INTRODUCTION

Most disorders are principally related to oxidative stress because of availability of reactive oxygen species. Widely recognized sorts of free radicals include, for example, superoxide anion (\(\text{O}_2^-\)) and hydroxyl radicals (\(\text{OH}\)) and also some non-free radical species, for example, hydrogen peroxide (\(\text{H}_2\text{O}_2\)) which are engaged with the pathophysiology and pathogenesis of diseases such as viral infections and inflammation [1]. Some of them have a positive impact in vivo, as they are used for energy generation, phagocytosis, direction of cell development, and growth as well as signaling between cells [2]. The free radicals are extremely harmful and cause damage when they attack the unsaturated fatty acids and cause oxidation that results in peroxidation of membrane lipid and DNA mutation which leads to cancer [3]. The scavenger of these free radical species might be conceivably counteracting and preventing interventions of the diseases caused by free radical damage [4]. There has been expanding interest for discovering natural antioxidants from plant sources to replace non-natural synthetic ones [5]. Natural antioxidants that are broadly present in plants can end the oxidative stress and useful to the human body [7]. These intense heterogeneous antioxidants interfere with the oxidative or antioxidative capability of the cell [8]. Traditional drug practitioners believe that plant components contain dynamic constituents that are important in enhancing the well-being of their patients and can be an alternative treatment [9]. Food antioxidants are very important due to their protective and defensive effect against oxidative stress, which involved in causing conditions such as anemia, diabetes, cardiovascular disease, and cancer. Portulaca quadrifida L. is a little, scattered, and succulent herb found all through the Mediterranean countries, and it belongs to the Portulacaceae family. It is utilized as a vegetable and also efficient in asthma, coughing, urinary discharge, infections, and ulcers as well as abdominal disorders, allergic dermatitis, and hemorrhoids [10]. This plant demonstrated antifungal activity against Candida albicans and Aspergillus fumigates [11]. Despite the fact that there are different secondary metabolites in various extracts of the plant that was uncovered and revealed in some studies [12]. The current examination is to identify the antioxidant cancer prevention effect of ethanol and polyphenolic extracts from P. quadrifida Linn. In the current study, oxidative stress is induced by limited restricted mobility and was estimated in terms of free radicals enzyme activities as catalase (CAT), superoxide dismutase (SOD), thiobarbituric acid reactive substances (TBARS), and glutathione (GSH)-transferase (GST). Antioxidant activity of aqueous extract of P. quadrifida Linn. leaves and their active ingredients were additionally examined beside the pre- and post-immobilization stress triggered oxidant or prooxidant status. The results are probably going to add to the understanding of P. quadrifida Linn. Extract effect in preventing or reducing stress-induced conditions that include oxidation damage to cellular damage, especially the brain. The objective of our study is to illustrate the impact of P. quadrifida extract on prooxidant changes induced by immobilization stress and identify the effect of active constituents of P. quadrifida on stress.

METHODS

Aqueous extract preparation from P. quadrifida leaves

Fresh leaves were collected locally and dried at room temperature. Aqueous powder was prepared and set up by reflux with deionized water at 80 °C. An amount of 100 mg/ml was set for use in oral administration.

Alkaloid and flavonoid fractions preparation

Dried leaves were powdered (500 g) and soaked in 1 l of methanol for 1 week under reduced pressure; it was dissolved in 1 l of ethyl acetate extracted with a (5%) aqueous hydrochloric acid (3×/100 ml). The latter
steps were done several times until a weak reaction with Dragendorff’s reagent was shown. After extraction with diethyl ether (500 ml×3), 25% NH₄OH was used to neutralize the acidic solution to a pH of 9–10 and then treated with chloroform. To obtain the alkaloid extract for the intragastric administration to rats, the samples were dried over sodium sulfate after being washed twice with water. To get rid of all phenolic components, the concentrated HCl was added to neutralize remaining part of the extract and treated several times with ethyl acetate until negative reaction observed for ferric chloride (ethyl acetate extract) with soluble extract was administered intragastrically to rats.

Animal model

Wistar male rats were selected weighing (180–200 g). The animals were in cages and divided and partitioned into groups; the Purina diet was supplied and provided with tap water ad libitum. Before starting, the animals were kept at room temperature 24±2 °C and light/dark cycles 12 h.

Experimental groups

To identify the ideal therapeutic dose which can modulate the free radical metabolism, a preliminary dose-dependent study (n=3) was done (results not shown). It was seen that the extract at 100 mg/kg dose for 2 h had the most efficient preventive activity on oxidative stress changes in the brain.

A total of 54 rats were chosen and separated into nine groups (one control group, one stress group, one group for crude extract, and six groups for active constituents). Only six experimental rats were kept in each group according to the latest guidelines for animal reduction in experiments.

