Effect of Aqueous Extract of Truffle on Some Antioxidants in Rats Exposed to Oxidative Stress induced by Hydrogen peroxide

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Abstract

The current study dealt with using of hydrogen peroxide with 0.5% with drinking water to induce state of oxidative stress in male rats and estimate the ability of different concentrations of the aqueous extract of Truffle Water Extract to reduce the effects of oxidative stress during periods 0,15,30 days by measuring the following parameters in serum: Glutathione (GSH), Malondialdehyde (MDA), the activity of super oxide dismutase enzyme (SOD) and the peroxynitrate radical (ONOO). Fifty male rats with the age of 3-4 months and weight of 300-400 gm were distributed into 5 groups: group (1): control group received drinking tap water, group (2): treated with H2O2 0.5% in drinking water, group (3): treated with H2O2 and Truffle Water Extract 20%, group (4): treated with H2O2 and Truffle Water Extract 40%, group (5): treated with H2O2 and Truffle Water Extract 60%. Hydrogen peroxide treatment led to a significant decrease (P ≤0.05) of the GSH and SOD activities and increase of the MDA and ONOO levels in the serum during treatment periods compared with the control group. The results of using different concentrations of Truffle Water Extract on rats revealed slight protection from the oxidative stress induced by Hydrogen Peroxide, because its effect was affected on the level of GSH, SOD, MDA and ONOO levels in the blood serum in different experiment period.

Keywords: Truffle Water Extract, Oxidative stress, Glutathione (GSH) Malondialdehyde (MDA).

1. Introduction

The first reference of traditional medicinal plant use, dates back to 4000 years ago, where it was depicted on a Sumerian clay table that records remedies for various diseases [1]. Desert truffles have been documented as medicinal food in Chinese, Greek and Egyptian civilization, and were called the miracle of nature in Mesopotamia [2,3,]. Truffle is one of the oldest forms of food, it has been used as a meat substitute and consumed in large quantities due to their highly delicious taste and musky aroma [4,5]. It has a unique nutritional profile of unsaturated fatty acid, vitamins, minerals, and protein [3], and has been used for eye treatment in folk medicine [6]. Terfezia claveryi is among the various known edible truffles in the world, including Iraq where it grows naturally in the central, southern and western parts [7]. The quantity of truffles is usually varied from season to season depending on the amount of rainfall [5]. Several studies have reported the nutritional value of Iraqi truffles. [8], investigated the amino acid compositions of T. claveryi. [5], reported that the black truffle T. claveryi contains 19.60% of saturated fatty acid with a high amount of linoleic acid. Different chemical constituents were reported in Iraqi truffles of the T. claveryi species as (17.6 2%) protein and (62% linoleic acid) [9]. Another study conducted by Bokhary and [3], on the Saudi T. claveryi reported the presence of (16%) protein, (28%) carbohydrate and (78%) total moisture. Noteworthy, the same species of truffles from different regions may not exhibit the same chemical composition; the diversity of the chemical profile is probably controlled by many environmental factors, such as amount of rain and time, soil types and climatic changes [10]. Despite its nutritional importance, the biological activities and phytochemicals of T. claveryi did not receive much attention. In addition, no reports on cytotoxicity, apoptosis, or antiangiogenic properties of the truffle T. claveryi were found. The phytochemical classes have a great role in the prevention and treatment of several diseases such as cancer, aging, and inflammation [11]. Therefore, the aim of this study was to investigate the antioxidant, anticancer and antiangiogenic properties of the T. claveryi truffle. In addition, the apoptosis mechanism and the main chemical constituents of the most active extracts using (GC–MS) will be identified.
2. Material and Methods

2.1. Preparation of the Truffle Water Extract
The dry slice samples were ground to powder in order to increase the efficiency of extraction. The sample powder was twice pretreated with 70% ethanol in order to remove small molecular compounds, which can dissolve in ethanol. After being extracted by ethanol, the mushroom sample was mixed with DI water in the ratio of 1:5. The mixture was boiled for 2 h and the extract was collected and filtered. Rotary evaporator (Heidolph, Germany) was used to concentrate water extract to get a dry solid form for animal study.

