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Effects of Forest Bathing (Shinrin-Yoku) in Female Subjects with Depression/Depressive Tendencies

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Abstract: Background: We previously found that forest bathing significantly reduced the scores for negative emotions and increased the score for vigor in the profile of mood states (POMS) test in both males and females and increased the serotonin level in males, indicating the potential for beneficial effect on depressive status. In the present study, we investigated the effects of forest bathing on the levels of serotonin, oxytocin and insulin-like growth factor I (IGF-1) in blood, Self-Rating Depression Scale (SDS) scores, POMS, subjective fatigue symptoms and subjective sleep quality in female subjects with depression/depressive tendencies. **Methods:** Thirty-one female subjects aged 40.1±2.4 years with depression/depressive tendencies were recruited after obtaining informed consent. These subjects took day trips to a forest park, the birthplace of forest bathing in Japan named Akasawa Shizen Kyuyourin in Nagano Prefecture, and to a city area of Nagano Prefecture as a control in June 2023. On both trips, they walked 2.2-2.5 km for two hours each in the morning and afternoon on Saturday and Sunday, respectively. Blood was sampled in the afternoon before and after each trip. Concentrations of serotonin, oxytocin, IGF-1 and lactic acid in blood were measured. The SDS scores, POMS test, and questionnaires for subjective fatigue symptoms and subjective sleep quality were conducted before and after the trips. Ambient temperature and humidity were monitoring during the trips. The Nippon Medical School Central Ethics Committee approved this study.**Results:** Forest bathing significantly decreased SDS scores and the effect lasted for one week after forest bathing. Forest bathing also significantly increased the level of serotonin in serum in subjects who were not taking antidepressants, significantly increased the levels of oxytocin and IGF-1 in blood, significantly increased the scores for vigor-activity and friendliness and decreased the scores for anger-hostility, confusion-bewilderment, fatigue-inertia, tension-anxiety, depression-dejection and total mood disturbance compared with city walking (all $p<0.05$) in the POMS test. In addition, forest bathing reduced subjective fatigue symptoms and improved sleepiness on rising and sleep length. **Conclusions:** Forest bathing reduces depression symptoms and may have potential preventive effects against depression.

Keywords: depression; female subjects; forest bathing; insulin-like growth factor I (IGF-1); Oguri-Shirakawa-Azumi sleep inventory MA version (OSA-MA); oxytocin; POMS; SDS; serotonin; shinrin-yoku

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1. Introduction

We previously found that forest bathing reduced stress hormones such as adrenaline and noradrenaline in urine and in cortisol in serum in males and/or females [1–3]. Forest bathing also reduces sympathetic nervous activity, blood pressure, heart rate and increases parasympathetic nervous activity, leading to a relaxation effect both in male and female subjects [4–9]. In addition, forest bathing reduced negative emotions such as tension–anxiety, anger, depression, fatigue and confusion and increase in feelings of vigor in the profile of mood states (POMS) test, and showed a relaxation effect in both male and female subjects [2–14] suggesting that forest bathing may have a potential preventive effect on depression. On the other hand, it has been reported that patients with major depressive disorder (MDD) show lower levels of serotonin in serum [15–20]. Recently, we found that forest bathing significantly increased the level of serotonin in serum and significantly improved sleepiness on rising and feeling refreshed in healthy males [14]. These findings suggested that forest bathing has the potential to improve depressive status. However, there has been no study so far on the effects of forest bathing on serotonin in patients with depression/depressive tendencies.

Oxytocin plays a central role in human social behavior, social cognition, anxiety, mood, stress modulation, and fear learning and extinction. The relationships between oxytocin and psychiatric disorders including depression, anxiety, and autism spectrum disorder have been extensively studied [21–23]. These findings indicate an association between depressive symptomatology and oxytocin levels. It has been reported that insulin-like growth factor I (IGF-1) increases the number of new neurons in the hippocampus, contributing to antidepressant effects [24–27]. However, there has been no study on the effects of forest bathing on oxytocin and IGF-1 levels in patients with depression so far.

In addition, there has been no study on the effects of forest bathing on Self Rating Depression Scale (SDS) [28] in subjects with depression/depressive tendencies so far. The novelty of this study is to examine the effects of forest bathing in depressed subjects.

Against this background, we hypothesized that forest bathing may have beneficial effects for patients with depression, and thus in the present study we investigated the effects of forest bathing on female subjects with depression/depressive tendencies.

2. Methods

Subjects

The Self Rating Depression Scale (SDS) test is a tool developed in 1965 for assessing emotional disturbance including depression [28]. Since the SDS is simple, it can be used for assessment in a variety of patients. It is now covered by Japanese medical insurance and therefore has become a useful tool for assessing emotional disturbance in daily clinical practice in Japan. Patients with SDS scores less than 40 are categorized as normal, those with scores between 40 and 49 as borderline, those with scores between 50 and 59 are considered depressive, and those with scores more than 59 as severely depressive [29].

