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Talbinah (Barley Porridge): neurotransmitters modulatory effect and antidepressant-like action in experimentally depressed rats

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Abstract. – OBJECTIVE: Talbinah is a traditional barley porridge used as food and brain tonic by Arabs to alleviate depression and reduce stress. It is a food product with the high potentiality to be used as a functional food. The present study was designed to explore the antidepressant-like effects of Talbinah (Barley porridge).

MATERIALS AND METHODS: Thirty-two adult rats were assigned into four groups: negative control group (normal environment), stress-model group, Prozac-treated stress group, and Talbinah treated stress group. The last three groups were exposed to chronic unpredictable mild stress for three weeks. Urine samples were collected at days 0, 21 (after stress induction), 36 (15 days after stress induction), and 51 (one month after stress induction) and were investigated for serotonin, norepinephrine, and dopamine.

RESULTS: Following the establishment of the CUMS protocol, urine serotonin levels decreased with increased levels of dopamine and noradrenaline at 21 days ($p<0.001$). Talbinah substantially restored all the above parameters, indicating an antidepressant-like effect, which might have been attributed to the modulation of monoaminergic pathways and the possible amelioration of oxidative stress.

CONCLUSIONS: These results provide important pharmacological insights into the therapeutic and protective effects of Talbinah against depression. Studies in the future should be directed towards investigating the mechanism underlying the antidepressant activity of these food products.

Key Words:
Talbinah, Barley porridge, CUMS, Urine, Monoamines, Antidepressant.

Introduction

Depression is one of the major leading causes of disability in public health, with a global incidence of over 18%. Depression remains a critical public mental health issue and has become a significant contributor to the worldwide burden of disease[1]. Depression is widely suggested to be biochemical-based or emotionally driven. The biochemical alterations underlying depressive illness are unknown, and comprehensive global research of neurotransmitters in major depressive disorder (MDD) has yet to be completed. Neurotransmitters are chemical messengers in the neurological system that are critical for mental wellness. The monoaminergic system is one of the key mechanisms driving depression and antidepressant effects[2]. Alternative therapies that target different biological pathways for depression are investigated to enhance treatment outcomes since existing medications are somewhat limited.

Nutrition can significantly impact the onset, intensity, and duration of depression. The nutrition approach is a significant component in treating mental illnesses[3]. The impacts of nutrition on mental health and how it interacts with stress have long attracted scientists' attention; nevertheless, the nature of these clinical associations is not well known. Investigating the link between food, stress, mood, and behavior could significantly improve the treatment of stress-related mental disorders[4,5]. As a result, improving one's nutritional state should be a treatment for depression-related disorders and a critical factor in preventing them. Furthermore, recent research[6-8] has revealed the role of dietary supplements, polyphenols, and vitamins as a potential antidepressant treatment, despite the lack of randomized controlled trials in this sector.

Since ancient times, barley (*Hordeum vulgare L*, Poaceae family) has been a staple food throughout the Arabian Peninsula. Due to its high level of bioactive phytocompounds, it is gaining popularity as a functional food ingredient. It contains non-starch polysaccharides, proanthocyanidins, quinones, lignins, minerals, vitamins folic

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acid, flavonoids, and polyphenolic compounds, which possess various pharmacological activities[9-11]. Talbinah is a barley-based porridge with a wide range of possible utilization as a functional food made from soaked barley, milk, and almonds that have been sweetened with honey[12]. Therefore, barley porridge, which contains antioxidative compounds, β-glucans, and flavonoids, may also exhibit an antidepressant-like effect.

Urine has long been the preferred bodily fluid for neurotransmitter measurements due to its non-invasive collection method and the fact that it is the principal mechanism of neurotransmitter excretion[13]. Some scholars[14] evidence that urine monoamines may be useful as biomarkers of nervous system function. Thus, urine excretion of monoamines and their measurements act as a biomarker in diagnosing and managing patients with depression. Moreover, it has been consistently shown[15] the significant relationship of depression disorders with urine monoamine excretion levels. The present ELISA provides a convenient and sturdy method for monitoring urine monoamines, including serotonin. To our knowledge, there is no study yet to investigate the effects of Talbinah on urine monoamines. Therefore, this study was designed to determine the impact of Talbinah (barley porridge) on levels of urine neurotransmitters in experimental rats, utilizing a chronic unpredictable stress model of depression.

