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Research article

The analyse of the antioxidant effect of natural peloidotherapy in aging process

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Abstract: Medical research has developed remarkably in recent years, including the involvement of the glutathione peroxidase (GPx) family of enzymes in the course of human aging, with numerous clinical studies published in the literature reporting this particular fact. Thus, mud therapy and its effect on biological aging have been represented in papers that have been published to date. Papers published in the literature analyzing GPx vari-ation during sapropelic mud therapy suggest the beneficial effect of this family of en-zymes in diseases with an important inflammatory component, mainly monitored in patients with osteoarthritis. This study investigated the effects of sapropelic mud treatment on GPx values in patients receiving treatment with sapropelic mud at the Balneal and Rehabilitation Sanatorium of Techirghiol, Romania. We included 52 patients, split into two groups, who received treatment with cold mud baths and warm mud baths. Values close to statistical significance were found in patients who received treatment with cold mud baths in terms of mean GPx values at the four-time points studied. Fur-ther studies evaluating GPx in patients receiving sapropelic mud treatment are needed.

Keywords: glutathione peroxidase (GPx), sapropelic mud, peloidotherapy, balneotherapy

1. Introduction

1.1. General considerations

Ageing is the result of progressive accumulation of cellular changes that reduce an organism's ability to withstand stress, causing a decrease in survival potential [1,2], thus, ageing has four characteristics [1]: *1) progressive*, *2) endogenous*, *3) universal* and *4) detrimental*.
Within the ageing process, accelerated functional decline has been shown to occur, the exact mechanisms causing this functional decline are unclear [3]. The free radical theory of ageing shows that an increase in oxygen radical production by mitochondria with age leads to an increase in cell damage [1,3]. According to this, researchers have shown that oxygen utilization by mitochondria of aerobic organisms can generate several reactive

radicals, such as superoxide (O_2-), hydrogen peroxide (H_2O_2) and hydroxyl radical (HO-; 9-11) [1].

Aerobic organisms are well protected against oxidative challenges by antioxidant defence systems [3]. However, it appears that an imbalance between oxidants and antioxidants, called oxidative stress, can occur during the ageing process. Oxidative stress induced by oxidative species occurs under conditions where antioxidant defences are depleted or when the rate of radical reaction constants are higher than those of antioxidant defence mechanisms [3].

Oxidative damage to biomolecules increases with age and this phenomenon is postulated to be a major causal factor of biochemical cellular senescence [4,5] .There is much support for this hypothesis, including the following observations: studies by Sohal and colleagues using transgenic *Drosophila*, in which the antioxidant enzymes, superoxide dismutase (SOD) and catalase (Cat) - two enzymes that scavenge highly reactive oxidative radicals, superoxide and hydrogen peroxide, respectively- are overexpressed, this showing increases in mean and maximum lifespan and a reduction in oxidative damage [4].

Further evidence linking oxidative generation and ageing is provided by the strong, inverse correlation between mitochondrial oxidative generation rate and maximum lifespan among different species, e.g. animals with an accelerated mitochondrial metabolism have a short lifespan because they have higher oxygen consumption and therefore higher oxidative production [3]. In addition, there is a strong correlation between the maximum lifespan of a species and its SOD activity (first defense against reactive oxygen species). The increase in SOD activity may provide more protection against superoxide radicals. This suggests that lifespan may, in part, depend on the activity of this enzyme [3].

1.2. Glutathione peroxidase (GPx)

Glutathione peroxidase (GPx) is found in both cytosol and mitochondria. Together with its substrate GSH (L-γ-glutamyl-L-cysteinyl-glycine), it forms an important defence against hydrogen peroxide and lipid peroxides [3].

