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Effect of melatonin treatment on serum levels of mineral elements, total oxidant and antioxidant capacity in mustard exposed patients

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ABSTRACT

Sulfur mustard (SM) is a mutagenic compound that causes oxidative stress, antioxidant depletion even several years after exposure. Melatonin is an alternative medication that has antioxidant peroperties. The aim of this study was to investigate the effect of melatonin treatment on serum levels of several mineral elements, total antioxidant (TAC) and total oxidant status (TOS) in sulfur mustard-exposed patients. Victims with lung and sleep disorders was divided randomly to placebo and melatonin groups. They received melatonin or placebo for 56 days. Blood samples were taken before and after drug usage. The concentrations of serum trace elements (manganese, zinc, copper, and iron) and one other essential element (magnesium) were determined by graphite furnace and flame atomic absorption spectroscopy. TAC and TOS in serum were determined colorimetrically. Results showed that melatonin administration increases the magnesium and TAC concentrations. After the drug usage, placebo and melatonin groups had the highest TOS and TAC contents, respectively. Therefore, melatonin can be considered as a compound suitable for suppression of oxidative stress and helps to decrease oxidative damages induced by mustard gas.

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Keywords
Antioxidants,
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INTRODUCTION

Melatonin is a hormone and a derivative of the amino acid tryptophan produced primarily by the pineal gland. It is secreted in the dark during night and modulates sleep, reproduction, circadian rhythm, and immunity [1]. Beside hormonal effect, melatonin is a powerful antioxidant and has protective effects against both reactive oxygen and nitrogen species. Melatonin and it's oxidation products, 6-hydroxymelatonin, 3-hydroxymelatonin and N-acetyl-N-formyl-5-methoxykynurenamine, are antioxidants. Therefore, melatonin can be considered as a compound suitable for suppression of oxidative stress and generation of reactive oxygen and nitrogen species [2, 3].

Regarding the nutritional role of micronutrients (calcium, iron, zinc, etc.) in different groups, especially children and pregnant women, the probably effect of melatonin on the absorption of micronutrients and risk of nutritional deficiency and nutritional diseases is extremely important.

On 22nd September 1980, the war between Iran and Iraq was occurred. This invasion was one of the longest conflicts in the 20th century, resulting in deaths and injuries of many military and nonmilitary people. Iraq extensively used chemical weapons during the war and about 44 000 Iranian soldiers lost their lives during the 242 chemical attacks [4]. Despite passing 28 years after the ceasefire, the injured people are one of the main health challenges in both countries and we occasionally see deaths among them due to their illnesses. Numerous studies performed by Iranian researchers showed that the chemical agents could lead to a

wide variety of early and late complications including respiratory, ocular, dermatological, immunohematological, psychological disorders and other toxic effects in exposed people. The late effects can continue even 40 years later after the initial exposure [5, 6].

Sulfur mustard (mustard gas) is a potent alkylating agent. It was used as a chemical weapon in the Iraq–Iran war. The victims are still suffering from late toxic effects of this warfare that respiratory disorders are the most lethal and disabling consequences [7].

The goal of this study was to evaluate whether the treatment of mustard gas victims with melatonin resulted in alteration of the serum level of some trace elements (manganese, zinc, copper, and iron) and one other essential element (magnesium). The second purpose of the study was to evaluate the ability of melatonin to influence the total antioxidant capacity (TAC) and total oxidant status (TOS) in the serum of victims.

MATERIALS AND METHODS

Study population

The patients were individuals who had a documented encounter with SM during the Iran-Iraq war. The exclusion criteria were as follows: (1) cigarette smoking or a history of exposure to any other respiratory pollutants; (2) history of allergic rhinitis or other allergic diseases before exposure to sulfur mustard; (3) history of asthma, lung cancer and pulmonary tuberculosis, acute inflammation at upper and lower respiratory system; (4) history of drugs consumption that are associated with lung injuries; (5) history of systemic diseases or other chronic abnormalities which are associated with lung problems (such as heart disorders, kidney diseases, hepatitis, cirrhosis); (6) history of diabetes and hypertension.

Study design

This is a double-blind clinical trial study (Clinical trials registration number: 75673) on mustard gas victims with mild or mediate lung disorder and poor sleep quality. Patients were randomized to the melatonin or placebo group. All patients were male and above 50 years old. The control group were selected from the same age and sex.

Fast-release 3 mg melatonin (Natural Wealth, USA) or placebo was supplied in identical capsules to be taken in a single dose 1 h before bedtime for 56 consecutive days. Other medications were maintained as prescribed by the attending physician. Patients and investigators were unaware of treatment allocation at all times. The research project was approved by the Human Research Ethics Committee of the Baqiatallah University of Medical Sciences in Iran.