Based on the results of our previous studies, with numbers of subjects between 9 and 20 [1–9,11,13,14], in the present study thirty-one female subjects with SDS scores between 40 and 59 and ranging in age from 24 to 66 years (mean ± standard error: 40.1±2.4 years) were recruited from three clinics (Ichimiya Mental Clinic in Tokyo, Tan Clinic in Tokyo and Hojo Clinic Mizonokuchi in Kawasaki, Japan). Information gathered from a self-administered questionnaire, including age and lifestyle habits that asked about cigarette smoking, alcohol consumption, sleeping hours and physical exercise, have been reported previously [13]. Thirty-one subjects were randomly divided into groups A and B (Table 1). There is no significant difference between groups A and B in age, SDS, depression-related medications, lifestyle habits on cigarette smoking, alcohol consumption, sleeping hours and physical exercise, white blood cell counts (WBC), red blood cell counts (RBC), hemoglobin (Hb), hematocrit (Ht) and platelet (PLT). Written informed consent was obtained from all subjects after a full explanation of the study procedures. The subjects consumed the same number of calories during the two trips since lunch on June 16th (Friday). To control for the effects of alcohol, the subjects did

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not consume alcohol during the study period. This study was conducted in accordance with the Declaration of Helsinki, and was approved by Nippon Medical School Central Ethics Committee.

Table 1. Basic information of the subjects in groups A and B.

	Group A (n=15)		Group B (n=16)		p level
	Mean	SD	Mean	SD	
Age (year)	40.7	14.0	39.6	12.5	>0.05
SDS (Recruit)$	50.3	6.7	50.3	5.7	>0.05
Depression-related medications#	11/15		11/16		>0.05
Smoking#	2/15		2/16		>0.05
Alcohol#	4/15		5/16		>0.05
Daily exercise habits#	7/15		9/16		>0.05
Sleep time (h)	6.6	1.1	6.8	1.1	>0.05
WBC (×10^3/μL)	5.9	1.4	6.3	1.9	>0.05
RBC (×10^6/μL)	4.3	0.3	4.3	0.3	>0.05
Hemoglobin (g/dl)	12.4	1.0	12.8	0.9	>0.05
Hematocrit (%)	37.8	2.9	38.8	2.3	>0.05
Platelet (×10^3/μL)	264	83.5	231.4	52.0	>0.05

$: "Recruit" means the SDS at the time of recruiting subjects. #: number of "Yes".

Walking in a forest environment and city area

The subjects took a three-day trip to a forest park named Akasawa Shizen Kyuyourin (Akasawa Natural Recreation Forest), Agematsu, Nagano Prefecture (situated in central Japan), which is the birthplace of forest bathing in Japan and to a city area of Nagano Prefecture where there were almost no trees in June 2023 as a control. We used a crossover design as shown in Figure 1 and as reported previously [14].

On the first day (Friday), all participants departed from Tokyo in the morning and arrived at a hospital near Akasawa Shizen Kyuyourin where blood samples were taken at 4 pm as the baseline measurements before the forest bathing. The SDS scores, POMS test, questionnaires for subjective fatigue symptoms and subjective sleep quality were conducted before blood collection as baseline controls of forest bathing and city walking. Then the subjects stayed at a hotel near the hospital.

On the second day (Saturday), group A was randomly assigned to the city site and group B was assigned to the forest site. In both trips, they walked 2.2-2.5 km for about 90 min in the morning and afternoon, respectively for a total 4.5-4.8 km per day on Saturday guided by forest therapists as shown in Table 2. During the walks, the subjects took two short breaks. Blood samples were taken at 4 pm at the same hospital after walking in the forest and city sites. Then the subjects stayed at the same hotel.

Table 2. Altitude and walking time, distance, and speed for forest and city walks of guides.

		Forest area		City area	
		AM	PM	AM	PM
June 17 (Day 1)	Time	10:01-11:26	12:45-14:09	09:59-11:25	12:44-14:13
	Walking time	1:25:16	1:24:44	1:26:01	1:29:36
	Distance (km)	2.2	2.3	2.4	2.3
	Speed (km/h)	1.6	1.6	1.7	1.6
	Altitude (m)	1110-1158	1101-1166	633-646	632-645
June 18 (Day 2)	Time	10:00-11:28	12:45-14:12	10:01-11:26	12:45-14:12
	Walking time	1:28:52	1:27:03	1:25:09	1:27:47
	Distance (km)	2.5	2.3	2.3	2.3
	Speed (km/h)	1.7	1.6	1.6	1.6
	Altitude (m)	1110-1165	1112-1171	634-647	631-645

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Both the city walking route (Figure 1) and the forest walking route (Figures 2 and 3) are flat roads, and the forest walking route is wheelchair accessible (Figure 3). Therefore, the effect of the differences in the ups and downs between city walks and forest walks should be limited.

Figure 1. City walking route and walking scene.

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Figure 2. Forest walking route and walking scene.

