression can be described as a state of mood or energy level that includes lack of motivation, a sense of hopelessness and a loss of physical energy. It is an emotional status that can result from many parts of our life. It is often a debilitating disease that affects a person’s work, family, sleeping and ability to assess life. A state of constant depression may suggest a biochemical imbalance or continual stress [4].

The common animal model of depression is Forced swimming test (FST) because it induces a depressive-like manner to conceal antidepressant effect of chemicals and can conclude depressive-like behavior in rats after exposure to other stressors [5]. FST explains a stressing situation which capable of creating a state of lower habit, reflected in the motionlessness induced in animals. The suggested mechanism by which stress causes its effects is via the hypothalamic–pituitary–adrenal axis and/or via the sympathoadrenomedullary system [6].

When free radicals are generated in extreme amounts in brain or the enzymatic and non-enzymatic antioxidant defense systems are ineffective, some chain reactions generating oxidative damage to lipids, proteins and DNA are activated and neurons are impaired or even dead [7]. Depressive condition has been accompanied to brain oxidative stress [8]. Free radicals can be evaluated indirectly by determination of the activities of some antioxidant enzymes such as Catalase (CAT), glutathione reductase.
(GSH-Rx) and the levels of the products of lipid peroxidation such as malondialdehyde (MDA) [9]. Moreover, there is evidence that oxidative damage to lipids, including lipid peroxidation, and proteins are important factors in the pathophysiology of neurodegenerative disorders [10].

Acetylcholine (ACH) is the principal vagus neurotransmitter and its action is finished by hydrolysis catalyzed by acetyl cholinesterase (AChE) (E.C.3.1.1.7) and butyrylcholinesterase (BuChE) (E.C.3.1.1.8) [11]. In humans, AChE is more abundant in the CNS, end plate of skeletal muscle, and erythrocytes membranes, while BuChE is more abundant in serum [12]. Although the exact physiological function of BuChE is unclear, it has been shown that it can promptly hydrolyze acetylcholine and to substitute AChE in maintaining the structural and functional integrity of central cholinergic pathways [13]. In addition, reports from the literature suggest a relationship between BuChE activity and risk factors for coronary artery disease [14] and that heart disease and depression are highly co-morbid [15].

Focusing in the usage of natural antioxidants is a new strategy for mitigate oxidative damage. Many of the negative effects of oxidative stress are decreased after supplementation with dietary antioxidants [16]. There is an increasing interest in total medicinal plant extracts, the largest value of which may be due to its constituents that subscribe to the modulation of the oxidative balance in vivo. Additionally, the special importance of total plant extracts is that they are easily available products, without purification to apply them in possible prevention of diseases [17]. Reasonably, the application of large quantities of plant extracts is not to be recommends before evaluation of important health issues regarding use of plant phenolics and flavonoids in particular.

The medicinally important species of *Salvadora persica* L. (SP) also known as Miswak, mustard tree and toothbrush tree, distributed mainly in tropical and sub tropical Asia. Miswak belong to family of Salvadoraceae and every part of the plant is used as a medicinal purpose among global Muslim community. Various phytochemical studies on *Salvadora persica* reported the presence of alkaloids salvadoreine, flavonoids, steroids, trimethyllyene and salvadoreinone. Flavonoids are responsible for antimicrobial, antiallergic, antioxidant, anti-inflammatory and antiploriferative activity. The leaves, roots and stem bark contain an alkaloid trimethylxilamine [18]. Various ingredients of *Salvadora persica* have valuable important biological properties, including critical antibacterial and antifungal activity. But till now no scientific works have been reported on its antidepressant activity. In light of above information, it is necessary to investigate and develop more effectual antidepressants with lower adverse-effect such as natural product extract (*Salvadora persica*) especially on the biochemical changes associated with the depression in rats induced by FST.

The mechanism of stress-induced depression is very complex and many actually beneficial artificial chemical antidepressants have decreasing rates of response and even acute adverse-effects [19].

**2. Materials and Methods**

**2.1. Plant Material**

The dried roots of SP extract were purchased from a local market in Jeddah, Kingdom of Saudi Arabia, and authenticated by Herbarium, King Abdulaziz University.

