NEUROPROTECTIVE ANTIOXIDANT EFFECT OF PORTULACA QUADRIFIDA LINN. LEAVES EXTRACT ON IMMobilization STRESS-INDUCED CHANGES IN RAT'S BRAIN

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ABSTRACT

Objective: The current study was established to illustrate the effect of this plant’s active constituents on stress-induced diseases that include oxidation damage to cellular components, especially the brain.

Methods: Immobilization stress method is induced and initiated by putting separated animals in specially prepared mesh cages on a wooden plank. The animals were exposed to 6 h of stress. Wistar male rats were selected weighing (180–200 g). A total of 54 rats were selected and separated into nine groups and only six experimental rats were kept in each group.

Results: The post-stress oral treatment of extract (100 mg/kg body weight) was more efficient in restricting stress-induced decline of superoxide dismutase (p<0.05), glutathione (GSH)-S-transferase (p<0.02), catalase (p<0.05), and GSH (p<0.05) and an enhanced level of thiobarbituric acid reactive substance (p<0.01) compared to stress alone or pre-stress extract treatments.

Conclusion: The extract 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).

Keywords: Antioxidant, Immobilization, Neurodegenerative diseases, Portulaca quadrifida, Stress.

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 (O₂⁻) and hydroxyl radicals (OH) and also some non-free radical species, for example, hydrogen peroxide (H₂O₂) 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 response intervened by free radicals and will have advantageous roles in protecting the body from these diseases [6]. Polyphenolic compounds are exceptionally efficient in counteracting 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)-S-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 proxidant 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 proxidant 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% NH₂OH 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 HC1 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.

The groups were divided as follows:
- First group (control group): Received normal saline orally
- Second group (stressed): Was subjected to immobilization stress
- Third group (crude extract): Received the aqueous extract orally (100 mg/kg)
- Fourth group: Received alkaloid extract of the same dose
- Fifth group: Received alkaloid extracts of the same dose 1 h before (pre-stress treatment) the 6 h session of stress
- Sixth group: Received alkaloid extracts of the same dose 1 h after (post-stress treatment) the 6 h session of stress
- Seventh group: Received flavonoid extract of the dose (100 mg/kg body weight)
- Eighth group: Received flavonoid extracts of the same dose 1 h before (pre-stress treatment) the 6 h session of stress
- Ninth group: Received flavonoid extracts of the same dose 1 h after (post-stress treatment) the 6 h session of stress.

Immobilization stress method
Immobilization stress induced and initiated by putting separated animals in specially prepared mesh cages on a wooden plank. The animals were deprived of water and food during exposure to stress [13]. The animals were exposed to 6 h of stress. Control group of rats treated at the same time as stressed and placed in separated cages in a timely manner.

Experimental protocol
After the termination of experiment, the rats were sacrificed by injecting sodium pentobarbital (50 mg/kg) and exsanguinated. Blood was collected and centrifuged at 5000 rpm for 15 min; plasma was separated and quick-frozen at −40 °C until assay.

The plasma was subjected for the assay 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).

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 (p<0.001), GST (p<0.01), CAT (p<0.001), and GST (p<0.001) with significantly increased levels 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) 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<0.02), CAT (p<0.05), and GSH (p<0.05) and an enhanced level of TBARS (p<0.01) compared to stress alone or pre-extract treatments.

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

| Groups          | SOD (U/mg protein) | GST (U/mg protein) | CAT (U/mg protein) | MDA (nmol/mg protein) | GSH (nmol/mg protein) |
|-----------------|--------------------|--------------------|--------------------|-----------------------|-----------------------|
| Control (6)     | 13.11±2.3.12       | 98.04±2.4.12       | 14.23±3.3.1        | 15.57±2.1.23          | 9.73±2.4.12           |
| Stressed (6)    | 6.115±2.1.11       | 33.04±6.3.2       | 4.83±4.3.2         | 26.02±1.2.6           | 3.526±2.0.9          |
| Crude extract (6) | 14.17±3.0.5   | 92.908±8.22       | 13.241±1.09        | 14.613±2.0.1          | 8.127±3.16          |

