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**Research Article**

# The Effects of MIGUN Therapy on Gene and Protein Expression in High Stress Volunteers

**Maryam Salehi[1], Hedayat Sahraei[1], Zeinab Akhtari[1*], Hossein Imani[1], Mahvash Jafari[2], Hamideh Mahmoodzadeh Hosseini[3], Raheleh Halabian[3]and Mahdi Jafari[4]**

[1]Neuroscience Research Center,
Baqiyatallah University of Medical Sciences, Tehran, Iran
[2]Department of Biochemistry, Faculty of Medicine,
Baqiyatallah University of Medical Sciences, Tehran, Iran
[3]Applide Microbiology Research Center,
Baqiyatallah University of Medical Sciences, Tehran, Iran
[4]Department of Clinical psychology, School of Medicine,
Shahid Beheshti University of Medical Sciences, Tehran, Iran
***Corresponding Author:**Zeinab AkhtariZ. Akhtari, PhD.
Neuroscience Research Center, Baqiyatallah University of Medical Sciences,
Niavaran, Arajst., Tehran, Iran.POBx: 19395-6558,
Telfax: (+9821) 26127286Email: zeakhtari@yahoo.com

## ABSTRACT

**Introduction:** Stress and disease originating from it are among the problems of human society. Stress has varied external and internal symptoms, but one of the most significant medical symptoms isan increase in geneexpression of glucocorticoidreceptor (cortisol hormone). Thus, researchers have been always attempting new therapeutic techniques to decrease the level of stress. Accordingly, Migun Thermal Bed System or Migunbed, which is used an instrument for physiotherapy andreducing blood pressure and sugar, functions based on the principles suggested for stress alleviation. Therefore, in the present study its functions wereexploredas an alternative and complementary therapy for stress management.

**Aim:** This study aimed to examine molecular,cognitive, and hormonal changes in male volunteers with high stress level before and after using Migun Thermal Bed System.

**Materials and Methods:** Twenty healthy men were tested in the study. Participants were recruited using Depression, Anxiety and stress Scales (DASS). Before and after using the bed, the participants' saliva and blood samples were collected for measuring salivary cortisol, α-amylase concentrations, as well as molecular cellular changesincluding gene expression and protein concentration by PCR and Dot blot methods. The Paced Auditory Serial Addition Test (PASAT) was for used perceptive and cognitive performanceevaluation. SPSS was used for data analysis.

**Results:** The results showed that the α-amylase and cortisol levels in the participants' saliva have significantly decreased after using the bed in comparison with the levels before using the bed; however, the one-session treatment group did not show significant changes. Migun bed therapy reduced glucocorticoid gene expression and glucocorticoid protein ($p<0/05$)(groupe of four sessions).This effect occurred after four sessions. The cognitive test results indicated that mental health, reaction time and sustained attention increased while mental fatigue decreased after using the bed.

**Conclusion:** The present study suggested that Migun can affect stress and perceptual-cognitive systems and also it might improve the functioning of chief components of the stress axis.

**Keywords:** cortisol, glucocorticoid gene expression, Migun, PASAT, α-amylase

## INTRODUCTION

Today, stress and disease originating from it are among the problems of human society.Scientists and researchers have attempted presenting new ways and treatments to decrease the level of

stress in individual, family and society [1]. That is why today, stress management, alternative and complementary therapies have important roles in reducing stress. The term *stress management* is extremely well known and has commonly been used by the lay-public as well as mental health specialists for numerous years. Today, this term has become a lesson that helps a person understand that everyone has stress and it is a part of life. This lesson focuses on the feelings of the learners and how they can control the stress they might have as parents, citizens as well as workers[1]. Progressive muscle relaxation (PMR) is a method for reducing stress and anxiety by alternately tensing and relaxing the muscles [2,3].Studies have shown that the followings are reduction of salivary cortisol levels and generalized anxiety are among the long-term benefits of PMR[3,4,5,6,7,8]. Massage therapy is a technique for relaxation. The study of McKechnie and colleagues indicated that this method of relaxation was beneficial in reducing the symptoms associated with anxiety [9,10, 11]. Researches have shown that yoga is one of the stress manage methods [12,13].