**2.1.1. Preparation of Aqueous Extract**

The root sticks were cut into small pieces and ground in grinding machine to fine powder, mixed with distilled water, and extracted for 24 h at 150 rpm at 25°C in a shaker. The mixture was then centrifuged at 3000 rpm for 20 min. The supernatants were subsequently filtered through Whatman No. 1 filter paper and the filtrate was concentrated in rotary evaporator (Buchi Rota vapor R-200) at 70°C and was lyophilized. The resulting powder was packed in a glass bottle and stored at 4°C until needed. It was dissolved in distilled water to prepare the exact aqueous dose (900mg Kg⁻¹ body weight) for orally injection [20]. The extract obtained (20.54% yield) was prepared in distilled water each time prior to experimentation.

**2.1.2. Quantitative Determination of Flavonoids by HPLC**

HPLC analyses were performed with Dionex Ultimate 3000 liquid chromatography (Germany) with four solvent delivery system quaternary pump (LPG 3400 SD) including a diode array detector (DAD 3000) with 5 cm flow cell, a manual sample injection valve equipped with a 20 µl loop and Chromeleon 6.8 system manager as data processor. The separation was achieved by a reversed-phase Acclaim TM 120 C18 column (5 µm particle size, 4.6 x 250 mm). A modified method of [21] was used. The mobile phase contains 1% aq. acetic acid solution (Solvent A) and acetonitrile (Solvent B), the flow rate was adjusted to 0.7 ml/min, the column was thermostatically controlled at 28°C and the injection volume was kept at 20 µl. A gradient elution was performed by varying the proportion of solvent B to solvent A. The gradient elution was changed from 10% to 40% B in a linear fashion for duration of 28 min, from 40 to 60% B in 39 min, from 60 to 90% B in 50 min. The mobile phase composition back to initial condition (solvent B: solvent A: 10: 90) in 55 min and allowed to run for another 10 min, before the injection of another sample. Total analysis time per sample was 65 min. HPLC chromatograms were detected using a photo diode array UV detector at three different wavelengths (272, 280 and 310 nm) according to absorption maxima of analyzed compounds. Each compound was identified by its retention time and by spiking with standards under the same conditions. The quantification of the sample was done by the measurement of the integrated peak area and the content was calculated using the calibration curve by plotting peak area against concentration of the respective standard sample. The data were reported with convergence limit in triplicate.
2.1.3. Quantitative Determination of Phenolic Compounds by HPLC

Phenolic compounds were determination by HPLC according to the method of [22] as follow: 5 gm of samples were mixed with methanol and centrifuged at 10000 rpm for 10 min and supernatant was filtered through a 0.2 μm Millipore membrane filter then 1-3 ml was collected in a vial for injection in to HPLC Hew lett Packared (series 1050) equipped with autosampling injection, solvent degasser, ultraviolet (UV) detector set at 280 nm and quaternary HP pump (series 1050). Hewlett Packard using a column Altman C18, 5mm (150mm x 4.6mm Alltech) the column temperature was maintained at 35°C. Gradient separation was carried out with methanol and acetonitrile as a mobile phase at flow rate of 1 ml/min. Phenolic acid standard from sigma Co. were dissolved in a mobile phase and injected into HPLC. Retention time and peak area were used to calculation of Phenolic compounds concentration by the data of Hewlett Packared software.

2.1.4. Determination of Vitamin C by HPLC Fraction

Chromatographic measurement was made using water 717 plus Auto sampler (Miford, USA), a UV-vis detector. The analytical column used was a Tracer Spheris orb C18 (250 x 4.6 mm 5 μm particle sizes). To analyze Vitamin C, 300 μl of sample mixed with 300 μl of 0.56% (w/v) meta-phosphoric acid solution were added to the same special centrifuge and filtration tube, shaken for 30 sec. and centrifuged at 1000 rpm for 10 min. 50 μl of the filtrate was directly injection into HPLC system. Isocratic chromatographic separation was carried out using a mobile phase of Milli-Q water with acetic acid 0.1% and methanol in a relative proportion the eluent flow rate was 0.7 ml/min and the column temperature was 25°C. Vitamin was identified by comparing the retention time of the sample peak with that of the vitamin C standard at 254 nm. Quantification was carried out using external standardization [23].