2.2. Preparation of hydrogen peroxide
Hydrogen peroxide were used at a concentration of 50% from a company (Laboratory reagents, India) and diluted by drinking water to a concentration of 0.5%. The solution was prepared daily and given to experimental animals in special bottles for drinking water throughout the 30-day of experiment.

3. Experimental Animals
Fifty Albino male rats with aged (3-4) months and weight ranged (400-300) g were raised in a special room with the suitable conditions for raising of animals like feeding, temperature, lighting and ventilation. the animals were randomly distributed to (5) groups (n=10) animals and treated for 30 days as follows:
Group 1: drinking water was given during the experimental period and consider as Control group.
Group 2: Drinking water containing 0.5% hydrogen peroxide was given during the experiment.
Group 3: Drinking water containing 0.5% hydrogen peroxide and 20% Truffle Water Extract was given during the experiment.
Group 4: Drinking water containing 0.5% hydrogen peroxide and 40% Truffle Water Extract was given during the experiment.
Group 5: Drinking water containing 0.5% hydrogen peroxide and 60% Truffle Water Extract was given during the experiment.

3.1. Collection of blood samples
Blood samples were collected from animal groups during periods: before treatment (time 0) and 15 to 30 days after treatment. The blood was collected from the vein of the eye by a capillary tube containing heparin implanted in the inner corner of the eyeball. The blood was allowed to flow into a dry and clean test tube. Then the blood was clotted and the tubes were placed in the centrifuge at 3000xg for 15 minutes to separate the clotted part from the serum and the serum was used to conduct the biochemical tests.

3.2. Biochemical parameters
a. Superoxide Dismutase activity: determination of it follows the method described by [12].
b. Glutathione concentration: the determination of glutathione concentration follows the method described by [13].
c. Malondialdehyde concentration: measurement of it follows the method described by [14].
d. Peroxynitrite radical concentration: estimation the level in the serum follows the method described by [15].

3.3. Statistical analysis
The results were analyzed using the SPSS program for values representing the mean and the standard error, the data were analyzing using Two way analysis of variance. The differences between the groups were determined using the Duncan multiple range test, at a probability level (P ≤ 0.05).

4. Result and Discussions
Significant change with high concentration and leads to increase level of superoxide dismutase. Tables 1-4 manifested with the main results of this work.

4.1. Effects of Truffle Water Extract on the level of glutathione in serum
It is noted from Table (1) that there is a significant decrease in the level of GSH in the group of animals treated with hydrogen peroxide in all treatment periods compared with the period of (0 time) and with control group, these results are agreed with the findings of the researcher [15], who mentioned that GSH level in the blood of male rats exposed to oxidative
stress may be due to the role of GSH in the oxidation and reduction reactions, and giving of hydrogen peroxide in drinking water leads to depletion of GSH in blood and tissues. [16], mentioned that the occurrence of oxidative stress leads to an increase in the oxidation of GSH by inhibiting the converting pathway of the five-sugar (Pentose phosphate shunt) which determines the production of necessary NADPH to activation of the enzyme glutathione reductase which is necessary to re-manufacture of GSH from its oxidize shape. These results indicate that hydrogen peroxide is a powerful oxidizing agent that leads to oxidative stress by increasing free radical formation or lacks of defensive system in the body. Our results agreed with [7], who reported that fungi are well known to contain different bioactive polyphenolic compounds. These compounds act as effective antioxidants based on their excellent ability to scavenge free radicals and act as reducing agents. Thus, it was proposed by many authors that there are strong correlations between the antioxidant activities of certain type of fungi and the type and concentration of polyphenolic compounds. Different groups of desert truffles and mushrooms showed strong antioxidant activities based on their high polyphenolic and ergosterol contents.

4.2. Effects of Truffle Water Extract on the activity of superoxide dismutase in serum

The treatment with hydrogen peroxide showed a significant decrease in the activity of SOD enzyme during the treatment periods compared with its control group and the treated groups with the Truffle Water Extract. These results were agreed with the finding of [17], which showed that oxidative stress effects on the enzymatic and non-enzymatic antioxidants. The results agreed with [18], who reported in his study that the famous white desert truffle T. nivea showed high antioxidant activities. It was claimed that the fungal antioxidant capacity is attributed to the presence of various chemicals such as ascorbic acid, carotenoids, esterified phenolics, and free- and nonflavonoid phenolics and flavonoids. Another research showed that the small truffle Picoa lefebvrei is also among the most attractive mushrooms in folk medicine based on its high antioxidant properties.