Migun Thermal Bed System is another type of treatment considered in today's world. The principles behind the Migun bed include acupuncture, energy therapy, the principles of Chiropractic, massage therapy and therapeutic pressure. It is proposed that the main mechanism of this bed is based on its effect on the spinal cord [14]. Further, this bed is effective in dealing with many diseases such as diabetes, hypertension and inflammatory diseases related to the immune system as well as digestive system problems, sleep problems and muscle pain [14]. Autonomic nervous system (ANS) and hypothalamus-pituitary- adrenal axis (HPA) is what the body's response to stress is done through. Sampling of salivary cortisol has been utilized previously as a measure for the HPA axis. Salivary alpha amylase (sAA) levels has been previously utilized as a measure for levels of salivary alpha amylase (sAA) [15]. It is indicated that cortisol is a main glucocorticoid of human, which is very functional in the metabolism of glucose, fat, and protein. Cortisol which is also named as stress hormone is a determinant in stressful situations [16]. Glucocorticoid hormones assume a critical part in the adaptation of an organism to stressful events in its life [17, 18]. Research has provided ample evidence that chronic hyper- as well as hypo-secretion of glucocorticoid hormones are involved in the development of a range of metabolic, immune endocrine and neuro-psychiatric disorders[19,20].

Reul and de Kloet indicated that corticosterone binds to the mineralocorticoid receptor (MR; also termed 'Type 1' in the early days) and the glucocorticoid receptor (GR) (also termed 'Type 2') in the high-speed soluble fraction ('cytosol') of hippocampus homogenates [21].In addition highest levels of MRs are typically found in dentate gyrus, CornuAmmonisareas 1 and 2 (CA2 and CA1) of the hippocampus, lateral septum and central amygdala, whereas GRs are found throughout the brain with high concentrations in the hippocampus, neocortex and hypothalamic nuclei such as the paraventricular nucleus (PVN) and the supraoptic nucleus [21,22]. However, to measure these receptors in human, peripheral blood lymphocyte should be used [23]. Moreover, studies have indicated that, activation of the sympathetic adrenal medullar system (SAM), as a portion of the stress response, is observed by thesize of the levels of salivary alpha amylase (sAA) in numerous analyses [17,24].

Moreover, a series of studies have shown that stress management can have effects on gene expression [25].Many studies have been conducted on stress gene alterations. For instance, Bower and colleagues' study showed that the inflammation-related gene expression in survivors of breast cancer with fatigue that is persistent can be reduced by yoga interventionas well as another study confirmed content [26,27]. Moreover, previous studies have indicated that stress can disrupt the individual's cognitive abilities and memory [28].

As mentioned in the introduction, nowadays, the effects of stress management techniques on molecular and cellular levels has been of interest to scientists. For this purpose, and in order to

investigate the cellular and molecular effects of stress management methods, the present study examined the effects of Migun bed therapy on the molecular cell biology, which is a symptom of high stress levels.Migun bed is usually used for physiotherapy and reducing blood sugar and pressure. However, the reasons for proposing Migun bed as a stress-alleviating instrument exceling such methods as yogaare as follows:

1. Great convenience;
2. High accessibility;
3. Functioning within the framework of principles suggested for stress alleviation;
4. No need for any specific knowledge on the part of the patient;and
5. dearth of research on the effects of Migun therapy on gene and protein expression

## MATERIALS AND METHODS

The present study was conducted at Neuroscience Research Centre, Baqiyatallah University of Medical Sciences, Tehran, Iran. The research process lasted for 6 months (2016/3/20-2016/9/29). In addition, the ethics committee of the university approved the process under the ethical clearance code ir.bmsu.rec.1394.112.