A single dose of P. quadrifida extract alone (100 mg/kg body weight) has not caused a significant change in the previously mentioned parameters (results not shown) in unstressed control rats. Oral administration of P. quadrifida extract both before (pre-stress treatment) and after (post-stress treatment) immobilization stress treatment resulted in a significant modification of these parameters as compared to stress-treated rats and reverted these parameters toward their control values.

However, the post-stress oral treatment of extract (100 mg/kg body weight) was more efficient in restricting stress-induced decline of SOD (p<0.05), GST (p<0.01), CAT (p<0.001), and GSH (p<0.001) and an enhanced level of thiobarbituric acid reactive substance (TBARS) (p<0.001) in comparison to non-stressed (control) rats (Table 1 and Fig. 1). A single dose of P. quadrifida extract alone (100 mg/kg body weight) was more efficient in restricting stress-induced decline of SOD (p<0.05), GST (p<0.02), CAT (p<0.05), and GSH (p<0.05) and an enhanced level of TBARS (p<0.01) compared to stress alone or pre-stress extract treatments.

Statistical analysis

A thorough measurable analysis was held to decide the contrasts between control levels of the enzymes under examination with respect to treatments given to animals. A one-way ANOVA test was utilized at p<0.05 because the data were obtained by repeated investigation. A paired t-test was additionally performed at p<0.05 to confirm if the results had changed significantly (followed by a conjugal comparison with explicit Tukey’s analysis). A similar statistical analysis was performed to assess the difference in enzyme activity in non-stressed control rats that received treatments with extract before and after stress.

RESULTS

The 6 h of immobilization stress resulted in a significant decrease in the brain activities of SOD [14], CAT [15], and GST [16], the protein content was identified by the method [17], malondialdehyde and GSH. All the experimental protocols have adhered to the guidelines of the University Animal Welfare Committee (Research Ethics Committee A-17-06-04).

Table 1: Immobilization stress effect on brain tissue activities of enzymes compared to control rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>SOD (U/mg protein)</th>
<th>GST (U/mg protein)</th>
<th>CAT (U/mg protein)</th>
<th>MDA (nmol/mg protein)</th>
<th>GSH (nmol/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (6)</td>
<td>13.11±3.12</td>
<td>98.04±2.41</td>
<td>14.23±3.31</td>
<td>15.57±1.23</td>
<td>9.73±0.78</td>
</tr>
<tr>
<td>Stressed (6)</td>
<td>6.115±2.11</td>
<td>33.04±6.32</td>
<td>4.83±3.32</td>
<td>26.02±1.26</td>
<td>3.526±1.09</td>
</tr>
<tr>
<td>Crude extract (6)</td>
<td>14.17±3.05</td>
<td>92.98±8.22</td>
<td>12.24±1.09</td>
<td>14.61±2.01</td>
<td>8.127±3.16</td>
</tr>
</tbody>
</table>

The number of experimental rats is indicated in the parenthesis. *p<0.05, **p<0.01, ***p<0.001, as compared with control rats. SOD: Superoxide dismutase, GST: Glutathione-S-transferase, CAT: Catalase, MDA: Malondialdehyde, GSH: Glutathione.

Fig. 1: Effect of immobilization stress on brain tissue activities of superoxide dismutase, glutathione-S-transferase, catalase, and glutathione content compared to control rats.
No significant alterations in the parameters of controls were shown after the intragastric administration of flavonoid and alkaloid fractions. However, the treatment with the active constituents of *P. quadrifida*, both before and after the immobilization stress, caused a significant reversion of the stress-induced altered parameters toward their normal values but with a relative dominance by the latter (Tables 2 and 3).

In Fig. 2, the results show that the active constituents of this plant, both before and after the immobilization stress, altered biochemical parameters toward their normal values. However, the post-stress oral treatment of extract was found more effective in restricting stress.

**DISCUSSION**

The cells in the body are exposed to oxidants from various sources but are also equipped with an antioxidant system [18,19]. There are numerous explanations for the failure of the antioxidant defense system which could be either due to the excess production of free radicals or declined activities of scavenging enzymes or both of them that leads to lipid peroxidation and the oxidation of only few lipids can result in serious tissue damage and disease since lipid peroxidation is a self-propagating chain reaction [20,21]. Plant extracts can provide an essential aspect of the antioxidant system by attenuating free radicals [22]. A vital importance of the antioxidants present in *P. quadrifida* plant’s extracts in stress modulation can show the use of this extract as a therapeutic supplemental nutritional agent in the disorders related with the free radical damage.