Materials and Methods

Materials

Serotonin (5-HT), norepinephrine (NE), and dopamine (DA) ELISA assay kits were purchased from Abnova company (Baverly, MA, USA). Prozac (Fluoxetine hydrochloride) was purchased from a local pharmacy. Fresh barley grains were bought from the local market. The experiments were done at the physiology labs of the College of Medicine, King Khalid University, from September 2021 till November 2021.

Animals

Thirty-two adult Wistar male rats, from the same lineage and genetic pool, aged six months and weighing 230-250 g, were obtained from the animal house at King Khalid University, Abha, Saudi Arabia.

The animals were housed in polypropylene cages of standard dimensions (50×26×16 cm) in groups (4 rats per cage) at 25±1°C with a standard 12 h day/night cycle. During the experimental period, all rats had free access to their drinking water and selected diet. The experimental design employed in the present study was approved by the Research Ethics Committee (REC), King Khalid University (ECM #20-0862). The study was carried out following the National Institutes of Health and National Society for Medical Research protocols for the care and use of laboratory animals. Every effort has been made to ensure minimal animal suffering and the number of animals used.

Preparation of Talbinah

Fresh barley grains from the local market were grounded into a coarse powder, then, the barley flour was added to water (1:10 w/v) according to Youssef[16], 2008. The mixture was then heated at 80 ± 5°C for 5 min with constant stirring until a porridge-like consistency was reached, and then it was left to cool. Talbinah was prepared fresh each day before being given to the test animals. Barley (200 mg/kg/day, p.o.) doses were used in this experiment.

Experimental Design

After two weeks of acclimation, all rats were sorted into four groups randomly (n = 8). 1) Control group: fed regular rat's diet for 51 days (no stress and no drug); 2) stress-model group: fed regular diet for 51 days but were exposed to chronic unpredictable mild stress (CUMS) during the first 21 days; 3) positive drug-treated stress group: Fed regular diet for 51 days, exposed to CUMS during the first 21 days, and daily administered fluoxetine (dissolved in normal saline) (2 mg/kg, i.p) for 30 days post the CUMS; 4) Talbinah treated stress group: fed regular diet for 21 days, exposed to CUMS during the first 21 days, and then, fed Talbinah (200 mg/kg/day, p.o) as a diet for the next 30 days post the CUMS.

Chronic Unpredictable Mild Stress (CUMS) Induced Depression Model

CUMS were used to induce irreversible depression in rats and were as follows: cage tilting and damp sawdust for 24 h (200 ml of water per individual cage, which is enough to make the sawdust bedding wet), 5 min cold swimming at 4°C cold water, noises for 1 h (alternative periods of 60 dBA noise for 10 min and 10 min of silence), 5 min thermal stimulation in an experimental room at 50°C, 48 h of food deprivation and 24 h of water deprivation, respectively, 15 electric shocks to

the foot (15 MA, one shock/5 s, 10 s duration), a 1 min tail pinch, and restricted movement for 4 h. During a period of 3 weeks, one of the stimuli was selected randomly and applied per day for each rat[17]. Under the same circumstances, the healthy control group of rats was accommodated undisturbed in another experiment room. The electrical shock was induced in the rats using the PowerLab stimulator (model No. 26T, ADinstruments, Sydney, Australia).

Urine Collection and Biochemical Analysis

Urine samples were collected from rats at day 0, 21 (after stress induction), 36 (15 days after stress induction), and 51 (one month after stress induction) by the use of metabolic cages (1 rat/cage) and were filtered using 0.2-µm filters. Special ELISA kits (Abnova, Beverly, Massachusetts, USA) were used for the determination of NE (Cat No. KA1891; Novus Biologicals, CO, USA), DA (Cat NO. KA1887; Novus Biologicals, CO, USA), and 5-HT (Cat. No. KA 1894; Novus Biologicals, CO, USA), using Multiskan FC microplate reader (Thermo Scientific, Dallas, TX, USA).