### Participants

The participants were recruited according to their score on the Depression, Anxiety and Stress Scales (DASS) (score < 25) (Theywere students of Baqiyatallah University of Medical Sciences)( According to the DASS questionnaire, the score is less than 25 participants with mild, and moderate stress, and we chose this group, and because participants has a score of over 25,they have severe andvery severe stress that we did not consider). Accordingly, 20 healthy young menweighing 50-70 kg and aged 20-25 years (highest levels of health factors could be found in this age range) were selected using Cochran's formula. This cross-sectional study followed an experimental (randomized clinical) design, based on which two groups, each with 10 participants, were randomly formed. It is worth noting that the exclusion criteria included:

1. history of systemic diseases such as diabetes and rheumatoid arthritis;
2. substance abuse/dependence;
3. smoking;
4. history of chronic low back pain during the past year; and
5. history of fracture and surgery in areas of the spine

### Experimental Design

The participants were randomly divided into two groups: the first group used the bed in "ON" state in one session of treatment (the one-session treatment group) and the second group used the bed in "ON" state in four sessions of treatment (the four-session treatment group). Each treatment session lasted 35 minutes. Before and after using the bed, 20 cc of saliva and 10cc of blood were collected from all of the participants. Accordingly,each participant put his saliva sample in a test tube.Then the tubes were closed and kept a -20°C for subsequent steps. In addition, blood lymphocytes (extracted using Ficoll method (Ficoll solution))were separated and stored at -80 °C. This procedure was completed over a couple of days.

environment in which the participant used the bed should be a calm and quiet environment. It's also better if the participant does not talk when using the bed and do not use electronic equipment until it is completely relaxed. It is also best to have light in the room to be more comfortable .Every factor that can disturb the peace and quiet of the environment should be avoided from the environment TheMigun Thermal Bed System

The hy-5000 model bed was manufactured by the Migun Company. The design of the Migun Thermal Massage Bed applies pressure as well as heat (Helium lamp, Infrared and jadestone) to the muscles alongside the spine and causes the massaging of the tendons as well as muscles that are located around the spine, which helps relax nerves, facilitate blood flow, and relives tension.

### Data Collection

On the day of experiment, first, the salivary samples were thawed at room temperature and after centrifuging, the supernatant was collected for testing. Then, Cortisol ELISA kit (IBL international, Germany, catalogueno: RE52611, standard range: 0 - 40ng/mL and Functional Sensitivity 0.030 µg/dL: Mean Conc. < 20 %

CV) and α-amylas kit (Pars Azmun Company, Tehran, Iran) were employed for the measurement of human salivary cortisol and α-amylas enzyme. Blood lymphocytes were used to check the status of GRs (glucocorticoid receptors). Blood lymphocytes were separated using Ficollesolution and were stored at -80°C. In the next step, the lymphocytes were used for RT-PCR [reverse transcription polymerase chain reaction:The concentration of RNA was measured by the nanodroop, then CDNA was made and replicated with PCR, gelled and Image J software interpreted bands density] and Western blot methods to evaluate the look of the glucocorticoid gene and the glucocorticoid protein [25].Moreover, the PASAT was used to assess the participants' cognitive performance, before and after using the bed. PASAT software was used to assess cognitive perceptual activity of the participants before and after using the bed[29]. The average response time (response speed), the longest chain of correct answers (sustained attention), and the longest chain of incorrect answers (mental fatigue) were also examined in this study.

**PCR Method**

The semi-quantitative reverse transcriptase-polymerase chain reaction (semi-RT-PCR) was utilized to assess the impact of the use of Migun bed on the expression of GR gene involved in stress condition. Primer used GRs were as follows:

- Forward: 5_-CAC TGG ACC TTA GAA GTT GAT A-3
- Reverse: 5_-ATA CAG TCC CAT TGA GAG TGA-3