Glutathione peroxidase (GPx) was discovered in 1957 by Gordon C. Mills. Glutathione peroxidase (GPx) is the general name of a family of enzymes with peroxidase activity whose main biological role is to protect the body against oxidative damage. The biochemical function of glutathione peroxidase is to reduce lipid hydroperoxides to their corresponding alcohols and to reduce free hydrogen peroxide to water. Several types of isoenzymes are encoded by different genes, which vary in cell locus and substrate specificity. To date, eight different isoforms of glutathione peroxidase (GPx1-8) have been identified in the human species.

Clinical significance: low serum glutathione peroxidase levels have been shown to be a contributing factor in vitiligo [5]. Lower plasma GPx levels have been observed in type 2 diabetes patients with macroalbuminuria, and this has been correlated with the stage of diabetic nephropathy [6]. In a clinical trial, glutathione peroxidase activity, together with other antioxidant enzymes such as superoxide dismutase and catalase, was not associated with a risk of coronary heart disease in women [7]. GPx activity was reported to be significantly lower in patients with relapsing-remitting multiple sclerosis [8]. A scientific paper suggested that glutathione peroxidase and superoxide dismutase polymorphism are involved in the etiopathogenesis of celiac disease [9].

From the perspective of scientific research direction resulting from studying animal models: mice genetically lacking glutathione peroxidase 1 (GPx1- mice) are phenotypically normal and have a normal lifespan, indicating that this enzyme is not vital [10]. However, GPx1- mice developed cataracts at a young age and showed defects in muscle cell proliferation [11]. Gpx1- mice showed an auditory brainstem response up to 16 dB higher than control mice. Mice lacking GPx3 (GPx3-/-) or GPx2 (GPX2-/-) also developed normally [11,12]. However, mice lacking GPx4 died during early embryonic development [11].

Some evidence, however, suggests that reduced levels of GPx4 may increase life expectancy in mice [12,13].

1.3. Antioxidant therapy

In humans, the first line of antioxidant defence are antioxidant enzymes, mainly SOD, glutathione peroxidase (GPX) and, to a lesser extent, catalase, as well as glutathione tripeptide (GSH) [14]. These enzymes will help to destroy reactive oxygen species (ROS), H_2O_2 and lipid peroxides, while GSH protects against oxidised proteins. There is not known the existing of a direct enzymatic defence against supertoxic organic hydroperoxide resistance (OHR), although, any antioxidant whitch represents a defence against ROS and H_2O_2 represents indirectly a defence against OHR, because they can combine to generate OHR [15]. Therefore, an important antioxidant role is left to a group of nutrients, including vitamins C and E, selenium, coenzyme Q_{10} and lipoic acid [16-18]. There is a positive correlation between coenzyme Q_{10} levels and superoxide production [18]. When coenzyme Q_{10} levels are experimentally manipulated, both in vivo and in vitro, neither superoxide production nor lifespan is altered. This clearly shows that coenzyme Q_{10} levels cannot control free radical generation or lifespan, despite the negative correlation between them.

The pineal gland hormone, melatonin, also plays several antioxidant roles and various drugs including the centrophenoxins and piritinol have also shown serious antioxidant potency [18,19].

Since the impact of free radicals on biological molecules has been highlighted as a primary cause of ageing, several attempts have been made to attenuate free radical reactions by exogenous antioxidants [3]. By mitigating these damaging reactions, scientists have theorized that diseases associated with aging and the aging process itself would be slowed, ultimately prolonging life expectancy [3].

2. Experimental Design

2.1. Details of the study

The present study represent a prospective, non-randomised study, that investigated the effects of peloid therapy on the ageing process. The study was carried out in the Techirghiol Rehabilitation and Balneary Sanatorium, for a period of 6 months. 52 patients (both males and females) were included in the study, who met the inclusion and exclusion criteria. All the patients were evaluated for serum GPx levels and also from quality of sleep at the time of admission and discharge. The GPx levels were assessed at admission, discharge, 1 month and 4 months after discharge. All the data were statisticaly analysed.

The inclusion and exclusion criteria for participation in the study are presented in Table 1.