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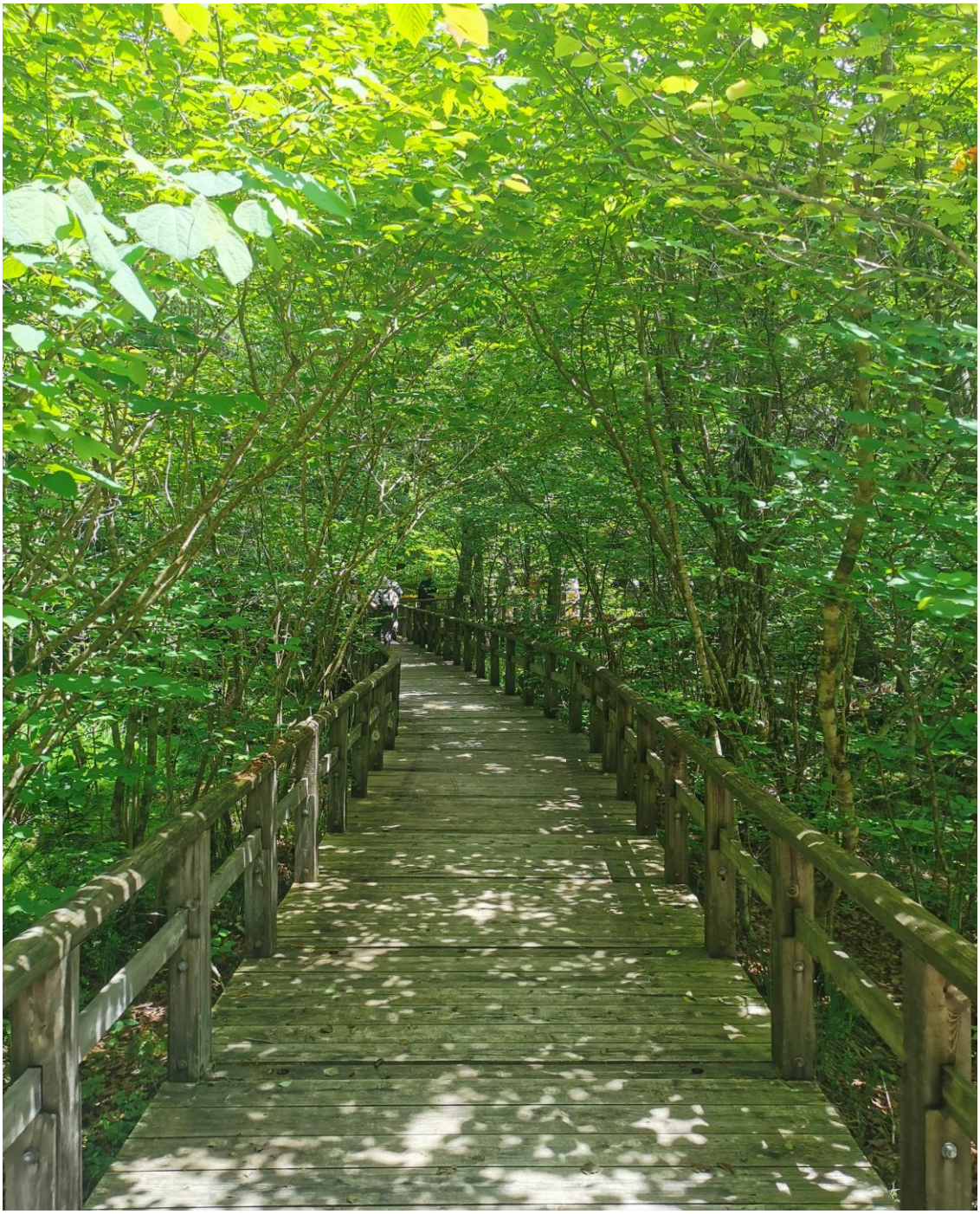

Figure 3. Wheelchair-accessible forest walking route.

On the third day (Sunday), the subjects switched field sites. The experiment protocol was the same as on the second day (Saturday). The blood samples were taken at 4 pm at the same hospital after walking in the forest and unban sites. Then the experiment was complete and the subjects returned to Tokyo.

During these three days, all participants stayed in identical single rooms at the same local hotel. Intake of all foods and physical activity were controlled, and smoking and drinking alcoholic or caffeinated beverages were prohibited. All participants took the same diet and there was no variation in the type of food they consumed during the experiments.

Ambient illuminance, temperature, and humidity were monitored during the trips as reported previously [6,14]. It was sunny on both days in the city and in the forest. As shown in Table 3, on

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both days, the city environmental illuminance and temperature were significantly higher than in the forest areas, whereas the humidity in the forest areas was significantly higher than in the city areas. Although we did not measure wind speed, we collected data from local meteorological observatories. The wind speeds on the 17th around the forest and city were 1.7 m/s and 1.4 m/s and the difference was 0.3m/s; on the 18th, around the forest it was 0.61 m/s, and in the city, it was 1.1m/s, and the difference was 0.5 m/s. Therefore, the wind power in both the forest and the city on both days was light air/light breeze. The influence of the difference in wind speed between city walks and forest walks was quite limited.

In fact, the effects of forest bathing are the total effect of the forest environment including the quiet atmosphere, beautiful scenery, calm climate, pleasant aromas, and clean fresh air compared with city environments.

Table 3. Environmental illuminance, temperature, and humidity in city and forest areas during walks.

			Illuminance (lx)		Temperature (°C)		Humidity (%)	
Sites	Groups	N	Mean	SD	Mean	SD	Mean	SD
Forest on June 17	B	360	16173.2**	28937.2	23.8**	2.9	49.7**	10.3
City on June 17	A	338	56398.4	49372.2	34.2	4.3	23.4	8.4
Forest on June 18	A	386	12096.1**	20389.8	24.1**	3.6	58.8**	14.8
City on June 18	B	335	58500.1	33539.2	34.9	4.2	26.6	7.1

**: $p<0.01$ forest vs city by t-test.

Physiological and Psychological Indices

Serotonin in serum

The concentration of serotonin in serum was measured by the Bio Medical Laboratories, Inc. in Tokyo, Japan (BML) by high performance liquid chromatography (HPLC) as described previously [14]. The normal range of serotonin levels in serum is 81.0-262.0 ng/mL. The detection rate of serotonin was 100% in the present study.

Oxytocin in plasma

The concentration of oxytocin in plasma was measured by enzyme immunoassay with an Oxytocin ELISA kit (Enzo Life Sciences) [30]. The detection limit is 2.5pg/mL and the detection rate of oxytocin was 100% in the present study.