Mineral analysis

Blood samples were taken at the beginning and at the end of the trial on day 0 and 56, respectively. After sampling, the blood was centrifuged at 750 *g* for 15 minutes and the serum kept at -80°C until analysis. The samples with hemolysis were discarded. Digestion of the samples was performed using a mixture of Perchloric and nitric acid (3:7 ratios respectively). The concentrations of magnesium, manganese, zinc, copper and iron in the serum were determined by atomic absorption spectrophotometry (Shimadzo AA-670, Kyoto, Japan) in an acetylene–air flame. The samples were digested by a mixture of nitric acid and perchloric acid (70: 30 ratio). 500 µl of serum and 500 µl of digestion solution were mixed in a tube and incubated in a water bath (83°C) for 16 h. Argon was used as the purging gas. The background absorption was automatically corrected by the Zeeman effect. One thousand micrograms per milliliter standard solutions of each mineral were used in the measurements.

Determination of total antioxidant and oxidant status in the serum

Total antioxidant and oxidant status were determined colorimetrically (PowerWave XS, BioTek, Instruments, Winooski, VT, USA) using a commercial kit (ZellBio GmbH, Assay kit, Ulm, Germany). Antioxidants in the sample reduce dark blue-green colored 2,22-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) radical to colorless reduced ABTS form. The change of absorbance at 660 nm is related with total antioxidant level of the sample.

Oxidants present in the sample oxidize the ferrous ion–chelator complex to ferric ion. The oxidation reaction is prolonged by enhancer molecules, which are present in the reaction medium. The ferric ion makes a colored complex with choromogen in an acidic medium. The color intensity is related to the total oxidant molecules present in the sample at 530 nm. Trolox and hydrogen peroxide standards were used for total antioxidant and total oxidant status [8].

Statistical analysis

The significance of differences between the treatments was established by the ANOVA and t test procedure of SPSS statistical software (version 20) and using Duncan's multiple range test post hoc. Significance level was set at $p<0.05$.

RESULTS

Mean concentrations of magnesium, manganese, zinc, copper, iron, TAC and TOS in the serum of all groups before and after study can be seen in Tables 1 and 2, respectively. There was significant difference in mean of these parameters between all groups before the study. A trend was observed for increased contents of serum Zn, Mg and TAC after melatonin treatment compared to the other groups (Table 2; $p<0.05$). The comparison of the studied minerals, TAC and TOS before and after study in melatonin and placebo groups is shown in Figs. 1-7. Non-significant difference was observed in mean of Fe, Cu, Mn, Zn, and TOS before and after treatment in the both groups. While, mean of Mg (Fig. 3) and TAC (Fig. 6) was significantly ($p<0.05$) increased after melatonin therapy. In after usage, TAC content of the melatonin group and TOS of the placebo group were higher compared to other groups.

Table 1. The mean (±SEM) concentrations of some minerals, TAC and TOS in the serum before drug usage

Group	*Mn (µg/mL)*	*Cu (µg/mL)*	*Zn (µg/mL)*	*Fe (µg/dL)*	*Mg (µg/mL)*	*TAC (mmol/L)*	*TOS*
Melatonin	0.036 ± 0.004	0.68 ± 0.05	1.84 ± 0.13	0.82 ± 0.07	19.83 ± 1.59	0.43 ± 0.01	51.28 ± 3.94
Placebo	0.034 ± 0.004	0.59 ± 0.06	2.18 ± 0.72	0.91 ± 0.15	21.80 ± 1.56	0.42 ± 0.02	52.92 ± 5.62
Control	0.035 ± 0.007	0.84 ± 0.07	1.73 ± 0.24	0.75 ± 0.10	20.57 ± 1.55	0.44 ± 0.01	48.71 ± 1.08

Table 2. The mean (±SEM) concentrations of some minerals, TAC and TOS in the serum after drug usage

Group	*Mn (µg/mL)*	*Cu (µg/mL)*	*Zn (µg/mL)*	*Fe (µg/dL)*	*Mg (µg/mL)*	*TAC (mmol/L)*	*TOS*
Melatonin	0.037 ± 0.004 [a]	0.77 ± 0.08 [a]	2.05 ± 0.23 [a]	0.96 ± 0.11 [a]	29.75 ± 4.59 [b]	0.48 ± 0.01 [b]	46.65 ± 2.15 a
Placebo	0.033 ± 0.004 [a]	0.71 ± 0.09 [a]	1.69 ± 0.19 [a]	0.91 ± 0.07 [a]	26.12 ± 4.83 [a]	0.43 ± 0.02 [a]	51.86 ± 1.36 b
Control	0.035 ± 0.007 [a]	0.84 ± 0.07 [a]	1.73 ± 0.24 [a]	0.75 ± 0.10 [a]	20.57 ± 1.55 [a]	0.44 ± 0.01 [a]	48.71 ± 1.08 [a]