Table 1. *Inclusion and exclusion criteria*

Inclusion criteria	*Exclusion criteria*
- age of patients over 50 - patients with degenerative osteo-articular pathology - patients who have signed the patient's informed consent after having previously explained to them the purpose and treatment to be performed	- inflammatory osteo-articular pathology - all contraindications of complex balneotherapy (cardiovascular diseases, oncologic pathology, respiratory diseases, recent surgery, infectious diseases, etc) - refusal to sign the informed consent.

The study started after receiving the approval of the Ethics Council of Balneal and Rehabilitation Sanatorium of Techirghiol. All participants were informed about the nature and

purpose of the study before signing the patient's informed consent. Subjects could withdraw from the study at any time. All subjects signed the informed patient consent.

2.2. Intervention

The 52 study participants were admitted to the Balneal and Rehabilitation Sanatorium of Techirghiol for a period of 2 weeks (10 sessions of treatments). They were divided into 2 intervention groups: the "cold mud baths" (CMB) group and the "warm mud baths" (WMB) group. Patients in the CMB group underwent progressive heliotherapy, mud application and immersion in lake water at the "Cold Baths Department" of the Balneal and Rehabilitation Sanatorium of Techirghiol, while patients in the WMB group underwent warm mud baths inside the sanatorium. In conjunction with this method of treatment, both groups of patients also underwent electrotherapy procedures, massage therapy and kinetotherapy of the same type.

2.3. Adherence to treatment

Participants were asked to maintain their daily food intake, prescribed medication and usual lifestyle throughout the study period. They were advised to avoid any strenuous physical activity beyond that required for the purpose of the study. Following discharge, patients were contacted by telephone to ensure compliance with the instructions.

4. Results

A total of 98 subjects admitted to the Balneal and Rehabilitation Sanatorium of Techirghiol entered the research study. Of these, only 52 completed the protocol and could be analysed at admission, discharge, one month and 4 months after discharge. The CMB group was composed of 15 patients (28.8%) and the WMB group of 37 patients (71.2%). The remaining 46 subjects did not meet the inclusion criteria or withdrew during the study for personal reasons. No difference was observed between the two groups in terms of age and anthropometric characteristics (height, weight and body mass index).

4.1. Variation in GPx levels of the patients included in the research in the cold mud bath (CMB) group analysed compared to admission, discharge, one month and 4 months after discharge – Table 2 and Figure 1

***Table no. 2** – Descriptive statistics for the GPx level recorded at different time intervals (admission, discharge, 1 month and 4 months after discharge) in CMB group*

GPx [U/gHb]

	No	Mean (M)	Standard Deviation (SD)	Standard Error	95% Confidence Interval for Mean		Minimum	Maximum
					Inferior	Superior		
Admission	15	45,28	18,55	4,79	35,00	55,55	22,90	90,10
Discharge	15	41,97	12,26	3,16	35,18	48,77	23,06	70,50
One month	15	55,03	18,16	4,68	44,97	65,09	29,60	90,30
Four months	15	57,63	20,28	5,23	46,39	68,86	28,00	99,80

For patients of the CMB group: at admission the mean GPx value is $M_{Admission}$ = 45.28 U/gHb and the standard deviation of $SD_{Admission}$ = 18.55 U/gHb; at discharge the mean GPx value is $M_{Discharge}$ = 41.97 U/gHb and the standard deviation of $SD_{Discharge}$= 12. 26 U/gHb; at 1 month the mean GPx value is M_{1Month}= 55.03 U/gHb, and the standard deviation of SD_{1Month} = 18.16 U/gHb; at 4 months the mean GPx value is M_{4Month} = 57.63 U/gHb, and the standard deviation of SD_{4Month} = 20.28 U/gHb.

There is no statistically significant difference between the mean values of the GPx variable measured at the four-time points determined by one-way ANOVA ($F(3,56) = 2.754$, $p = 0.057 > \alpha = 0.05$).