As described earlier, lymphocytes were collected from peripheral blood of each participant in due time and the total mRNA was purified by the RNX-Plus kit (Cinnagen, Iran) in accordance with the manufacturer's guideline. The quantity and quality of each isolated RNA was evaluated using the nanodrop spectrophotometer (Thermos, USA) and agarose gel electrophoresis (1% gel), respectively. cDNAsynthesized from each sample usingBioneer kit (Takara, Japan) was applied. briefly, one µg each RNA was transformed to cDNA by the master mix containing M-MLV reverse transcriptase, 1 random hexamer, oligodT and related buffer. Finally, the GR gene expression was detected using PCR and related specific primer set. The mRNA appearance of β-actin was surveyed as an internal control. All PCR reactions were performed in a thermocycler (Techne, UK) containing 1.5 µlcDNA, 2.5 mM MgCl2, 0.2 mM of the deoxynucleosidetriphos-phates (dNTPs), 10 pmol of all primers as well as 1.5 U of Taq DNA Polymerase (Cinnagen, Iran). The program, PCR, was 6 min initial denating at 94 C, 35 cycles of 45 s at 95 °C, 45 s at 58 °C for both primers as well as 1 minute s at 72 °C following a 7 minute conclusive extension at 72 °C. To measure the density of amplicons, each PCR product was runed on 2% agarose gel electrophoresis, staine by etidium bromide and and visualized under UV gel document. Conclusively, Image J software measured each product bands density[30].

**Western Blot**

The dot blot protocol, a type of Western blot technique, was used to examine GR expression when the participant used Migun bed for 4 and 1 sessions.Briefly, lymphocyte samples of the whole blood were used for the experiments. Then samples (0.07 mm) were spotted onto nitrocellulose strips, which were allowed to dry at room temperature. The strips were rinsed briefly in phosphate-buffered saline (PBS; pH 7.2) and were incubated overnight at 4°C in 5% skimmed milk (BBL) in PBS with 0.05% sodium azide to block the residual binding sites on the strip. The stripswere rinsed for 10 min in PBS and werethen incubated with sera diluted 1:80 in 5% skimmed milk in PBS for 30 min at 37°C. After incubation, the strips were washed by the use of four changes of PBS and were further incubated for 10 min at 37°C with a rabbit anti-human serum (Dako-Immunoglobulins, Carpinteria, Calif.) diluted 1:500 in 5% skim milk. The strips were washed four times with PBS(3min) and were incubated with a protein A-gold complex (protein A labelled with 20-nm colloidal gold; A520 5 5.3; Sigma Chemical Co., St. Louis, Mo.) for 5 min at room temperature. The strips were washed four times and were allowed to dry on a filter

paper. A clearly defined red spot at the site where the antigen was spotted was considered a positive result. A trace reaction or the absence of any reaction was considered a negative result [31].

## DATA ANALYSIS

PASAT data analysis, the cortisol and α-amylas enzyme levels were analyzed by SPSS(version 24)and Image J software measured each product bands density (for PCR) . The mean and standard deviation (Mean ± SD) of the data were presented. Paired t-test was used to determine the significance of the within-group differences. $p< 0.05$ was set as the statistical significance level.

## RESULTS

### The impact of Migun Thermal Bed System on salivary cortisol

The results showed that the cortisol level in the participants' saliva (four-session treatment group) has significantly decreased after using the bed in comparison with the levels before using the bed ($p < 0.05$) (Table/Fig-1) but the one-session treatment group did not show significant changes. The impact of Migun Thermal Bed System on salivary cortisol concentration in the participants before and after using the bed is shown in Table1.

| Testing trail | Salivary Cortisol (ng/ml) (Mean± SD) (one-session treatment group) | Salivary Cortisol (ng/ml) (Mean± SD) (four-session treatment group) |
|---|---|---|
| Pre-test | 81.4±12.65 | 75.39±9.8 |
| Post-test | 71.26±4.4 | 64.59±12.76* |

**[Table/Fig-1]:** The participants' salivary cortisol concentration before and after using Migun Thermal Bed System
*Note:*The four-session treatment group's salivary cortisol concentration shows a significant decrease after using the bed in comparison with Pre-treatment ($^{*}p < 0.05$).