2.2. Animals

Adult male rats (150-200 g) were kept under the constant temperature (25±2°C) and light. They were give available ad libitum and tap water. Rats were randomly divided into two groups (12 animals in each group): rats received distilled water orally and served as controls and rats received a single dose of Salvadora persica extract (900 mg/kg PO) for two weeks (extract group). After completion of two weeks, animals were further classified into four equal groups (six animals each): Undepressed control group, depressed rats were daily exposed to FST for 10 min, extract plus undepressed rats received a single dose of Salvadora persica extract orally (900 mg /kg) and extract plus depressed rats were daily exposed to FST for 10 min and orally administered with plant extract at 900 mg/kg b. wt. for next two weeks. Following FST, rats were returned to their cages and access food and tap water freely. The weight of the animals was measured every week. All behavioral procedures were carried out in animal models of depression for the evaluation of antidepressant-like effects of extract in groups of rats orally supplemented with aqueous extract following exposure to repeated stress for two weeks.

2.3. Depression Induced by FST

The FST [24] was done by immersing rats individually in a 60×50×40 cm (L×W×H) filled with water (23–25°C) from which it could not escape out for 10 min for 7 days to cause behavioral depression. The rats initially were swam energetically but gradually became immobile; floating in the water with minimum movements of paws and legs to keep their head above the water level [25]. The total duration of immobility was recorded during the next 4 min of total 10 min test. The changes in immobility duration were studied after administrating extract in separate groups of animals. Each animal was used only once. All behaviors of rat in the test were recorded by video camera (Sony-SD/DIGITAL VIDEO CAMERA).

2.4. Blood Samples

At the end of the experimental period (28 days), animals were fasted for 8 h before blood collection in order to cause no interference in the analysis of blood glucose. Blood samples were withdrawn by end tail vein cutting method from overnight fasted animals and blood glucose was measured by one touch electronic glucometer ACU check.

2.5. Vital Organs Weights

Vital organs (brain, liver, and kidneys) were harvested from scarified rats. They were washed with ice-cold saline solution (0.9%w/v), blotted, and weighted. The weight of each organ was standardized to 100 g body weight of each animal.

2.6. Determination of DNA Fragmentation

DNA fragmentation assays for apoptotic changes in the brain were evaluated calorimetrically by DNA fragmentation and by agarose gel electrophoresis according to Burton, 1956 [26]. Brain samples were homogenized in 700 μl hypotonic lysis buffer and centrifuged for 15 min at 11,000 rpm. The supernatants containing small DNA fragments were separated; one-half of the volume was used for gel electrophoresis and the other half together with the pellet containing large pieces of DNA were used for quantification of fragmented DNA by the diphenyl amine (DPA) assay. The samples were treated with equal volumes of absolute isopropyl alcohol and NaCl to precipitate DNA. Extracted DNA was electrophoresed on 1% agarose gels containing 0.71 μg/ml ethidium bromides. At the end of the runs, gels were examined using UV transillumination. The diphenyl amine (DPA) assay reaction was applied according to Perandones et al. 1993 [27]. The colorimetric reaction was measured spectrophotometrically at 575 nm and the percentage of DNA fragmentation was calculated as follow:

\[
\text{DNA fragmentation} \text{(%)} = \frac{\text{OD of supernatant} \text{+ OD of pellet}}{\text{OD of supernatant} \times 100}
\]
2.7. Enzymatic Assay

After blood collection, all rats of each group were sacrificed under ether anesthesia; the brains from the different experimental normal and other groups were removed immediately, washed with saline then weighed and homogenized in 4 volumes of ice cold phosphate buffer saline 50 M pH 7.4 using an electrical homogenizer CH-6010 KRIENS-LU (The Northern Media Supply LTD, England). The homogenates were centrifuged at 4000 rpm for 15 minutes at 4°C using HERAEUS MULTIFUGE X3R CENTRIFUGE (Thermo Fisher Scientific, Germany). The supernatant was collected and aliquoted in eppendorf tubes and stored at - 80°C (Lab Tech, Indonesia). The supernatants were used for enzymes biochemical tissue analysis.