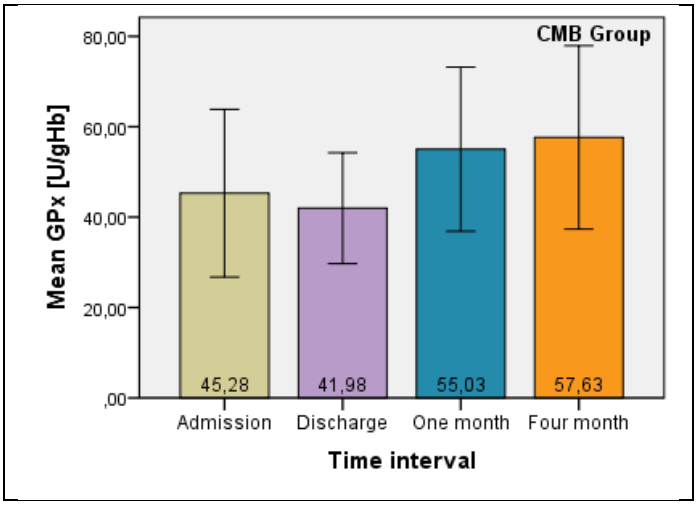

Figure no. 1 - *Graphical representation for the GPx variable at the four time points in the patients who made up the cold mud bath (CMB) group*

4.2. Variation in GPx levels of the patients included in the research in the warm mud bath (WMB) group analysed compared to admission, discharge, one month and 4 months after discharge are shown in Table 3 and Figure 2

Table no. 3 *- Descriptive statistics for the GPx level recorded at different time intervals (admission, discharge, 1 month and 4 months after discharge) in WMB group*

GPx [U/gHb]

	No	Mean (M)	Standard Deviation (SD)	Standard Error	95% Confidence Interval for Mean		Minimum	Maximum
					Inferior	Superior		
Admission	37	48,70	16,44	2,70	43,22	54,18	23,40	94,40
Discharge	37	47,49	16,28	2,67	42,06	52,92	18,60	78,70
One month	37	50,86	16,90	2,77	45,22	56,49	21,40	104,40
Four months	37	54,12	21,20	3,48	47,05	61,19	19,70	120,30

For patients of the WMB group: at admission the mean GPx value is $M_{Admission} = 48.70$ U/gHb and the standard deviation of $SD_{Admission} = 16.44$ U/gHb; at discharge the mean GPx value is $M_{Discharge} = 47.49$ U/gHb and the standard deviation of $SD_{Discharge} = 16.28$ U/gHb; at 1 month the mean GPx value is $M_{1Month=} 50.86$ U/gHb, and the standard deviation of $SD_{1Month} = 16.90$ U/gHb; at 4 months the mean GPx value is $M_{4Month} = 54.12$ U/gHb, and the standard deviation of $SD_{4Month} = 21.20$ U/gHb.

There is no statistically significant difference between the mean values of the GPx variable measured at the four-time points determined by one-way ANOVA ($F(3,144) = 0.987$, $p = 0.401 > \alpha = 0.05$).

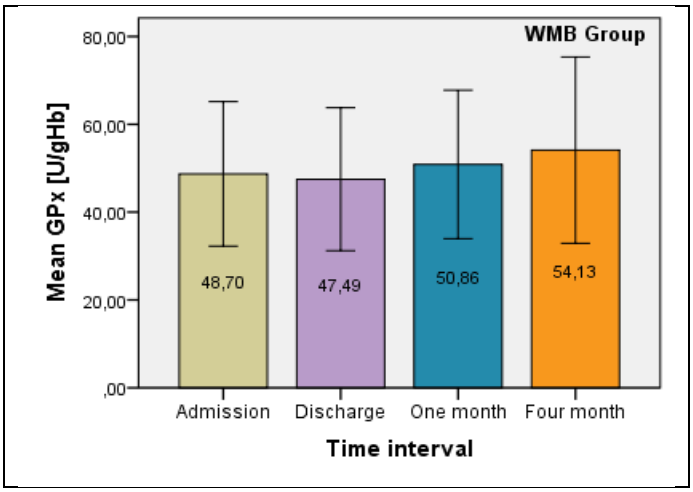

Figure no. 2 *- Graphical representation for the GPx variable at the four time points in the patients who made up the warm mud bath (WMB) group*