### The impact of Migun Thermal Bed System on α-amylase enzyme

The results indicated that the α-amylase enzyme level in the participants' saliva after using the bed for four sessions significantly decreased in comparison with the level before using the bed ($p < 0.05$), but, the one-session treatment group did not show significant changes in α-amylase enzyme activity (Table/Fig-2).

| Testing trail | Salivary alpha amylase (U/L) (Mean± SD) (one-session treatment group) | Salivary alpha amylase (U/L) (Mean± SD) (four-session treatment group) |
|---|---|---|
| Pre-test | 3.1±204.5 | 4.9±2.3 |
| Post-test | 2.9±161.29 | 2.01±1.4* |

**[Table 2/Fig-2]:** The participants' salivary alpha amylase concentration before and after using Migun Thermal Bed System
*Note:* The four-session treatment group's salivary alpha amylase concentration shows a significant decrease after using the bed in comparison with Pre-treatment ($^{*}p < 0.05$).

### The impact of Migun Thermal Bed System on mental healthand response time

To investigate the participants' mental health, the PASAT was administered. The test evaluates cognitive performance through examining the number of correct answers, response time, sustained attention, mental fatigue. The findings indicated that Migun Thermal Bed System altered mental health (number of correct answers) in the PASAT before and after using the bed, as shown in Table/Fig-3. The results showed that the number of correct answers given by the four-session treatment group noticeably increased after using the bed in comparison with Pre-treatment ($p < 0.001$). However, the one-session treatment group did not show significant changes in the number of correct answers (Table/Fig-3). Moreover, there was a significant decrease in the four-session treatment group' response time after using the bed in comparison with Pre-treatment ($p < 0.001$); however, the one-session treatment group did not show significant changes in response time (Table/Fig-3).

| Testing trail | Mental health (Number) (Mean± SD) (one-session treatment group) | Mental health (Number) (Mean± SD) (four-session treatment group) | Response time (Sec) (Mean± SD) (one-session treatment group) | Response time (Sec) (Mean± SD) (four-session treatment group) |
|---|---|---|---|---|
| Pre-test | 51.11±8.03 | 40.85±15.86 | 2.39±0.91 | 2.8±0.28 |
| Post-test | 54.77±3.8 | 54.71±4.1* | 2.05±1.42 | 2.1±0.69* |

**[Table/Fig-3]:** The participants' mental health and reaction time before and after using Migun Thermal Bed System

*Note:* The four-session treatment group's mental health noticeably increased after using the bed in comparison with Pre-treatment. It is also shown that the four-session treatment group's reaction time significantly decreased after using bed in comparison with Pre-treatment (* $p < 0.001$)

**The impact of Migun Thermal Bed System on sustained attention and mental fatigue**

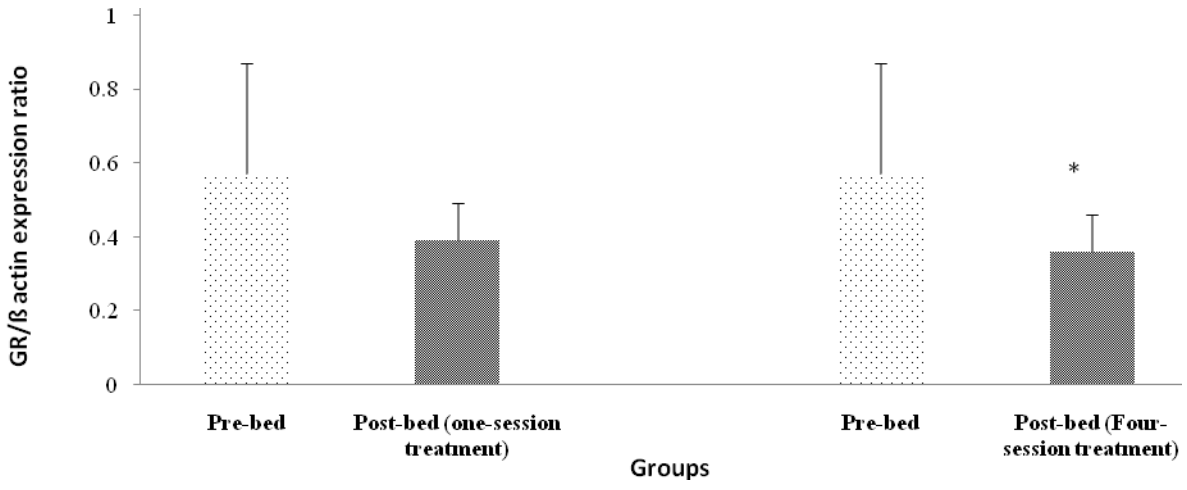
As mentioned earlier, the longest chain of the correct answers in the PASAT was considered as a measure of sustained attention. The results showed that the four-session treatment group's sustained attention has significantly increased after using the bed in comparison with Pre-treatment ($p < 0.001$), but the one-session treatment group did not show significant changes in the longest chain of the correct answers (Table/Fig-4). The longest chain of the wrong answers in the PASAT was considered as a sign of mental fatigue. The results indicated that the four-session treatment group's mental fatigue significantly decreased after using the bed in comparison with Pre-treatment ($p < 0.001$), but the one-session treatment group did not show significant changes in the longest chain of the wrong answers (Table/Fig-4).