The number of experimental rats is indicated in the parenthesis. *p<0.05, **p<0.02, ***p<0.01, ****p<0.001, as compared with control rats. SOD: Superoxide dismutase, GST: Glutathione-S-transferase, CAT: Catalase, MDA: Malondialdehyde, GSH: Glutathione.
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 supplement 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 changes in the brain of the rats [23]. In our study, 6 h of restraint stress resulted in a significant decrease in CAT, GST, SOD and activities, and GSH levels along with a significant rise in TBARS levels, which is a sign of lipid peroxidation. The observed changes in the above-mentioned parameters are due to the generation of ROS in the rat’s brain. The depletion of GSH content in rat brain may also result in increased lipid peroxidation, which serves as one of the guarding factors against oxidative stress [24]. GSH decreased levels might also be because of the declined activities of GST, SOD, and CAT. The GST enzymatic machinery also has peroxidase activity, which can directly attack the peroxides generated through oxidative reduction recycling [19]. The reduced GST activity observed in our study might also have contributed to the increased lipid peroxidation and the treatment of rats both before and after stress with crude extract of P. quadrifida leaves and its active constituents resulted in a significant increase in the antioxidant enzymes activities and GSH level along with a decrease in LPO (Figs. 1 and 2). Post-stress treatments of extract and its active constituents were found more effective in combating stress-induced prooxidant changes compared with pre-stress extract treatments (Tables 2 and 3).

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 antioxidant property of this plant extract recorded in our study could be due to the presence of the β-carotene, Vitamin C, and polyphenolic antioxidant property of this plant extract recorded in our study could be due to the presence of the β-carotene, Vitamin C, and polyphenolic antioxidant property of this plant extract recorded in our study could be due to the presence of the β-carotene, Vitamin C, and polyphenolic antioxidant property of this plant extract recorded in our study could be due to the presence of the β-carotene, Vitamin C, and polyphenolic antioxidant property of this plant extract recorded in our study could be due to the presence of the β-carotene, Vitamin C, and polyphenolic antioxidant property of this plant extract recorded in our study could be due to the presence of the β-carotene, Vitamin C, and polyphenolic antioxidant property of this plant extract recorded in our study could be due to the presence of the β-carotene, Vitamin C, and polyphenolic antioxidant property of this plant extract recorded in our study could be due to the presence of the β-carotene, Vitamin C, and polyphenolic antioxidant property of this plant extract recorded in our study could be due to the presence of the β-carotene, Vitamin C, and polyphenolic antioxidant property of this plant extract recorded in our study could be due to the presence of the β-carotene, Vitamin C, and polyphenolic

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

| Groups                              | SOD (U/mg protein) | GST (U/mg protein) | CAT (U/mg protein) | MDA (nmol/mg protein) | GSH (nmol/mg protein) |
|-------------------------------------|--------------------|--------------------|--------------------|-----------------------|-----------------------|
| Alkaloid fraction (6)               | 12.23±5.1.15       | 87.16±9.32         | 10.74±1.24         | 14.60±1.62            | 9.09±1.37             |
| Pre-stressed alkaloid treatment (6) | 10.32±1.24         | 52.73±6.62         | 6.73±1.01          | 19.21±1.83            | 3.76±1.03             |
| Post-stressed alkaloid treatment (6)| 10.19±1.66         | 73.78±13.83        | 10.72±1.46         | 22.18±2.81            | 8.61±1.56             |

The number of experimental rats is indicated in the parenthesis. *p<0.05, **p<0.02, ***p<0.01, ****p<0.001, 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

| Groups                              | SOD (U/mg protein) | GST (U/mg protein) | CAT (U/mg protein) | MDA (nmol/mg protein) | GSH (nmol/mg protein) |
|-------------------------------------|--------------------|--------------------|--------------------|-----------------------|-----------------------|
| Flavonoid (6)                       | 15.32±2.10         | 107.35±15.19       | 13.46±3.08         | 16.73±3.01            | 10.61±1.92            |
| Pre-stressed flavonoid treatment (6)| 10.12±2.19         | 83.12±10.33        | 10.36±2.28         | 13.62±1.97            | 9.51±2.13             |
| Post-stressed flavonoid treatment (6)| 14.27±1.13         | 91.12±9.13         | 14.73±2.97         | 11.71±2.86            | 13.71±2.14            |

The number of experimental rats is indicated in the parenthesis. *p<0.05, **p<0.02, ***p<0.01, ****p<0.001, 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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