4.3. Analysis of GPx level of patients in the two groups according to the time of admission and sleep quality

Sleep quality was subjectively assessed by patients reporting any sleep disturbance or presence of restful sleep. Patients rated sleep quality on a scale from 0 to 5, where 0 = major sleep problems, 5 = restful sleep. The results at the time of admission are shown for CMB group in Table 4 and Figure 3 and for WMB group in Table 5 and Figure 4).

Tabel no 4 *– Descriptive statistics for the GPx level recorded in the CMB group at the time of admission(A) according to the patient's sleep quality*

	Sleep quality (A)	No	Mean (M)	Standard Deviation (SD)	Standard Error of Mean
GPx (I) [U/gHb]	Restful	9	47,86	21,29	7,09
	Sleep disturbance	6	41,40	14,45	5,90

GPx: CMB group: mean value of GPx for patients who found the presence of restful sleep is $M_{\text{Restful sleep}}$=47.86 U/gHb, and the standard deviation of $SD_{\text{Restful sleep}}$ =21.29 U/gHb; for patients who found the presence of sleep disturbances the mean GPx value is $M_{\text{Sleep disturbance}}$=41.40 U/gHb, and the standard deviation of $SD_{\text{Sleep disturbance}}$ =14.45 U/gHb.

There is no statistically significant difference between the mean values of the GPx variable measured in patients with restful sleep compared to patients with sleep disturbances: M_{Dif} = 6.466 U/gHb; t=0.647; df=13; p = 0.529 > α = 0.05; 95%IC_{Dif} =(-15.11, 28.05) U/gHb.

Table no 5 *– Descriptive statistics for the GPx level recorded in the WMB group at the time of admission (A) according to the patient's sleep quality*

	Sleep quality (A)	No	Mean (M)	Standard Deviation (SD)	Standard error of Mean
GPx (I) [U/gHb]	Restful sleep	24	48,58	16,91	3,45
	Sleep disturbance	13	48,92	16,21	4,49

GPx: WMB Group: The mean GPx value for patients who found the presence of restful sleep is $M_{Restful\ Sleep}$=48.58 U/gHb, and the standard deviation of $SD_{Restful\ Sleep}$ =16.91 U/gHb; for patients who found the presence of sleep disturbance the mean GPx value is $M_{Sleep\ disturbance}$=48.92 U/gHb, and the standard deviation of $SD_{Sleep\ disturbance}$ =16.21 U/gHb.

There is no statistically significant difference between the mean values of the GPx variable measured in patients with restful sleep compared to patients with sleep disturbances: M_{Dif} = -0.339 U/gHb; t=-0.059; df=35; p = 0.953 > α = 0.05; 95%IC_{Dif} =(-11.99, 11.31) U/gHb.