Insulin-like growth factor 1 (IGF-1) concentration in plasma

The concentration of IGF-1 in plasma was measured by BML with *an* electro chemiluminescence immunoassay (*ECLIA*). The normal range of IGF-1 levels in plasma is 59-245 ng/mL. The detection rate of IGF-1 was 100% in the present study.

Lactic acid concentration in serum

To monitor the physical activity of subjects, lactic acid concentration in serum was also measured in the laboratory of Nagano Prefectural Kiso Hospital by enzyme method with a range of 5.0-20.0 mg/dL as described previously [14]. The detection rate of lactic acid was 100% in the present study.

SDS scores

SDS scores were evaluated using Japanese version of the SDS evaluation sheet as described in the section of subjects.

POMS test

Before and after the trips the POMS test was conducted with the POMS 2 questionnaire in Japanese for adults, which is a short version with 35 questions [5–9,11,13,14].

Questionnaire for subjective fatigue symptoms

The questionnaire for subjective fatigue symptoms consists of 30 items and is divided into Groups I, II, and III. Group I (10 items) evaluates sleepiness and sluggishness (decreased vitality), group II (10 items) evaluates difficulty in concentrating attention (decreased energy), and group III (10 items) evaluates fatigue symptoms such as physical discomfort. The questionnaire has previously

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been applied to evaluate the effects of forest bathing [11,31]. This questionnaire was conducted before and after the trips.

Subjective sleep quality

Subjective sleep quality was assessed using the Oguri-Shirakawa-Azumi sleep inventory MA version (OSA-MA) before and after the forest bathing and city walking in the morning [14]. The OSA-MA consists of 16 items measured according to a four-point rating scale and consolidated into the following five factors: sleepiness on rising, initiation and maintenance of sleep, frequent dreaming, feeling refreshed (recovery from fatigue), and sleep length. The OSA-MA scores were calculated as corrected (Zc) scores, with higher scores indicating better quality of sleep [32].

Statistical analysis

If the samples are of equal variance, a paired t-test can be used. In this study, we performed a variance test (F-test) before performing a t-test. We confirmed that the data were of equal variance, and so a paired t-test was used to compare the differences between forest bathing and city walking or between before and after the forest bathing and city walking in lactic acid concentrations, levels of serotonin, oxytocin and IGF-1 in blood, and scores in the POMS and SDS tests and questionnaires for subjective fatigue symptoms and subjective sleep quality. Analyses were performed with the Microsoft Excel software package for Windows [14]. The significance level for p values was set at < 0.05.

3. Results

Guide's physical activity levels

As shown in Table 2, there were no significant differences in the walking time, distance, and speed of the guides who guided the participants between city and forest areas.

Lactic acid concentrations in serum during the forest bathing and urban area walking

As shown in Table 4, there were no significant differences in lactic acid concentrations between forest bathing and city walking (p=0.68), before and after forest bathing (p=.0.91) or before and after city walking (p=0.72).

Table 4. Information of basic statistics of lactic acid (mg/dL) (Mean±SD).

	Before (June 16th)	After city walking	After forest bathing
Mean	4.56±1.91	4.73±1.56	4.61±1.31
N	31	31	31

"Before" means before walking in the city area or before forest bating. p=0.68, forest vs city by paired t-test.

Effect of forest bathing on serotonin in serum

Twenty-two subjects took antidepressants while nine did not take antidepressants during the experiments as shown in Tables 5 and 6.

As shown in Tables 5 and 6, the serotonin levels in serum of the subjects varied greatly depending on whether they were taking antidepressants. Because of this, statistical analyses were conducted separately for subjects taking antidepressants and those not taking them.

Table 5 shows the results of serotonin in serum in subjects who took medications. According to data from a serotonin measurement company (BML), the normal range of serotonin levels in serum is 81.0-262.0 ng/ml. However, the average of serotonin concentrations in serum before walking in the subjects who took the medications was 30.25 ng/ml, which is significantly lower than 81.0 ng/ml, the lower limit of the normal range of serotonin in serum. In addition, there were 12 subjects (12/22) with levels below 10.0 ng/ml.

There were no significant differences in serotonin between forest bathing and city walking, before and after forest bathing, or before and after city walking (all $p>0.05$).

As shown in Table 6, the concentrations of serotonin in serum in subjects who did not take medications were 136.79±38.77 (mean±SD) ng/ml before forest bathing and city walking, 132.60±36.97 ng/ml after city walking, and 138.43±40.59 ng/ml after forest bathing. It is not possible to evaluate the effect of food on blood serotonin concentration the day before the forest walk (June 16th, Friday);

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therefore, in this study, we did not compare blood serotonin concentration before and after forest walk. Instead, we compared blood serotonin levels after forest bathing and after a city walk. All subjects ate the same meal starting from lunch on the first day (June 16th, Friday), so it is possible to rule out any influence of meal on the blood serotonin levels after forest bathing and after walking around the city. There was a significant difference between after forest bathing and after city walking ($p=0.042$), indicating that forest bathing significantly increased the level of serotonin in serum compared to city walking.

The serotonin concentrations in serum before walking in all subjects who did not take medications were higher than 81.0 ng/ml (Mean:136.79 ng/ml).

The average value for subjects who took antidepressant medications (30.25 ng/ml) was significantly lower than that of the subjects who did not take them (136.79 ng/ml) ($p<0.0001$). This suggests that antidepressants significantly reduce blood serotonin concentration.