DISCUSSION

Mustard gas and its analogs can increase oxidative stress by modulating intracellular antioxidants or enzymes that regenerate antioxidants [9]. Enhanced generation of reactive oxygen species (ROS) is considered as one of the main contributory factors in a wide variety of diseases. Free radical theory of degenerative diseases attributes the damage to cellular components through ROS imbalance as a major determinant of disease. Among the possible affecting organs, the brain and cell membranes possess high proportion of easily peroxidizable fatty acids; hence, they are the main targets for oxidative stress. Antioxidant therapy is a way for slowing the oxidative damage that is responsible for functional decline or death of the cells or organs. Endogenous antioxidant defense system reduces free radicals within the mitochondria. However, in extensive oxidative stresses, the endogenous antioxidants should be restored [10].

Melatonin is found widely in nature. It is a hormone of the pineal gland and also synthesized in various other organs, tissues, and cells. Melatonin shows properties of a powerful antioxidant, at sufficiently high concentrations as a direct radical scavenger, but, at lower, near-physiological levels, as a regulator of redox-relevant enzymes, suppressor of prooxidant excitatory and inflammatory processes and as a mitochondrial modulator [11]. Because of its amphiphilic properties, melatonin can cross all biological membranes, and thus it can indicate protective effects against oxidative stress. Melatonin scavenges several free radicals including