2.7.1. Total Antioxidant Capacity Level

Determination of the antioxidant capacity was performed by the reaction of antioxidants in the sample with a defined amount of exogenously provide hydrogen peroxide (H₂O₂). The antioxidants in the sample will eliminate a certain amount of the provided hydrogen peroxide. The residual H₂O₂ is determined calorimetrically by an enzymatic reaction which involves the conversion of 3, 5, dichloro–2–hydroxy benzensulphonate to a colored product [28].

2.7.2. Catalase Activity

It reacted with a known quantity of H₂O₂. The reaction was stopped after exactly one minute with CAT inhibitor. In the presence of peroxidase, the remaining H₂O₂ reacted with 3, 5-Dichloro–2–hydroxybenzene sulfonic acid and 4-aminophenazone to form a chromophore with color intensity inversely proportional to the amount of CAT in the original sample, each 1 unit =1µmol of H₂O₂ degraded for a minute, using a molar extinction coefficient of 43.6M⁻¹cm⁻¹ [29].

2.7.3. GSH-Reductase Activity

GSH-Red catalyzed the reduction of oxidized glutathione (GSSG) to reduced glutathione (GSH). GSH-Red is essential for the glutathione redox cycle that maintains adequate levels of reduced cellular GSH. According to the method of Goldberg and Spooner 1983, GSH-Red catalyzed the reduction of the oxidized glutathione (GSSG) in the presence of NADPH that oxidized into NADPH⁵. The decrease in absorbance was measured at 340mm [30].

2.7.4. Lipid Peroxidation Level

The thiobarbituric acid reactive substances (TBARS) as malondialdehyde were estimated by the method of Ohkawa et al., 1979 [31]. Briefly, to 0.2ml of brain homogenate, 0.2ml of 40% sodium dodecyle sulphate, 1.5ml of 20% acetic acid (prepared in 0.27M of HCl) and 1.5ml of 0.5% thiobarbituric acid were mixed together. The mixture was heated for 60min at 95°C in a water bath to give a pink color. The mixture was then centrifuged at 3500 rpm for 10min. Finally absorbance of the supernatant layer was read spectrophotometrically at 532nm, the molar extinction coefficient factor equal 1.56 x 105 M⁻¹cm⁻¹.

2.7.5. Total Protein Level

In the presence of an alkaline cupric sulfate, the protein produced a violet color, the intensity of which is proportional to their concentration [32]. The absorbance was read at 550nm.

2.7.6. Butyrylcholinesterase Activity

This assay depended on the ability of the enzyme to produce a thiocholine and form a complex with dithio-bis-nitrobenzoate [33]. The mean absorbance change was determined per 30 sec at 405 nm.

2.8. Statistical Analysis

All values are expressed as mean ± SD. Statistically significance was determined using one way ANOVA followed by Dennett’s comparison test. P values less than 0.05 were considered significant.

3. Results

3.1. HPLC Analysis

In the present study, qualitative analysis of the Phenolic compounds of root extract from SP was performed by HPLC analysis [Table 1]. A total of 23 Phenolic compounds were characterized by comparison to the retention times and UV spectra of authentic standards analyzed under identical analytical conditions. Under the optimized condition, a remarkable amount of pyrogallol, vanillic acid, ceumarin and salycilic acid were found in the aqueous extract. Traces of 4-aminobenzoic acid, α-coumaric acid, and cinnamic acid were also detected.

The present study indicated the occurrence of large amount of luteo 6-arabinose 8-glucose, luteolin, hespinid, rosmanric acid, kaemp. 3, (2-p-comaroyl) glucose and acacetin as flavonoids compounds [Table 2].

Ascorbic acid is ubiquitous in fruits and vegetables and the boon of ascorbic acid as nutraceutical in human nutrition dates back from time immemorial. The aqueous extract of SP was found to contain highest amount of ascorbic acid (83.19 ppm), Table 3.