A known way for the production of chronic emotional and physical stress is restraint stress and is shown to bring about antioxidant supplemental nutritional agent in the disorders related with the free radical damage.

The rats that received extract of *P. quadrifida* before being exposed to stress showed a significant resistance toward the derangement of their oxidative metabolism triggered by restraint stress, though the post-extract treatment (therapeutic) was more effective in restoring the altered oxidative metabolism toward their control values compared with pre-extract treatment (prophylactic). *P. quadrifida* has been reported in scientific literature as an effective antioxidant for the protection against diseases as a result of oxidative stress [25-27]. The extract was reported to contain many polyphenolic compounds, mainly flavonoids such as quercetin and rutin and some of the other chemical constituents reported in leaves are riboflavin, α-tocopherol, ascorbic acid, and GSH. The antioxidant property of *P. quadrifida* extract observed in our study might be due to the presence of polyphenolic compounds, GSH, and ascorbic acid. Our results also indicate that *P. quadrifida* extract could be used as a dietary supplement to fight various neurodegenerative diseases.

**CONCLUSION**

The extract of *P. quadrifida* before being exposed to stress showed a significant resistance toward the oxidative metabolism triggered by restraint stress, though the post-extract treatment (curative) was observed to be more effective in restoring the altered oxidative metabolism compared with pre-extract treatment (prophylactic). The antioxidant property of this plant extract recorded in our study could be due to the presence of the β-carotene, Vitamin C, and polyphenolic compounds. Our results also indicate that this plant extract could be used as a dietary supplement to combat and fight various neurodegenerative diseases and in terms of effects of alkaloids, tannins

**Table 2:** Treatment with the active constituents (alkaloid fractions) of *P. quadrifida*, both before and after the immobilization stress

<table>
<thead>
<tr>
<th>Groups</th>
<th>SOD (U/mg protein)</th>
<th>GST (U/mg protein)</th>
<th>CAT (U/mg protein)</th>
<th>MDA (nmol/mg protein)</th>
<th>GSH (nmol/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloid fraction (6)</td>
<td>12.23±5.1.15</td>
<td>87.16±9.32</td>
<td>10.74±1.24</td>
<td>14.60±1.62</td>
<td>9.09±1.37</td>
</tr>
<tr>
<td>Pre-stressed alkaloid treatment (6)</td>
<td>10.32±1.24</td>
<td>52.73±6.62</td>
<td>6.73±1.01</td>
<td>19.21±1.83</td>
<td>3.37±1.03</td>
</tr>
<tr>
<td>Post-stressed alkaloid treatment (6)</td>
<td>10.19±8.16</td>
<td>73.78±13.83</td>
<td>10.72±3.16</td>
<td>22.18±2.81</td>
<td>8.61±1.56</td>
</tr>
</tbody>
</table>

The number of experimental rats is indicated in the parenthesis. *p<0.05, p<0.01, as compared with stress alone. SOD: Superoxide dismutase, GST: Glutathione-S-transferase, CAT: Catalase, MDA: Malondialdehyde, GSH: Glutathione, P. quadrifida: Portulaca quadrifida*

**Table 3:** Treatment with the active constituents (flavonoid fractions) of *P. quadrifida*, both before and after the immobilization stress

<table>
<thead>
<tr>
<th>Groups</th>
<th>SOD (U/mg protein)</th>
<th>GST (U/mg protein)</th>
<th>CAT (U/mg protein)</th>
<th>MDA (nmol/mg protein)</th>
<th>GSH (nmol/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flavonoids (6)</td>
<td>15.32±1.20</td>
<td>107.36±15.19</td>
<td>13.46±5.03</td>
<td>16.73±5.01</td>
<td>10.18±1.92</td>
</tr>
<tr>
<td>Post-stressed flavonoid treatment (6)</td>
<td>14.22±1.13</td>
<td>91.12±8.93</td>
<td>14.73±2.97</td>
<td>11.75±2.86</td>
<td>13.71±1.43</td>
</tr>
</tbody>
</table>

The number of experimental rats is indicated in the parenthesis. *p<0.05, p<0.01, as compared with stress alone. SOD: Superoxide dismutase, GST: Glutathione-S-transferase, CAT: Catalase, MDA: Malondialdehyde, GSH: Glutathione, P. quadrifida: Portulaca quadrifida*
and others, our current study results were in accordance with a study done in 2016 which provided a general evidence that the extract of this plant contains medicinally important compounds and our study discussed the detailed specific neurological effect which was proven in the current study [28].

AUTHORS' CONTRIBUTIONS

Moayad Shahwan designed the experiments, drafted, edited the manuscript, and interpreted the results, and Sabrina Ait Gacem did the experiments and statistical analysis.

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

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Nil.

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