Table 5. Serum serotonin concentration (ng/ml) in subjects taking antidepressants (Mean±SD).

	Age (years)	Before (June 16th)	After city walking	After forest bathing	Scores of SDS
Mean	36.4±11.3	30.25±39.88	28.75±38.38	28.50±37.80	51.64±6.15
N	22	22	22	22	22

$p>0.05$, forest vs city by paired t-test.

Table 6. Serum serotonin concentration (ng/ml) of subjects not taking antidepressants (Mean±SD).

	Age (years)	Before (June 16th)	After city walking	After forest bathing	Scores of SDS
Mean	49.2±13.0	136.79±38.77	132.60±36.97	138.43±40.59*	46.44±4.39
N	9	9	9	9	9

* : $p<0.05$ (forest vs. city), paired t-test (two-tailed).

Effect of forest bathing on oxytocin in plasma

As shown in Table 7, blood oxytocin concentrations after a walk in the forest and after a walk in the city were both significantly higher than before ($p=0.0014$, $p=0.011$, respectively). The concentration after forest bathing was significantly higher than that after city walking ($p=0.031$), suggesting that forest bathing increases the blood oxytocin concentration.

A significant negative correlation was observed between oxytocin and age ($r=-0.351$, $n=31$, $p<0.05$). In contrast, the concentrations of oxytocin were 8.80±2.90 pg/ml (mean±SD, n=22) in subjects who took antidepressants and 7.28±1.57 pg/ml (mean±SD, n=9) in subjects who did not take the antidepressants, with no significant difference between the two groups ($p=0.071$) indicating that antidepressants did not affect the level of blood oxytocin.

Table 7. Effect of forest bathing on the level of oxytocin in plasm (pg/ml) (Mean±SD).

	Before (June 16th)	After city walking	After forest bathing
Mean	8.36±2.65	9.29±3.00*	10.41±3.87**,$
N	31	31	31

"Before" means before walking in the city area or before forest bating. *: $p<0.05$, **: $p<0.01$ compared with before; $: $p<0.05$ forest bathing vs city walking by paired t-test.

Effect of forest bathing on IGF-1 in plasma

As shown in Table 8, the blood IGF-1 concentration after forest bathing was significantly higher than that after city walking ($p=0.016$), suggesting that forest bathing increases the blood IGF-1 concentration.

Table 8. Effect of forest bathing on IGF-I in plasma (ng/mL) (Mean±SD).

	Before (June 16th)	After city walking	After forest bathing
Mean	150.19±46.92	149.52±50.95	156.06±55.07*

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N	31	31	31

*: p<0.05 compared with city walking by paired t-test.

Effect of forest bathing on SDS scores

As shown in Table 9, there was no significant difference between SDS at the time of recruiting subjects and SDS from day before forest bathing (June 16th, Friday). We thus took the SDS from the day before forest bathing as the baseline SDS and compared it with the SDS after forest bathing and after city walking. Both forest bathing and city walking significantly reduced SDS scores compared to the baseline (all p<0.01). In addition, the SDS score after forest bathing was significantly lower than that after city walking (p<0.01) indicating that forest bathing has a greater effect than city walking. Moreover, the SDS score after one week remained significantly lower than that before forest bathing, suggesting that this effect was sustained for one week after forest bathing.

Table 9. Effect of forest bathing on SDS scores (Mean±SD).

	Recruit	Before (June 16th)	After city walking	After forest bathing	After 1 week
Mean	50.29±6.09	48.61±8.27	43.84±9.64**	40.74±8.72**,##	43.20±12.16**
N	31	31	31	31	31

*: p<0.05, **: p<0.01 compared with before; ##: p<0.01 forest bathing vs city walking by paired t-test. "Recruit" means the SDS at the time of recruiting subjects. "Before" means the SDS from the day before forest bathing, determined as before walking in the city area or forest bathing on June 16th. "City" means after walking in the city area. "Forest" means after walking in the forest (forest bathing). "After 1 week" means one week after forest bathing.

Effect of forest bathing on depressive symptoms in the POMS test

The effect of forest bathing on POMS scores is shown in Table 10. Scores for AH (anger-hostility), CB (Confusion-Bewilderment), DD (Depression-Dejection), FI (Fatigue-Inertia), TA (tension-anxiety), VA (Vigor-Activity), F (Friendliness) and TMD (Total Mood Disturbance) were significantly improved after forest bathing compared with before forest bathing (all p<0.01). Moreover, scores for AH, CB, FI, TA, VA, F and TMD were also significantly improved after forest bathing compared with after city walking (all p<0.05). scores for VA (Vigor-Activity) and F (Friendliness) were significantly higher after forest bathing than after city walking (p<0.01) indicating a beneficial effect of forest bathing in POMS scores. Scores for CB, TA, VA were also significantly improved after city walking compared with before (all p<0.05).

Table 10. Effect of forest bathing on scores in the POMS test (Mean±SD).