Table 1. Profile of phenolic compounds in SP

<table>
<thead>
<tr>
<th>Phenolic compounds</th>
<th>(ppm) of phenolic compounds</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pyrogallol</td>
<td>1898.6</td>
</tr>
<tr>
<td>Gallic</td>
<td>104.86</td>
</tr>
<tr>
<td>4-Amino-benzoic</td>
<td>19.63</td>
</tr>
<tr>
<td>Protocatechuic</td>
<td>196.43</td>
</tr>
<tr>
<td>Catechein</td>
<td>113.1</td>
</tr>
<tr>
<td>Catechol</td>
<td>40.18</td>
</tr>
<tr>
<td>Chlorogenic</td>
<td>117.65</td>
</tr>
<tr>
<td>Epicatachin</td>
<td>65.31</td>
</tr>
<tr>
<td>P-OH-benzoic</td>
<td>106.27</td>
</tr>
<tr>
<td>Caffeine</td>
<td>133.18</td>
</tr>
<tr>
<td>Caffeic</td>
<td>56.71</td>
</tr>
<tr>
<td>Vanillic</td>
<td>97.75</td>
</tr>
<tr>
<td>P-coumaric</td>
<td>78.98</td>
</tr>
<tr>
<td>Ferulic</td>
<td>166.65</td>
</tr>
<tr>
<td>Iso-ferulic</td>
<td>---</td>
</tr>
<tr>
<td>e-vanillic</td>
<td>729.34</td>
</tr>
<tr>
<td>Ellagic</td>
<td>279.22</td>
</tr>
<tr>
<td>Alpha-coumaric</td>
<td>23.33</td>
</tr>
</tbody>
</table>

In the presence of an alkaline cupric sulfate, the protein produced a violet color, the intensity of which is proportional to their concentration [32]. The absorbance was read at 550nm.
Table 2. Profile of flavonoids compounds in SP.

<table>
<thead>
<tr>
<th>Flavonoids compounds</th>
<th>Flavonoids (mg/100g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Luteo 6-arabinose 8-glucose</td>
<td>372.88</td>
</tr>
<tr>
<td>Luteo 6-glucose 8-arabinose</td>
<td>11.65</td>
</tr>
<tr>
<td>A pig 6-rhamnose 8-glucose</td>
<td>35.02</td>
</tr>
<tr>
<td>A pig 6-glucose 8-rhamnose</td>
<td>12.07</td>
</tr>
<tr>
<td>Naringenin</td>
<td>52.13</td>
</tr>
<tr>
<td>Luteolin</td>
<td>102.92</td>
</tr>
<tr>
<td>Hespirdin</td>
<td>108.96</td>
</tr>
<tr>
<td>Rutin</td>
<td>2.89</td>
</tr>
<tr>
<td>Quercetin 3-o-glucoside</td>
<td>8.84</td>
</tr>
<tr>
<td>Rosmarinic</td>
<td>66.54</td>
</tr>
<tr>
<td>Apig. 7-O-neohesperidoside</td>
<td>2.12</td>
</tr>
<tr>
<td>Kaemp. 3,7-dirhamoside</td>
<td>10.62</td>
</tr>
</tbody>
</table>

Table 3. Profile of Ascorbic acid in SP.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Concentration (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ascorbic acid</td>
<td>83.19</td>
</tr>
</tbody>
</table>

3.2. Effect of SP Extract on Body Weight and Vital Organ Weights

Body weight was evaluated before and after 28 days of treatment is shown in figure 1.

![Figure 1. Body weight change of the rats during the experimentation. The results are expressed as the mean ± SD for 6 animals in each group.](image)

Administration of SP extract did not bring significant differences in body weights following 28 days of extract administration [figure 1]. Body weight gain ranged between 41.1 and 69.6% for the four treatment groups [Table 4].

Concerning the relative organs weight to the body weight of rats, the results showed that force swimming test did not produce any significant effect on the weight of various vital organs of rats after daily administration for 28 days [Table 5].

Table 4. Effect of oral administration of Salvadora persica aqueous extract (900mg/kg b.wt.) after 4 weeks on body weight and body weight gain% against depression in rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Parameters</th>
<th>Initial weight (g)</th>
<th>Final weight (g)</th>
<th>Body weight gain (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td>146.6 ± 15.2</td>
<td>241.5 ± 3.0</td>
<td>65 ± 0.71</td>
</tr>
<tr>
<td>Depressed</td>
<td></td>
<td>158.8 ± 17.4</td>
<td>268.3 ± 5.8</td>
<td>69.6 ± 0.34</td>
</tr>
<tr>
<td>Extract +Depressed</td>
<td></td>
<td>192.6 ± 3.6</td>
<td>271 ± 2.7</td>
<td>41.1 ± 0.70</td>
</tr>
<tr>
<td>SP extract</td>
<td></td>
<td>185.6 ± 5.1</td>
<td>264.8 ± 5.4</td>
<td>42.7 ± 0.57</td>
</tr>
</tbody>
</table>

Values are presented as mean ± standard deviations.