Statistical Analysis

The data were analyzed in GraphPad Prism version 8.0.0 for Windows, GraphPad Software, (San Diego, CA, USA, www.graphpad.com) and the results were reported as mean ± the standard error of the mean (SEM). A two-way ANOVA using Tukey's honestly significant difference test was applied to evaluate the statistically significant changes between the treatments and intervals, with $p< 0.001$ considered statistically significant.

Results

A total of three 5-HT, NE, and DA were evaluated as potential urinary biomarkers for CUMS-induced depression in rats. ELISA method was used to measure the urinary neurotransmitter levels. The 5-HT concentration was significantly decreased in the urine samples of CUMS-induced animals than in normal rats, as seen in Figure 1. Lower urinary levels of 5-HT than in controls were also observed in the CUMS-exposed treat-

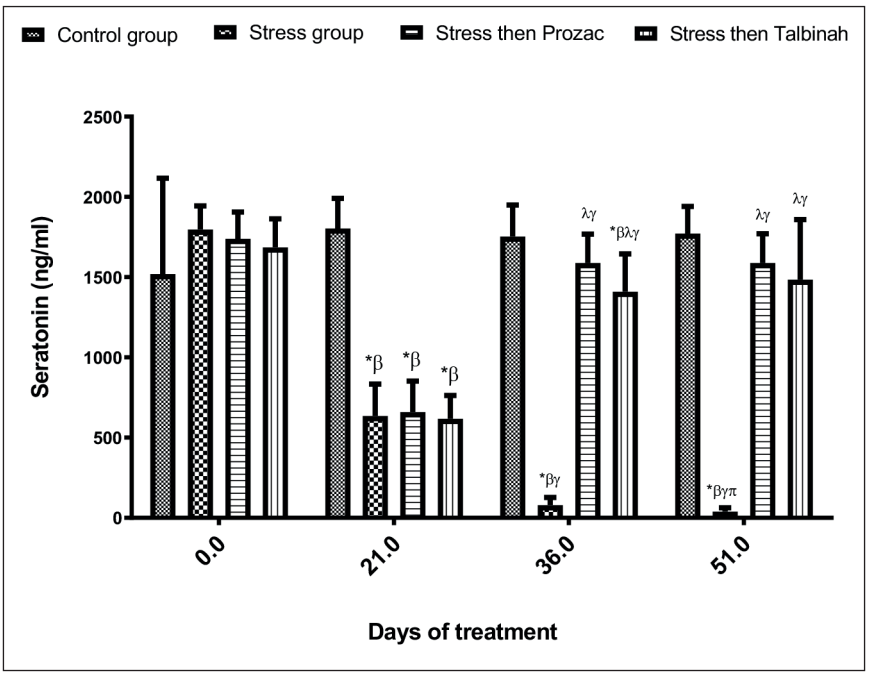

Figure 1. Effect of chronic oral administration of Talbinah on urine serotonin (5-HT) content in CUMS-indued depression on rats. The values are expressed mean ± SEM. Statistical analyses were performed between various groups and treatment intervals. Two-way ANOVA followed by Tukey's honestly significant difference test was used to analyze the data. *, β, λ, γ, $p<0.001$ are considered statistically significant. *: significantly different from the control group of day 0.0. β: significantly different from the control group of the same day. λ: significantly different when compared to the stress group of the same day. γ: significantly different when compared to day 21.