	AH	CB	DD	FI	TA	VA	F	TMD
Before June 16th	2.16 ±3.62	5.55 ±4.86	4.36 ±5.06	7.71 ±5.56	6.26 ±5.05	5.74 ±4.84	8.77 ±4.32	20.42 ±22.98
City	1.48 ±3.15	4.35* ±4.8	3.58 ±5.03	9.16 ±4.67	4.16** ±4.81	6.74* ±5.78	9.65 ±5.40	16 ±23.05
Forest	0.74**,$ ±2.77	3.26**,$ ±4.53	2.87* ±4.31	4.61**,$$ ±4.21	2.94**,$ ±4.12	10.55**,$$ ±5.21	11.03**,$$ ±5.06	2.90**,$$ ±21.43

*: p<0.05, **: p<0.01 compared with before; $: p<0.05, $$: p<0.01 forest bathing vs city walking by paired t-test (Mean+SE, n=31) "Before" means before walking in the city area or before the forest bathing on June 16th. "City" means after walking in the city area. "Forest" means after walking in the forest (forest bathing). AH: anger-hostility, CB: Confusion-Bewilderment, DD: Depression-Dejection, FI: Fatigue-Inertia, TA: tension-anxiety, VA: Vigor-Activity, F: Friendliness, TMD: Total Mood Disturbance.

Effect of forest bathing on subjective fatigue symptom scores

Table 11 shows the effect of forest bathing on subjective fatigue symptom scores. Group I evaluates sleepiness and sluggishness (decreased vitality), group II evaluates difficulty in concentrating attention (decreased energy), and group III evaluates fatigue symptoms such as

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physical discomfort, as reported previously [11,31]. It was found that forest bathing significantly lowered the scores of Groups I, II and III compared to before, and significantly lowered the scores of Groups I and III compared with city walking, indicating that forest bathing was effective in improving subjective fatigue.

Table 11. Effect of forest bathing on subjective fatigue scores (Mean±SD).

Groups	Before (June 16th)	After city walking	After forest bathing
Group 1	5.29±2.04	5.19±2.40	3.35±2.29**,$$
Group 2	4.10±2.96	3.26±3.51*	2.71±3.12**
Group 3	2.55±1.55	2.61±1.60	1.58±1.29**
N	31	31	31

*: p<0.05, **: p<0.01 compared with before; $$: p<0.01 forest bathing vs city walking by paired t-test. "Before" means before walking in the city area or before forest bathing on June 16th. "City" means after walking in the city area. "Forest" means after walking in the forest (forest bathing).

Effect of forest bathing on subjective sleep quality

As shown in Table 12a, forest bathing significantly improved sleepiness on rising (p=0.022) and sleep length (p=0.036). Forest bathing also showed trends of improvement in feeling refreshed (recovery from fatigue), initiation and maintenance of sleep and frequent dreaming, but the differences were not significant as assessed by the OSA-MA.

As shown in Table 12b, city walking also showed trends of improvement in sleepiness on rising, feeling refreshed (recovery from fatigue) and sleep length, but the differences were not significant. In contrast, city walking significantly worsened frequent dreaming as assessed by the OSA-MA.

Table 12a. Effect of forest bathing on subjective sleep quality assessed by the OSA-MA.

Forest bathing		Mean	SD	N
Factor Ⅰ	Before	39.15	6.83	16
	After	45.77*	10.84	16
Factor Ⅱ	Before	39.03	13.32	16
	After	42.06	11.77	16
Factor Ⅲ	Before	41.74	16.21	16
	After	43.26	12.59	16
Factor Ⅳ	Before	40.20	10.08	16
	After	45.79	9.30	16
Factor Ⅴ	Before	41.15	8.92	16
	After	51.14*	12.49	16

*: p<0.05 compared with before by paired t-test.

Table 12b. Effect of city walk on subjective sleep quality assessed by the OSA-MA.

City walking		Mean	SD	N
Factor I	Before	38.98	8.80	15
	After	43.91	10.88	15
Factor Ⅱ	Before	40.97	11.80	15
	After	40.68	11.24	15
Factor Ⅲ	Before	48.11	13.85	15
	After	40.27*	14.83	15
Factor Ⅳ	Before	42.34	7.77	15
	After	45.38	11.69	15
Factor Ⅴ	Before	41.85	15.75	15
	After	51.25	10.53	15

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*: p<0.05 compared with before by paired t-test "Before" means before forest bathing or city walking, "After" means after forest bathing or city walking, Factor I: Sleepiness on rising, Factor II: Initiation and maintenance of sleep, Factor III: Frequent dreaming, Factor IV: Feeling refreshed (recovery from fatigue), Factor V: Sleep length.

4. Discussion

This study was the first to comprehensively examine the effects of forest bathing on subjects with depression and depressive tendencies using indicators such as SDS, POMS, serotonin, oxytocin, IGF-1, subjective sleep quality, and subjective fatigue symptoms. We found that forest bathing improved SDS scores and that this effect lasted for one week after the forest bathing. Forest bathing also reduced negative emotions such as anger-hostility, confusion-bewilderment, depression-dejection, fatigue-inertia, tension-anxiety, total mood disturbance and subjective fatigue symptoms, while it increased positive feelings such as vigor-activity and friendliness in the POMS test, corroborating previous reports of such effects in healthy male and female subjects [1–9,11,13,14]. In addition, forest bathing also reduced the scores of subjective fatigue symptoms.

The etiology of MDD in recent decades has been linked to the pathophysiology of the serotonin system [33]. It has been reported that patients with MDD show lower levels of serotonin in serum [15–20]. Serotonin concentrations were significantly lower in the patients with severe atopic dermatitis, and there was an adverse relationship between the serotonin concentration and the score of depression. These features were not noticed in the control group [18]. Against this background, we previously found that walking in a forest park (forest bathing/shinrin-yoku) significantly increased the concentration of serotonin in serum in middle-aged males without MDD compared with walking in an urban area [14]. However, there had previously been no study on the effects of forest bathing on serotonin in serum in patients with depression.