Table 5. Effect of SP extract on relative organ weights of the rats.

<table>
<thead>
<tr>
<th>Organ weight (% body weight)</th>
<th>Control</th>
<th>Depressed</th>
<th>Depressed +Extract</th>
<th>Extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brain</td>
<td>0.69 ± 0.13</td>
<td>0.67 ± 0.06</td>
<td>0.66 ± 0.05</td>
<td>0.64 ± 0.18</td>
</tr>
<tr>
<td>Liver</td>
<td>3.4 ± 1.9</td>
<td>3.2 ± 1.2</td>
<td>3.17 ± 0.69</td>
<td>3.17 ± 0.6</td>
</tr>
<tr>
<td>Kidney</td>
<td>0.68 ± 0.28</td>
<td>0.62 ± 0.21</td>
<td>0.64 ± 0.06</td>
<td>0.61 ± 0.29</td>
</tr>
</tbody>
</table>

Each value represents the mean ± SD, n=6.
### 3.3. Effect of SP Extract on Depressant Behaviors in Response to the FST

We examined the antidepressant-like effects of SP extracts in the FST. SP extract treatment in rats reduced the duration of immobility, reducing immobility by a maximum of 32.4% when administered at a dose of 900 mg/kg [figure 2a]. SP extracts also significantly increased the swimming time (16.8%) without any significant change in climbing [figure 2b].

**Figure 2.** Antidepressant-like effects of treatment with SP extracts on depressive behavior in response to the FST. Immobility (a); and swimming (b) were recorded during FST. Columns show the means ± SD (n = 6). *p < 0.05 vs. the depressed group.

Exposure to force swimming test for 28 days resulted in an significantly increased (p < 0.001) blood glucose level in depressed rats [figure 3], which was significantly decreased by SP extract at a dose of 900mg/kg when compared to depressed rats.

**Figure 3.** Effects of SP extract (900mg/kg bw) on blood glucose level in control and depressed rats (28 days). The results are expressed as the mean ± SD for 6 animals in each group. *p < 0.001 compared with control.

### 3.4. DNA Fragmentation

The current study revealed that force swimming test resulted in a significant increase in the percentage of DNA fragmentation in brain tissue by 25.6% compared to the control group [figure 4]. On the other hand, treatment with SP extracts decrease fragmentation to 5.7%.

**Figure 4.** DNA fragmentation in control and treated groups.
There was a significant elevation (p<0.001) in fragmented brain DNA level by 85.55% in depressed group when compared to the control group. Administration of extract, reduced the level of DNA fragmentation level by 42.53% (p<0.001).

The figure clearly shows DNA ladders that characterizes apoptosis in depressed brain [figure 5]. It seems that SP extract significantly decreased DNA fragmentation in brains in depressed rats.

3.5. Brain Antioxidants and Oxidative Stress in Different Groups of Rats

The depressed group in Table 6, showed very high significant decrease (P<0.001) in the brain antioxidant enzymes activities when compared with control group. Also the decrease (P<0.001) in total antioxidant capacity level was registered in depressed group. The oxidative stress was estimated as increment in TBARS level in the depressed group. The aqueous extracts of SP ameliorate the antioxidants status as they increase the antioxidant enzymes activities and also decrease the TBARS level in the depression & SP groups when compared with depressed group.
Table 6. Brain antioxidants and oxidative stress in different groups of rats.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>SP extract</th>
<th>Depressed</th>
<th>Depressed+ Extract</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Groups</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TAC (mM/L)</td>
<td>0.56±0.03¹ᵃ</td>
<td>0.56±0.03²ᵃ</td>
<td>0.47±0.008ᵇ</td>
<td>0.51±0.008⁴ᵃ</td>
</tr>
<tr>
<td>CAT (U/mg protein)</td>
<td>0.31±0.08ᵃ</td>
<td>0.32±0.01ᵃ</td>
<td>0.08±0.02ᵇ</td>
<td>0.32±0.02ᵃ</td>
</tr>
<tr>
<td>GR (U/mg protein)</td>
<td>16.5±3.9⁹ᵃ</td>
<td>13.5±0.8⁸ᵇ</td>
<td>6.8±2.1¹ᵇ</td>
<td>11.7±1.7³ᵇ</td>
</tr>
<tr>
<td>MDA (nmol/mg protein)</td>
<td>1.96±0.06ᵃ</td>
<td>1.86±0.12ᵃ</td>
<td>6.05±0.86ᵇ</td>
<td>2.28±0.45⁴ᵇ</td>
</tr>
<tr>
<td>Total Protein (g/dl)</td>
<td>2.79±0.15</td>
<td>2.78±0.55</td>
<td>2.27±0.56</td>
<td>2.32±0.16</td>
</tr>
</tbody>
</table>