4.3. Effect of Truffle Water Extract on the level of Malondialdehyde in the serum

In this study, we noted that using Hydrogen peroxide in drinking water caused significantly increasing in the level of MDA in all treatment periods compared with control group. [19], reported that the inducing of oxidative stress orally by hydrogen peroxide leads to occurrence of oxidative effects that acts on the peroxidation of unsaturated fatty acids in the lipid of membranes in the cells and eventually leads to the production a high amounts of cytotoxic compounds including MDA, and oxidative stress may be leads to release OH• radicals that very active in breaking down all tissues by increasing levels of lipid peroxidation, low-density lipoprotein (LDL), very low density lipoprotein (VLDL), as well as the oxidation of nitric oxide. These findings are what we noted in our study. Our results agreed with (Janakat et al., 2014) who revealed in his study that T. claveryi was reported to exhibit a higher oxidative inhibition on lipid peroxidation, and deoxyribose and has the ability to scavenge nitric oxide radical. [7], attributed that decreasing the level of Malondialdehyde during using Truffles due to its contains of antioxidants such as vitamin A, C, β-carotene and phenolic compounds, which can scavenge peroxyl radicals and chelate ferric ions, thus reducing lipid peroxidation.

4.4. Effect of Truffle Water Extract on the level of peroxy nitrite in the serum

The results showed that the treatment with hydrogen peroxide caused significantly increasing in the level of peroxy nitrite radical in the treatment periods in pilot groups compared with control group. These results are agreed with what the researcher [20], who showed a significant increase in the level of peroxy nitrite radical in the treated group with hydrogen peroxide. The giving of Hydrogen peroxide with drinking water causes an increase in the level of the negative superoxide radical which derived from its high level has the ability to bond with the proton to be HO2 which has highly effectiveness in peroxidation of lipids [21]. [22], revealed that eating of fruits and vegetables including leads to increases the antioxidants in the body, thereby reducing the harm caused by oxidative stress and reducing free radicals. These findings are what we found them in our study. Our results agreed with [23], who explained in his study that among the constituents of truffles and mushrooms are vitamins A, C, and b-carotene, all of which have protective effects because of their antioxidant and antiradical properties. Truffles and mushrooms also contain many phenols, which are very efficient scavengers of peroxyl radicals. Moreover, the action of phenolic compounds is related to their capacity to reduce and chelate ferric iron, which catalyzes lipid peroxidation. Several authors have observed the blocking effects of Hydnum mushrooms on induced liver lipid peroxidation [24]. Other study [25], explained that the role of truffle in the scavenging free radicals like peroxy nitrate through it’s contain a high amount of antioxidant which was demonstrated to have a very powerful hepatoprotective activity when evaluated in rats using the potent hepatotoxin carbon tetrachloride.
Table 1. Effects of Truffle Water Extract on Super oxide dismutase activity.

| Treating                               | Super oxide dismutase activity | Treating periods (day) |
|----------------------------------------|-------------------------------|------------------------|
|                                        |                               | 0                      | 15                      | 30                      |
| Control group                          | 0.042±0.0054                  | 0.040±0.002            | 0.038±0.0012            |
| Hydrogen peroxide 0.5 %                | a A                           | a A                    | a A                     |
| Hydrogen peroxide 0.5 % and 20 % Truffle Water Extract | 0.043±0.001              | 0.044±0.0013           | 0.050±0.0022            |
|                                        | a A                           | a A                    | b C                     |
| Hydrogen peroxide 0.5 % and 40 % Truffle Water Extract | 0.042±0.0081            | 0.048±0.0022           | 0.054±0.002             |
|                                        | a A                           | b C                    | c D                     |
| Hydrogen peroxide 0.5 % and 60 % Truffle Water Extract | 0.042±0.002              | 0.049±0.0002           | 0.059±0.0014            |
|                                        | a A                           | b C                    | c D                     |

- Values represent the mean ± standard deviations.
- Different letters in the columns and rows indicate a significant difference at a significant level (P ≥0.05).

Results presented in Table (2) revealed that treatment of Truffle Water Extract showed significant changes with high concentration and leads to increase level of glutathione.