| Testing trail | Sustained attention (Number) (Mean± SD) (one-session treatment group) | Sustained attention (Number) (Mean± SD) (four-session treatment group) | Mental fatigue (Number) (Mean± SD) (one-session treatment group) | Mental fatigue (Number) (Mean± SD) (four-session treatment group) |
|---|---|---|---|---|
| Pre-test | 29.55±19.13 | 16.71±16.07 | 8.88±8.03 | 21.14±16.65 |
| Post-test | 33.44±13.93 | 43.57±17.53* | 5.22±3.89 | 5.4±4.1* |

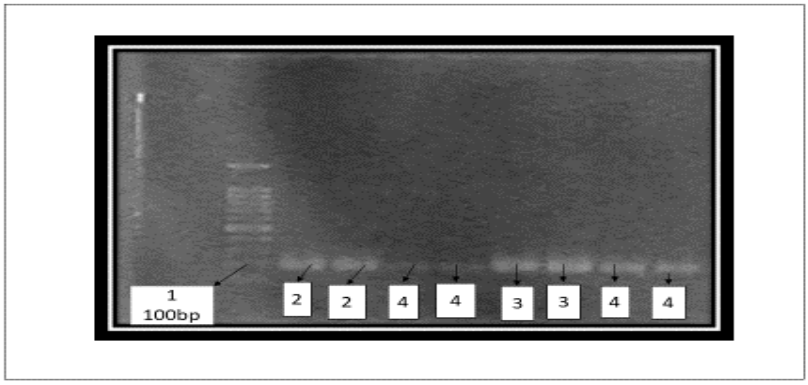
**[Table/Fig-4]:** The participants' sustained attention and mental fatigue before and after using Migun Thermal Bed System

*Note:*The four-session treatment group's sustained attention significantly increased after using the bed in comparison with Pre-treatment. In addition, the four-session treatment group's mental fatigue (the longest wrong answer chain) after using the bed significantly decreased in comparison with Pre-treatment (* $p < 0.001$).

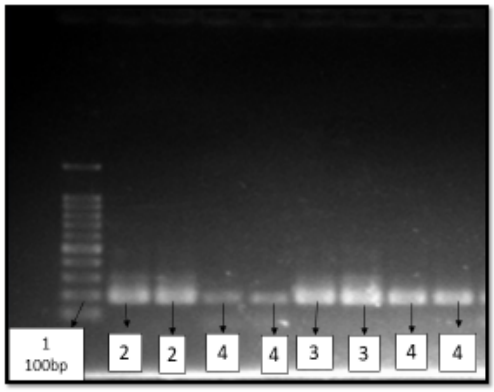
The results demonstrated that the use of Migun bed reduced glucocorticoid gene expression.This impact occurred after four sessions (Table/Fig-5, 6, 7). The densitometry analysis results are also provided in Table/Fig-5.