Data are expressed as means ± SD (n=6 rats per group). Comparison between groups was made using ANOVAs test. Values not sharing a common letter differ significantly at p < 0.05.

3.6. Butyrylcholinesterase Activity in Different Groups of Rats

As in figure 6, there was a significant decrease (P<0.001) in the activity of the butyrylcholinesterase in serum of the depressed group when compared with the control ones. The depressed & SP groups have the ability to ameliorate the decrease of BuChE activity when compared with the depressed group.

![Figure 6](image)

Figure 6. Effects of SP extract (900mg/kg bw) on serum butyrylcholinesterase enzyme activity in control and depressed rats (28 days). The results are expressed as the mean ± SD for 6 animals in each group. P < 0.001 compared with control.

4. Discussion and Conclusion

The forced swimming test represents a valid animal model for screening ant fatigue potency of various bioactive compounds [34]. According to results of HPLC and the literature, the antidepressants like potential might be due to the presence of phenols, glycosides and flavonoids. Flavonoids and glycosides are mostly hydrolyzed into their glycols by mucosal and bacterial enzymes in the intestines, and then converted to conjugated metabolites during the absorption process [35, 36]. Transportation of these metabolites into the brain tissues via the blood brain barrier and their effect on the central nervous system (CNS) has been recently argued [37, 38]. Therefore, one of the antidepressant mechanism of SP is thought to involve flavonoids and glycosides which reach the brain tissues through the metabolizing process, protecting brain function from CNS disturbance and consequently, exerting an antidepressant effect. Thus, extracts of SP may have potential therapeutic value for the management of depressive disorders.

Body weight gain ranged between 41.1 and 69.6% for the four treatment groups. This result was in contrast to the results obtained by Osman et al., 2012 [39] who reported up to 14% changes in body weight of rats given M. oleifera extract for 21 days, attributing these changes to the rich nutrient quality of the extract.

Animal models of depression play an important role in the screening and evaluation of antidepressants [40]. The FST is an effective screening tool with good reliability and predictive validity [41]; the state of immobility in the FST is reported to mimic the symptoms of depression in humans and can be reversed by treatment with antidepressant drugs [42]. In the present study, aqueous extract of SP produced significant antidepressant like effect in rat in Forced Swim Test (FST). This test is quite sensitive and relatively specific to all major classes of antidepressant drugs [43]. In FST, rats are forced to swim in restricted space from which they cannot escape. This induces a state of behavioral despair in animals, which is claimed to reproduce a condition similar to human depression [44]. Our results show that SP root extracts can decrease immobility time in forced swim test. It is found that
SP can produce antidepressant like activity at a dose of 900mg/kg body weight after 28 days of treatment. The decrease in the immobility time is accompanied with the increase in swimming time. The precise mechanisms by which SP extracts may produce antidepressant like effect are not completely understood.

The results showed that the swimming time to exhaustion of extract-treated group was significantly longer (p <0.05) than that recorded for the control group. The maximum forced swimming times were 172.50 ± 28.3 and 201.7714 ± 18.78 seconds for depressed and depressed + extract groups, respectively. The shortness of the length of the swimming time indicates the degree of fatigue [45]. The results therefore indicated that SP extract enhanced the swimming capacity by delaying the onset of physical fatigue in rats. Similar results have been obtained by other workers who tested the ant fatigue potential of various plant extracts [34, 46].