Table 2. Effects of Truffle Water Extract on Glutathione concentration.

| Treating                               | Glutathione concentration (μmol/L) | Treating periods (day) |
|----------------------------------------|-------------------------------------|------------------------|
|                                        |                                     | 0                      | 15                      | 30                      |
| Control group                          | 1.63±0.06                           | 1.79±0.05              | 1.98±0.03               |
| Hydrogen peroxide 0.5 %                | 1.54±0.08                           | 0.50±0.02              | 0.41±0.03               |
|                                        | a A                                 | b A                    | c B                     |
| Hydrogen peroxide 0.5 % and 20 % Truffle Water Extract | 1.57±0.10          | 1.82±0.06              | 2.03±0.12               |
|                                        | a A                                 | b C                    | b C                     |
| Hydrogen peroxide 0.5 % and 40 % Truffle Water Extract | 1.60±0.13          | 2.37±0.10              | 2.96±0.05               |
|                                        | a A                                 | b D                    | c D                     |
| Hydrogen peroxide 0.5 % and 60 % Truffle Water Extract | 1.53±0.10          | 2.88±0.03              | 3.66±0.09               |
|                                        | a A                                 | b E                    | c E                     |

- Values represent the mean ± standard deviations.
- Different letters in the columns and rows indicate a significant difference at a significant level (P ≥0.05).

Results presented in Table (3) revealed that treatment of Truffle Water Extract showed significant changes with high concentration and leads to modify level of Malondialdehyde.

Table 3. Effects of Truffle Water Extract on Malondialdehyde concentration.

| Treating                               | Malondialdehyde concentration (μmol/L) | Treating periods (day) |
|----------------------------------------|----------------------------------------|------------------------|
|                                        |                                       | 0                      | 15                      | 30                      |
| Control group                          | 0.52±0.022                            | 0.49±0.010             | 0.47±0.06               |
| Hydrogen peroxide 0.5 %                | 0.54±0.010                            | 0.68±0.020             | 0.95±0.050              |
|                                        | a A                                   | b A                    | c B                     |
| Hydrogen peroxide 0.5 % and 20 % Truffle Water Extract | 0.52±0.020          | 0.66±0.010             | 0.86±0.030              |
|                                        | a A                                   | b B                    | c B                     |
| Hydrogen peroxide 0.5 % and 40 % Truffle Water Extract | 0.54±0.010          | 0.54±0.012             | 0.47±0.083              |
|                                        | a A                                   | b C                    | b D                     |
| Hydrogen peroxide 0.5 % and 60 % Truffle Water Extract | 0.55±0.020          | 0.48±0.020             | 0.39±0.022              |
|                                        | a A                                   | b C                    | c E                     |
Values represent the mean ± standard deviations.
- Different letters in the columns and rows indicator to significant difference at a significant level (P ≥0.05).

Table 4. Effects of Truffle Water Extract on Peroxynitrite radical concentration.

| Treating                                      | Peroxynitrite radical concentration (μmol/L) |
|-----------------------------------------------|-----------------------------------------------|
|                                               | Treating periods (day)                        |
|                                               | 0    | 15   | 30   |
| Control group                                 | 49.26±2.21 | 50.34±1.89 | 49.73±1.27 |
| Hydrogen peroxide 0.5 %                       | a A  | a A  | a A  |
| Hydrogen peroxide 0.5 % and 20 % Truffle Water Extract | 50.93±1.24 | 51.69±1.66 | 52.75±1.85 |
| a A                                           | a A  | b B  | c B  |
| Hydrogen peroxide 0.5 % and 40 % Truffle Water Extract | 49.80±1.84 | 44.74±1.23 | 41.12±1.47 |
| a A                                           | a A  | b C  | c C  |
| Hydrogen peroxide 0.5 % and 60 % Truffle Water Extract | 50.27±1.26 | 42.03±1.89 | 37.90±1.76 |
| a A                                           | a A  | b C  | c D  |

Conclusion

The results of using different concentrations of Truffle Water Extract on rats revealed slight protection from the oxidative stress is induced by Hydrogen Peroxide, because its effect on the level of GSH, SOD, MDA and ONOO levels in the blood serum in different experiment period. Recommendation of using the truffel in nutrition and reliance on them and as researchers platform for studying the lipid profile and liver's enzymes.

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