**[Table/Fig-5]:** Comparison of the groups' GR/β-Actin expression ratio before and after using Migun Thermal Bed

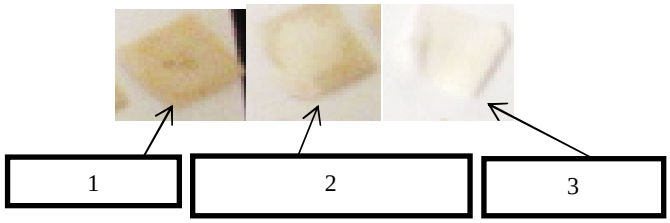



**[Table/Fig-6]:**GR expression (98kDa) (before and after using Migun Thermal Bed: 1. ladder,2. Pre-bed GR expression (GR expression before bed (98kDa)),3. Post-bed GR (One-session) (GR expression after bed- One-session(98kDa)), 4. Post-bed GR (Four-session) (GR expression after bed- Four-session(98kDa))




**[Table/Fig-7]:**β-Actin expression before and after using Migun Thermal Bed:1. ladder, 2. Pre-bed β-Actin (β-Actin expression before bed),3.Post-bed β-Actin (One-session) (β-Actin expression after bed- One-session), 4. Post-bed β-Actin (Four-session) (β-Actin expression after bed- Four-session)

The results of the dot blot protocol for the glucocorticoid protein in the participants' lymphocytes were proved to be similar to those of PCR(Table/Fig-8).




**[Table/Fig-8]:** The results of the dot blot protocol for the glucocorticoid protein(98kDa) in the participants' lymphocytes before and after using Migun Thermal Bed:1.Pre-bed (glucocorticoid protein before using bed), 2. Post-bed (One-session) (glucocorticoid protein after using bed), 3. Post-bed (Four-session) (glucocorticoid protein before using bed)

## DISCUSSION

The normal reaction that leads to a rise in arousal as well as the ability to deal with threat is known as the stress response [32]. The body relaxes and goes back to its usual state of tension in the lack of a resumption threat. A small dose of everyday stress is not unhealthy at all and it is part of life; the body is tailored to handle it. Intensive stress can cause serious and severe psychological as well as physical effects [32]. As mentioned earlier, there are various methods to reduce psychological and physical stress. One method is the use of alternative and complementary therapies. The alternative and complementary treatments have fewer side effects than do chemical drugs.

According to the results of the present study, a comparison of the level of cortisol in the participants' saliva with high stress
before and after using the bed showed a statistically significant decrease after four sessions of treatment. However, the results did not show significant changes after one session of treatment [Table/Fig-1]. Some studies have proposed that mindfulness-based therapy as an alternative and complementary therapy proved to reduce stress-related medical conditions. Mindfulness-based therapy can decrease cortisol level [32, 33]. Other studies have proposed that relaxation techniques are best ways to decrease tension [34, 35].Accordingly, the results showed that Migun Thermal Bed System can reduce the cortisol level, suggesting that the effect of these functions is centered on HPA axis. In line with many previous studies, according to the results of the present study a comparison of the level of α-amylase in subjects with high stress before and after using the bed showed a significant decrease after using the bed for four sessions of treatment in comparison with Pre-treatment (Table/Fig-2). Some studies have also proposed that the stress system activity causes sympathetic (or parasympathetic) responses in individuals [36, 37]. One of these responses is the increase in the secretion of salivary alpha-amylase enzyme. Further, this reaction occurs rapidly and shows its effect within a few minutes. Researches have shown that this increase occurs through an increase of the adrenergic system activity in the salivary glands [38, 39]. According to the results, the effect of Migun Thermal Bed System on public mental health (the number of correct answers in the PASAT) was positive, because the number of the four-session treatment group's correct answers significantly increased in Post-treatment in comparison with Pre-treatment (Table 3).

According to the results, the effect of Migun Thermal Bed System on response speed (response time on the PASAT) was positive, because the four-session treatment group's response speed significantly decreased after using the bed in comparison with Pre-treatment) (Table/Fig-3). The cognitive functions above are mediated by amygdala processing of the external stimulation of the mediate emotions, and then the output of the amygdala leads to the release of hormones in blood through the stimulation of hippocampus, frontal cortex and finally the hypothalamus and the activation of HPA stress axis. These hormones move towards the brain and attach to the neurons in the amygdala and ultimately connect with the hippocampus and improve the explicit memory [40]. In this study, the findings demonstrated that response speed decreased after using the bed, which is closely connected to reducing stress and improving short-term memory of individuals.