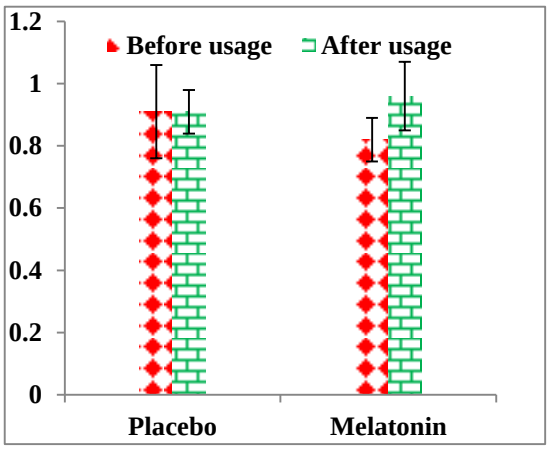

Figure 1. The comparison of iron's serum before and after drug usage

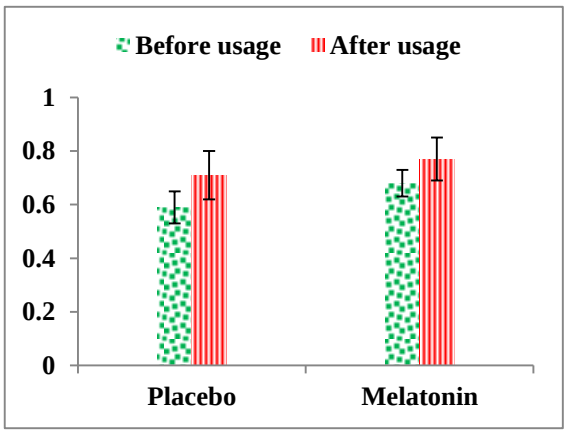

Figure 2. The comparison of copper's serum before and after drug usage

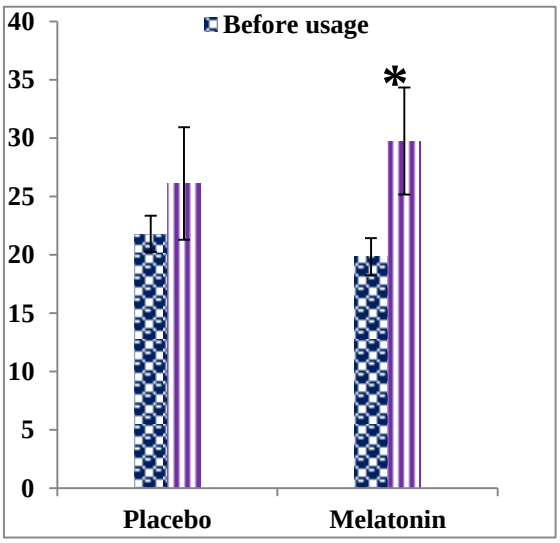

Figure 3. The comparison of magnesium's serum before and after drug usage

*Indicates $p<0.05$ between before and after

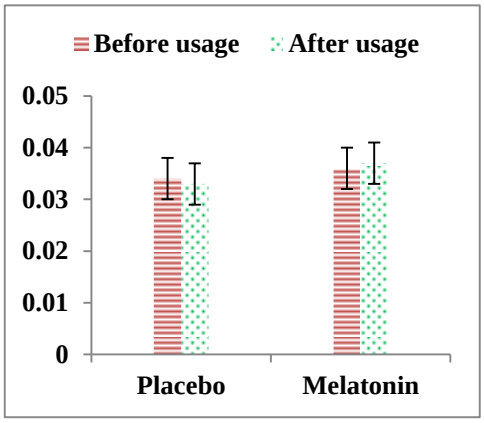

Figure 4. The comparison of manganese 's serum before and after drug usage

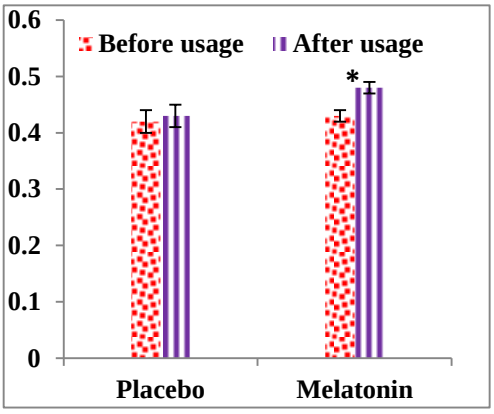

Figure 6. The comparison of TAC's serum before and after drug usage, *Indicates p<0.05 between before and after

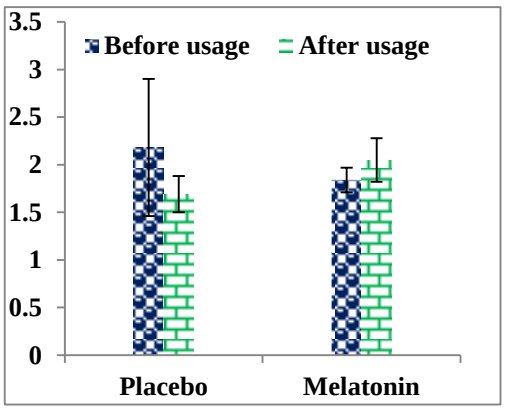

Figure 5. The comparison of zinc 's serum before and after drug usage

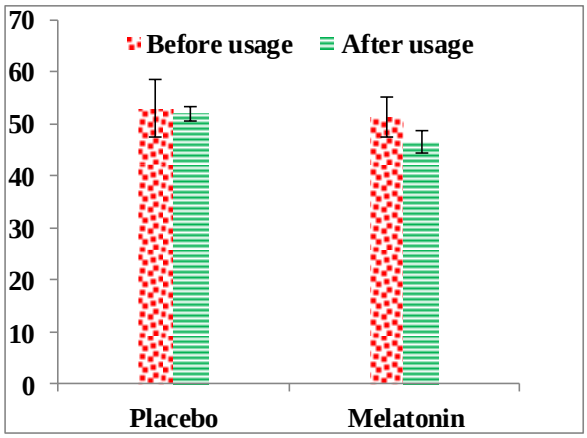

Figure 7. The comparison of TOS's serum before and after drug usage

the peroxyl and hydroxyl radicals. Both these radicals can initiate lipid peroxidation. Melatonin also increases the activity of glutathione peroxidase in the brain [12].

Melatonin by stimulating the activity of the enzyme γ-glutamyl-cysteine synthetase promotes synthesis of GSH. It also plays an important role in mitochondrial physiology through its effects on gene expression of GPx, GSH reductase, catalase, and dismutase, helping in maintaining the GSH/GSSG ratio and in high recycling of GSH. More importantly, melatonin is selectively taken up by mitochondria and acts as a powerful antioxidant. Furthermore, melatonin increases the permeability of membranes and acts as inhibitor of lipoxygenase. Melatonin also acts as stimulator of antioxidant enzymes including superoxide dismutase, GPx, GSH reductase, and catalase. Melatonin has been effective against a wide variety of pathological conditions [13].