In the present study, we found that patients with depression who took antidepressants had significantly lower concentrations of serotonin in serum compared with subjects who did not take antidepressants. It has been reported that treatment with SSRI antidepressants such as sertraline [34], fluoxetine [35] and citalopram or escitalopram [36] reduces serotonin levels in plasma and/or in serum and that plasma serotonin levels are lower in MDD subjects compared to controls. Baseline plasma serotonin levels did not correlate significantly with baseline HDRS scores in all MDD subjects [34].

In the present study, no increase in blood serotonin levels was observed either after forest bathing or after taking a walk in the city among subjects who had taken antidepressants, and no effect of forest bathing was observed. The effect of antidepressants on blood serotonin levels far exceeded the effect of forest bathing, so it is thought that any effects of forest bathing would have been masked by the antidepressants. Further study on this issue is required in the future.

In contrast, forest bathing significantly increased the level of serotonin in serum in subjects who did not take antidepressant medications compared to city walking, indicating a beneficial effect of forest bathing on the level of serotonin in serum and confirming our previous results in male subjects [14].

Subjects who took medication had significantly higher SDS scores (51.64) than those who did not take medication (46.44) ($p<0.05$), suggesting that subjects who took medications had stronger depressive symptoms.

Oxytocin, a neuropeptide synthesized by the hypothalamus, plays a central role in human social behavior, social cognition, anxiety, mood, stress modulation, and fear learning and extinction. The relationships between oxytocin and psychiatric disorders including depression, anxiety, schizophrenia, and autism spectrum disorder have been extensively studied [21]. Ozsoy et al. [22] reported that female patients with MDD had significantly lower oxytocin levels than control females, whereas no difference was found between male patients and male controls. Furthermore, antidepressant treatments appear to have no effect on serum oxytocin levels. Moreover, Veiga et al. (2022) [23] reported that depressive symptomatology was negatively associated with oxytocin serum levels in healthy female university students. These findings indicate an association between depressive symptomatology and oxytocin levels. In the present study we investigated for the first

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time the effect of forest bathing on oxytocin, and found that forest bathing significantly increased the level of oxytocin in plasma compared with city walking, indicating a beneficial effect of forest bathing on oxytocin in patients with depression. Improved friendliness (Table 10) in the POMS test after forest bathing supported this finding. This is a new finding among the effects of forest bathing in patients with depression. In addition, antidepressant treatments appeared to have no effect on blood oxytocin levels, which is consistent with previous research [22].

It has been reported that IGF-1 increases the number of new neurons in the hippocampus, contributing to antidepressant effects and preventing cognitive decline [24–27]. It is known that exercise-induced increase in serotonin promotes the release of IGF-1 in the hippocampus, increases hippocampal neurogenesis through the IGF-1 signaling pathway, and produces antidepressant effects [26,27]. Thus, in the present study, we investigated the effect of forest bathing on IGF-1 and found that forest bathing significantly increased the level of IGF-1 in plasma compared with city walking, indicating a beneficial effect of forest bathing on IGF-1 in patients with depression. It is a new finding that forest bathing increases the level of IGF-1 in patients with depression.

It is well known that patients with MDD have sleep disorders, and sleep disturbance is a common and key symptom that affects most patients with MDD [37,38]. We previously found that forest bathing improved subjective sleep quality in middle-aged males without MDD evaluated by the questionnaire of OSA-MA [14]. Thus, in the present study, we also investigated the effects of forest bathing on subjective sleep quality by the questionnaire of OSA-MA [32] in female subjects with depression and found that forest bathing significantly improved sleepiness on rising and sleep length. In contrast, city walking did not improve the subjective sleep quality. We previously found that forest bathing significantly increased sleep time [11]. Morita et al. [39] reported that two hours of forest walking improved nocturnal sleep conditions for individuals with sleep complaints, possibly as a result of exercise and emotional improvement. Forest bathing/shinrin-yoku improved subjective fatigue symptom scores including sleepiness.

To control for the effects of alcohol, the subjects did not consume alcohol during the study period. It has been reported that physical activity affects mental health and depression biomarkers [40], and this we also needed to control the effect of physical activity. To this end, subjects walked the same distance during the same period in both trips, guided by forest therapists. We also confirmed that there was no significant difference in lactic acid concentrations in serum between the forest bathing and city area walking. The lactic acid concentration in serum is an useful indicator to evaluate physical activity [14].

Patients with MDD show lower serotonin in serum [15–20], sleep disorders, and depressive symptoms [33,38]. Forest bathing could increase serotonin in serum and improve depressive symptoms and subjective sleep quality, suggesting that forest bathing may have a preventive effect on MDD. However, this effect needs to be confirmed in male patients with MDD in future studies.

The altitude differed between walking in the forest and walking in the city. Regarding the effect of the difference in the altitude between walking in the forest and walking in the city, multiple studies suggest that the risks of depression and suicide increase with increasing altitude of residence and elevation appears to be a significant risk factor for MDD [41,42]. Wang et al. (2019) [43] provide the first evidence that the prevalence of depression in Tibetans of the Qinghai-Tibet Plateau is higher than that in the general Chinese population and that reported in Western studies, a finding that may be related to cultural differences and chronic hypoxia caused by the high altitude. However, no studies were found regarding the effects of short stays (one day) in high-altitude areas on depressive symptoms.