Stress in optimum quantum acts as stimulator to achieve the best, but when it exceeds, it causes imbalance in some biochemical parameters which leads to suppression in physical endurance [47]. Increased blood glucose levels are reversed by antistress agents [48, 49]. During stress, blood glucose and cortisol level increases [50] which are found to be significantly reduced in SP extracts treated rats. The regular raised blood glucose like in condition of chronic stress depressed the cognitive functions and immune function. In this study the SP extract (900mg/kg) showed regulatory effect on circulating glucose significantly in depressed conditions. The anti diabetic activity of SP may be due to the presence of phytochemical (flavonoids, tannins, glycosides, sterols, and saponins) [51]. Plants that contain the active principals such as glycosides and flavonoids have antioxidant activity and are said to possess anti diabetic effect. Besides this, SP also contain several organic sulphur compounds and it is well known that sulphur derivatives show hypoglycemic effects. In fact, many plants containing sulphur are used traditionally as antidiabetic.

DNA fragmentation is very typical of the apoptotic process and its measurement with the diphenylamine colorimetric assay is preferentially used here to evaluate apoptosis in cells [52]. In the present study, it was shown that such depression by force swimming test induced apoptotic cell death including alterations in the immobility time and blood glucose level, could be prevented by the plant extract.

The animal experiments show that physical stress-induced depression are accompanied by lowered antioxidant levels and increased oxidative stress as a result of damage to fatty acids and proteins [8]. The brain is especially susceptible to such damage due to the large amount of lipids that compose its architecture. Lipid peroxidation targets the polyunsaturated fatty acids in the brain, thus, decreasing the membrane integrity. The decrease in membrane stability is especially important because the membrane contains receptor proteins and ion channel entities.

Along with its own deleterious effects, lipid peroxidation is also responsible for the inhibition of lipid repair enzymes such as lysophosphatidylcholine acyltransferase and fatty acyl CoA synhase [53]. Since neurons in the brain are strongly depend on aerobic respiration (consumes approximately 20% of the available oxygen), these neurons become much more vulnerable to oxidative stress. Oxidative stress has been related with depressive status [54]. It was reviewed that depression is associated with neurodegeneration and a reduced neurogenesis in the brain. The brain also contains high levels of lipids while possessing low amounts of antioxidants, thus further increasing its susceptibility to damage as the result of ROS and oxidative stress [55]. Along with their role in effecting the transcription of various proteins, ROS generated by reperfusion can itself cause direct cellular stress. So, the increase in TBARS levels in the brain as in may be inducing the neurodegeneration of the brain after exposing to depression induced by FST. This may be due to some chain reactions causing oxidative damage to lipids, proteins and DNA are activated and neuron are injured or even dead [7].

The increase in the oxidative stress was accompanied by the decrease in the antioxidants enzymes such as CAT, GSH-Red activity in depression group. On the other word the total antioxidant capacity was decreased by depression. Moreover some studies have shown that oxidative stress may play an important role in the pathophysiology of neuropsychiatric disorders [56].

Previous studies have shown that BuChE can be inhibited by homocysteine [57], and this inhibition is mediated by the generation of free radicals. Therefore, the reduced BuChE activity could be related to the increase oxidative stress [57]. On the other hand the antioxidant properties of SP extract play a vital role in restoring the balance of the antioxidants/oxidative stress induced by the FST in the brain of rats. As mentioned previously that Phenolic compounds that are present in the extract such as chlorogenic acid play a role in restoring the memory and may prevent Alzheimer disease. Also the rosamrinic acid in aqueous extract plays a role ant depressive agent in FST [58] that leads to imbalance between antioxidants and oxidative stress in rats’ brain.

Forced swimming test may be use as an experimental model for depression. The induced depression causes imbalance in the oxidants and antioxidant system in the brain of rats. Therefore, the changes in the brain neuronal signaling of depressed rats as reflected by the changes in the activity of butyrylcholine esterase.

The administration of aqueous extract of SP showed high ameliorative action in managing the biochemical and molecular changes accompanied by depression. As they have anti-apoptic and antioxidant action, it may be recommended as antidepressant agents. Further studies are needed to determine the effect of extract on chronic physical activity. These findings might be important in the development of new treatment strategies and in the medical practice.
References


REFERENCES