Melatonin can be metabolized nonenzymatically in all cells of the body. It is converted into 3-hydroxymelatonin when it scavenges two hydroxyl radicals. In the brain a substantial amount of melatonin can be metabolized to kynuramine derivatives, especially under brain inflammatory conditions. These metabolites of melatonin which are formed in the brain, namely, N1-acetyl- N2-formyl-5-methoxy kynuramine (AFMK) and N1-acetyl-5-methoxykynuramine (AMK), also share the antioxidant and anti-inflammatory properties of melatonin [14]. It appears that melatonin was able to modulate stressogenic reaction by metabolism rather than direct involvement in regulation of immunity or antioxidant barriers.

Oxidative stress is defined as the interruption of balance between oxidants and reductants within the body due to the excess production of peroxides and free radicals. It has been proposed that antioxidants would be consumed in the

reaction with free radicals. This imbalance will cause damage to cellular components and tissues in the body leading to oxidative stress [15].

In the present study, we assayed oxidative status of the serums by using TOS and TAC as indicator of oxidative stress, reflecting the redox balance between oxidation and antioxidation. Melatonin therapy could increase TAC of the serums. It is well known that oxidative stress can be defined as an increase in oxidants and/or a decrease in antioxidant capacity, and various oxidants and antioxidants have additive effects on oxidative status. Although the concentration of plasma level of oxidants and antioxidants can be measured individually, it may not accurately reflect the oxidative status [16].

Trace elements and the minerals play a vital role in the body to perform its functions properly. Trace elements, in low concentrations, are integral parts of the protein structures and should present in the body in appropriate amounts and must be available for reacting with other elements to form critical molecules as well as to participate in various important chemical reactions. For instance, zinc, copper, and manganese are essential elements that play a vital role as cofactors for enzymes [17]. Some studies have shown that the biological role of these elements in many physiological and pathological features as they play an important role in protection the body by inhibiting the generation of reactive oxygen free radicals [18].

In this study, melatonin administration didn't affect the trace elements levels but increase the magnesium concentrations of serums. Magnesium is involved in at least 300 enzymatic processes which is essential for maintaining proper body functions. The concentration of intracellular Magnesium is very high as compared with concentration of extracellular Magnesium for that reason can be attributed the increase in the output cell concentration to the damage done to the cells and then increase its concentration in the blood. It is vital for body's immune system, cardiovascular, and musculoskeletal systems [19].

Zinc is a part of every cell in the body and forms a part of over 300 enzymes that have functions ranging from proper action of the body hormones to cell growth. Zinc also has significant antioxidant properties thereby protecting the cells from damage due to free radicals [20].

Copper has been found to be an important constituent of vital Cu-dependent enzymes su-ch as lysyl oxidase, cytochrome oxidase, tyrosinase, dopamine-β-hydroxy-lase, peptidylglycine alpha-amidating monooxygenase, monoamine oxidase, ceruloplasmin, and copper-zinc superoxide dismutase (Cu-Zn SOD), functioning as antioxidants and as oxidoreductases and these enzy-mes act as antioxidant defense system . Thus as a part of powerful antioxidant it helps to protect the cell from damage [21].

Manganese is a component of enzymes that play a role in the formation of carbohydrates, amino acids, and cholesterol. Manganese is found as a free element in nature (often in combination with iron), and in many minerals. It is a cofactor for a wide range of enzymes including oxidoreductases, transferases, hydrolases, lyases, isomerases, ligases, lectins, and integrins. It is also a component of the polypeptide arginase and Mn-containing superoxide dismutase (Mn-SOD). As a part of a powerful antioxidant called manganese superoxide dismutase; it prevents damage by superoxide free radicals [22].

Iron is another essential trace element present in almost all cells of the body. Human body requires iron for the synthesis of oxygen carrying protein (haemoglobin and myoglobin), DNA and cell division. Furthermore, iron is used in the connective tissues, some of the neurotransmitters in brain, and to maintain the immune system. Iron played a potential role in oxidative stress mediated injuries and pathologies e.g. rheumatoid arthritis [23].

Despite the passage of so many years from the imposed war, one of the fundamental problems of veterans, especially chemical ones, is their post-war physical problems and the damage to their mental health and social performance [24]. Melatonin usage in combination of specific medication could improve cure rate of them.

CONCLUSION

Regarding the study findings and in comparison with similar researches, it is concluded that melatonin may emerge as a safe and low-cost therapy in devotees patients. Melatonin role as a powerful antioxidant may help to improve healthy condition of devotees. However, we didn't enter another group a positive control (an approved drug) because we didn't compare melatonin effect with another standard drug. It was one of the limitations of our study which can be investigated in further studies.

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CONFLICT OF INTEREST

The authors declare that this research does not have any conflict of interest with anyone or any institute.

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