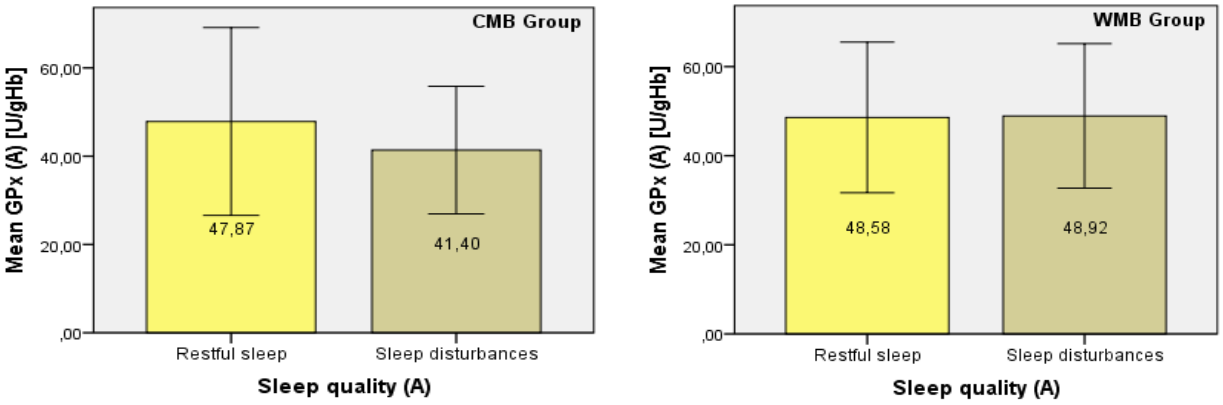

***Figure No. 3** and **Figure No. 4**- Graphical representations of sleep quality of patients included in the research from the CMB and WMB groups at the time of admission (A) according to GPx level variations.*

4.4. Analysis of GPx level of patients in the two groups according to the time of discharge and sleep quality are shown in Table 6 and Figure 5 for CMB group and in Table 7 and Figure 6 for WMB group.

***Table no 6** - Descriptive statistics for the GPx level recorded in the CMB group at the time of discharge (D) according to the patient's sleep quality*

	Sleep quality (D)	No	Mean (M)	Standard Deviation (SD)	Standard Error of the Mean
GPx (E) [U/gHb]	Restful sleep	11	44,61	12,21	3,68
	Sleep disturbances	4	34,71	10,44	5,22

GPx: CMB Group: the mean GPx value for patients who found the presence of restful sleep is $M_{Restful\ sleep}$=44.61 U/gHb, and the standard deviation of SD $_{Restful\ sleep}$=12.21 U/gHb; for patients who found the presence of sleep disturbance the mean GPx value is $M_{Sleep\ disturbance}$=34.71 U/gHb, and the standard deviation of $SD_{Sleep\ disturbance}$ =10.44 U/gHb.

There is no statistically significant difference between the mean values of the GPx variable measured in patients with restful sleep compared to patients with sleep disturbances: M_{Dif} = 9.903 U/gHb; t=1.434; df=13; p = 0.175 > α = 0.05; 95%IC_{Dif} =(-5.01, 24.82) U/gHb.

***Table 7** - Descriptive statistics for the GPx level recorded in the WMB group at the time of discharge according to the patient's sleep quality*

	Sleep quality (D)	No	Mean (M)	Standard Deviation (SD)	Standard Error of Mean
GPx (E) [U/gHb]	Restful sleep	30	47,75	15,48	2,82
	Sleep disturbances	7	46,35	20,73	7,83

GPx:WMB Group: the mean GPx value for patients found to have restful sleep is $M_{Restful\ Sleep}$ = 47.75 U/gHb and the standard deviation of $SD_{Restful\ Sleep}$ = 15.48 U/gHb; for patients found to have sleep disturbance the mean GPx value is $M_{Sleep\ disturbance}$ = 46.35 U/gHb and the standard deviation of $SD_{Sleep\ disturbance}$ = 20.73 U/gHb.

There is no statistically significant difference between the mean values of the GPx variable measured in patients with restful sleep compared to patients with sleep disturbances: M_{Dif} = 1.399 U/gHb; t=0.202; df=35; p = 0.841 > α = 0.05; 95%IC_{Dif} =(-12.66, 15.46) U/gHb.