The effect of Migun Thermal Bed System on sustained attention is depicted by the longest chain of the correct answers in the PASAT. The results showed that the four-session treatment group's sustained attention significantly increased after using the bed in comparison with Pre-treatment (Table/Fig-4). Making a decision is the result of an active memory to perform brain processing and the processes that are used to maintain and manipulate information in short term. Further, active memory deficiency causes disruption in efficiency, learning calculations and solving complex problems [41].

The results also indicated that the four-session treatment group's mental fatigue significantly decreased after using the bed in comparison with Pre-treatment (Table/Fig-4). In previous research works, it has been proposed that exposure to stress can weaken the ability to make decisions in animal or human models and disrupt different types of memory [39]. Therefore, in this study, the results showed that mental fatigue decreased after using the bed (four-session treatment group), which is closely connected to reduction of stress and memory enhancement in the subjects; thus, the bed might have a positive effect on brain functions.

Here it was shown that there were glucocorticoid gene expression modifications in peripheral blood mononuclear cells (PBMCs) of the participants during four sessions of using the bed. The data advises that when using Migun bed, an integral physiological component at the molecular level is needed, which is immediately

initiated throughout practice and might shape the foundation for long-term stable outcomes.

The presence of the glucocorticoid protein in lymphocytes of the participants was confirmed by Dot Blot method. An increase in the expression of GR beta in mononuclear cells from septic patients, as well as the serum from septic patients induces cell glucocorticoid resistance in vitro was shown by Guerrero and colleges' study. In the study, the western blot was used to study the GR alpha and beta expression from patients as well as from immune cell lines cultured in the occurrence of serum from septic patients [42].Another study revealed that the irregularities of expression as well as fastening of the GR might be connected with resistance of the tissue to steroids in patients with systemic lupus erythematosus (SLE). The persistence of the GR expression and binding by flow cytometry (FCM) may be beneficial in foreseeing the response for steroid treatment for patients with SL [43].

Gotovac and colleagues suggested that the Multipara meter flow cytometric determination of GCR expression in lymphocyte subpopulations might be a useful tool for observing the immunoregulatory action of glucocorticoids. This study examined post-traumatic stress disorder (PTSD) patients [44].

In the present study, the glucocorticoid protein concentration was determined using the Western blot technique. However, since there was a very small amount of this protein in lymphocytes, the dot blot method was used to detect the presence of this protein in lymphocytes. The result of the dot blot protocol for the presence of the glucocorticoid protein in the participants' lymphocytes was positive. Based on the results of the present study, Migun bed is suggested to be used in medical environments for all individuals with high levels of stress. In addition, further research studies on the topic might help disclose other unknown effects of this bed on patients with high stress. Overall, the results of the present study help explore the positive effects of Migun therapy on alleviation of stress among patients with severe effects of stress.

## LIMITATIONS

The present study encountered some limitations during the research process, some of which are as follows:

- Limited sample size
- Lack of timely budgeting required
- Participants' withdrawal from the testing procedure

To increase the generalizability of the results, researchers can conduct experimental research studies on larger sample sizes. In addition, using highly precise laboratory devices can help advance the accuracy of the results. Finally, stakeholders are recommended to provide financial supports for researchers in terms of laboratory equipment, high-tech medical environments and investment in research on complementary therapies for stress alleviation.

## CONCLUSION

The present study suggested that Migun Thermal Bed System could positively affect stress and perceptual-cognitive systems. Further, it might improve the functioning of chief components of the stress axis (the hypothalamic-pituitary-adrenal or HPA axis) and brain cognitive system in individuals with high stress level. The results of the present study demonstrated that the use of Migun bed could affect glucocorticoid gene expression and thereby reducing stress at the cellular and molecular levels.

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