In this study, the altitude of forests (1110-1171 m) is higher than that of urban areas (631-647 m), therefor although high-altitude may reduce the effect of forest bathing on improving depressive symptoms, it does not increase the effects of forest bathing. Namely, the effects of forest bathing on depression at high latitudes in the forest may be underestimated, they are not overestimated. Therefore, the difference in altitude between forest and urban areas does not affect the conclusions of this study.

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The city environmental illuminance was significantly higher than in the forest areas. It has been reported that bright light therapy (≥1,000 lx) can be regarded as an effective treatment for depression [44] indicating that although the effects of forest bathing on depression at lower level of environmental illuminance in the forest may be underestimated, they are not overestimated. Therefore, the difference in environmental illuminance between forest and urban areas does not affect the conclusions of this study.

On both days, the city environmental temperatures were significantly higher than in the forest areas, whereas the humidity in the forest areas was significantly higher than in the city areas. It has been reported that weather conditions influence depressive symptoms [45]. On the other hand, the effects of forest bathing are the total effect of the forest environment including the quiet atmosphere, beautiful scenery, calm climate, pleasant aromas, and clean fresh air compared with city environments which are affected by the weather conditions.

There were some limitations to the present study. Only female subjects were investigated, and male subjects with MDD should also be investigated. We intend to conduct a study including male subjects with MDD next time.

As another limitation of this paper, various factors such as deep breathing, differences between forest and urban in ambient illuminance, temperature, and humidity, the ups and downs during walking and the conditions of the underfoot while walking may affect blood serotonin concentration and symptoms of depression, but in this study, it was not possible to exclude the influence of some of these factors. In addition, this study was a field study, but not a laboratory experiment; therefore, it is difficult to control all confounding factors. This should be studied in the future research. However, both the city walking route (Figure 1) and the forest walking route (Figures 2 and 3 in) are flat roads, and the forest walking route is wheelchair accessible. Therefore, the effect of the differences in the ups and downs between city walks and forest walks should be limited. In fact, the effects of forest bathing are the total effect of the forest environment including the quiet atmosphere, beautiful scenery, calm climate, pleasant aromas, and clean fresh air compared with city environments.

Regarding the sample size, in our past published studies, we obtained statistically significant results despite the number of subjects being 9-20 [1–9,11,13,14]. The number of subjects in this study was 31, which is larger than previous studies.

Regarding generalizability, although this forest bathing study was conducted in a Japanese forest environment, forest bathing is possible in similar forest environments globally. Indeed, as a method of stress management, promoting health and/or preventing diseases, forest bathing/shinrin-yoku which originated in Japan is spreading all over the world now and becoming a focus of public attention [10–12,46–50].

Despite the above limitations, this study has the following strengths.

1. This study revealed for the first time that forest bathing increases the levels of serotonin, oxytocin and IGF-1 in blood in female subjects with depression/depressive tendencies.
2. This study is also the first to find that forest bathing improves SDS scores in female subjects with depression/depressive tendencies. The effect was sustained for one week after forest bathing.
3. This study was conducted in the birthplace of forest bathing in Japan, Akasawa Shizen Kyuyourin. This forest is one of the three most beautiful forests in Japan [10,47,48]. We have conducted several forest bathing experiments in this forest so far [1,6–9,14]. A good forest environment ensured the reliability of the data.
4. By adopting a crossover research design, this study eliminated order bias and improved the accuracy of statistical analysis [14]. Although the effects of the first forest bathing may have an impact on the later city walking next day, even in this situation, forest bathing was found to be more effective at improving depressive symptoms and other indicators than city walking; therefore, this design does not affect the conclusions of this study, as there may be an underestimation, but no overestimation, of the effects of forest bathing.

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The present article does not state that forest bathing cures depression, but rather suggests that forest bathing may have the potential contribution to improve depression due to various factors in addition to the beneficial effects of forest bathing itself.

5. Conclusions

Taken together, our study indicated that the forest bathing/shinrin-yoku program induced significant positive effects on serotonin, oxytocin and IGF-1 in blood, SDS and POMS depressive scores, subjective sleep quality and subjective fatigue symptoms in female subjects with depression/depressive tendencies. These findings indicate a potential preventive effect against depression (depressive status) in female subjects. Future clinical research will verify the improvement of depression through forest bathing.

Declaration

Ethics approval and consent to participate: This study was conducted in accordance with the Declaration of Helsinki. The Nippon Medical School Central Ethics Committees approved this study on March 24, 2023 (No. M-2022-079). Written informed consent was obtained from all subjects after a full explanation of the study procedures. UMIN-CTR registration number is R000058963 (UMIN000051668).

Data Availability Statement: The datasets used and analyzed during the current study are available on reasonable request to the corresponding author.

Conflicts of Interest: The authors declare that they have no competing interests.

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Author Contributions: Qing Li conceived and designed the study and contributed to data acquisition, interpretation of results, and manuscript preparation. Norimasa Takayama, Masao Katsumata, Hiroshi Takayama, Yukako Kimura, Ruri Tan conducted data acquisition. Takashi Miura and Shigeyoshi Kumeda contributed to preparation of the experimental sites and cooperated with data acquisition. Tetsuya Ichimiya, Ruri Tan, Haruka Shimomura and Amane Tatano participated in subject recruitment. Michiko Imai conceived the study and participated in the interpretation of results. Yoichiro Aoyagi and Amane Tatano participated in the interpretation of results. Tsunemi Kitagawa was in charge of monitoring the research. All authors have read and approved the final version submitted for publication.

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