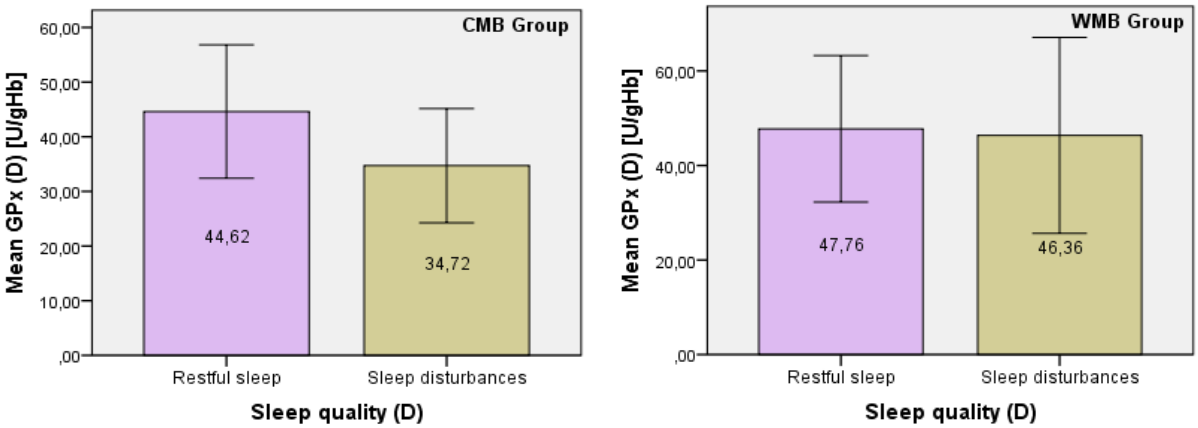

***Figure No. 5** and **Figure No. 6**- - Graphical representations of the sleep quality of the patients included in the research from the CMB and WMB groups at the time of discharge (D) according to GPx level variations*

5 .Discussions

A 2012 study by Gulec et al. investigated the effects of primary insomnia on certain markers of oxidative stress [20]. Thus, glutathione peroxidase, superoxide dismutase and myeloperoxidase activities and reduced glutathione and malondialdehyde (MDA) levels were measured in 30 patients with primary insomnia and 30 healthy volunteers. Results showed that patients with primary insomnia had significantly lower glutathione peroxidase activity compared to controls. Thus, it may indicate the important role of sleep in mitigating oxidative stress [20]. In the studied groups, GPX values and sleep quality did not correlate statistically. As the assessment was subjective, it was not supported by a diagnosis of insomnia by a specialist, insomnia being a major sleep disorder. None of the patients considered that the sleep disorder they had was a real medical problem for which they should seek specialist medical advice.

An important aspect is the value close to statistical significance (p=0.057) obtained in our study in the batch with cold mud bath therapy, regarding the mean values of GPx measured at the four time points. This may suggest an important link between GPx and cold mud therapy. Further studies are needed to confirm this link, on larger groups of patients and over a longer period of time.

6. Conclusions

For the antioxidant status, quantitative fluctuations of GPx can be noticed during the treatment and post balneal treatment in both therapeutic modalities with closed statistical significance (p=0.057) in the cold mud baths group and without statistical significance in the warm mud baths group. Low GPx values during treatment were considered as an adap-

tive mechanism to peloidotherapy, with activation of the antioxidant mechanism post balneal treatment. This finding underlines the importance of extensive evaluations in patients undergoing balneal treatment.

Author Contributions: Conceptualization, LES. and RAU.; methodology, LES, RAU, VMC, LI.; software, LP.; validation, LES., EVI. and LP.; formal analysis, ABU and AEU.; investigation, CM.; resources, CO.; data curation, LES.; writing—original draft preparation, LES and MGI.; writing—review and editing, MGI.; visualization, MGI.; supervision, LES.; project administration,LES and MGI. All authors have read and agreed to the published version of the manuscript. LES, MGI, LP, RAU, VMC and LI contributed equally to this work.

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Institutional Review Board Statement: The study was conducted in accordance with the Declaration of Helsinki, and approved by the Ethics Committee of Balneal and Rehabilitation Sanatorium of Techirghiol (No 15732/ 12.01.2022.

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.
Data Availability Statement: Not applicable.

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