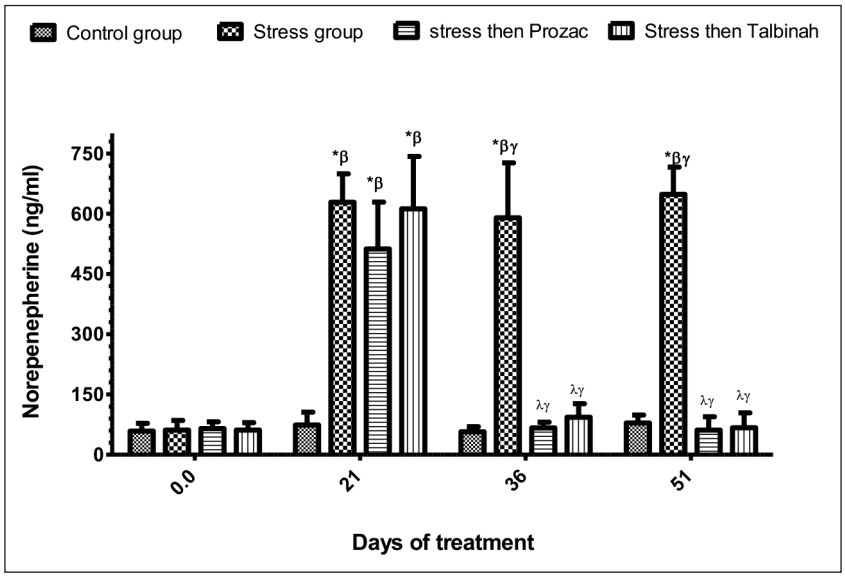

Figure 2. Effect of chronic oral administration of Talbinah on urine norepinephrine (NE) content in CUMS-indued depression on rats.
The values are expressed mean ± SEM. Statistical analyses were performed between various groups and treatment intervals. Two-way ANOVA followed by Tukey's honestly significant difference test was used to analyze the data. *, β, λ, γ, π $p < 0.001$ are considered as statistically significant. *: significantly different from the control group of day 0.0. β: significantly different from the control group of the same day. λ: significantly different when compared to the stress group of the same day. γ: significantly different when compared to day 21.

ment groups. However, long-term therapy with Talbinah significantly increased the concentration of 5-HT levels after 36 and 51 days ($p<0.001$) compared with CUMS-stressed rats. The 5-HT concentration in the Prozac group was also significantly increased after 36 and 51 days ($p<0.001$) than in the CUMS control group.

Figures 2 and 3 show a significant rise in the urinary levels of NE and DA after 21 days of CUMS induction compared to the unstressed group. When comparing the CUMS-stressed group to the treatment group, we found a sustained and substantial increase in NE and DA levels. Both Talbinah and Prozac-treated groups exhibited a significant decrease in NE (Figure 2; $p < 0.001$) and DA levels (Figure 3; $p < 0.001$) after 36 and 51 days.

Discussion

Urine samples have an advantage over blood samples when it comes to collecting, especially when dealing with small experimental animals. Moreover, non-invasive sampling can avoid interfering with the neuropsychological activities of these animals[18]. A total of three monoamines, DA, NE, and 5-HT were measured as potential urine biomarkers for chronic unpredictable mild stress-induced depression. The urine levels of NE and DA were significantly elevated, while the levels of 5-HT were significantly reduced at 21 days in all the experimental groups exposed to the CUMS. The altered levels of these urine monoamines after 21 days could be associated with the CUMS-induced depression. The levels of DA and NE were substantially decreased, along with elevation of 5-HT level in chronically stressed rats that received the Talbinah food, at 36 and 51 days. Chronic oral administration of Talbinah significantly reversed the clinical observations caused by CUMS, similar to those in rats given the Prozac. These results indicate that Talbinah can restore the altered levels of urine neurotransmitters, which signifies the increased monoamines in synaptic clefts. Normalization of neurotransmitter expression might indicate changes in stress pathways and neuromodulator function and induce the antidepressant effects of Talbinah. The exact mechanism for the inhibitory activity of Talbinah on urine DA and NE levels remains unclear and should be further explored.

Some experimental studies[19] have confirmed that chronic unpredictable mild stress models could induce depression-like behavior in the ex-

perimental rats. It is currently the most used, reliable, and effective rodent model of depression[20,21]. The brain monoamine systems play a primary role in depression and are supported by the antidepressant mechanism of action[22]. The 5-HT system's dysfunction plays a crucial part in the pathophysiology of depression[23]. It was previously shown[24] that oral administration of Talbinah led to a substantial increase in DA, 5-HT, and GABA contents in different brain regions of male rats. In a randomized cross-over study, the results demonstrated that daily morning consumption of Talbinah could reduce depression after three weeks of supplementation and enhance mental health in elderly depressed patients[25]. The depression score decreased in the intervention group[26]. Another possible mechanism of action of Talbinah is the attenuation of oxidative stress produced during CUMS depression by the polyphenols, minerals, and flavonoids present in the Talbinah diet. These effects could be ascribed to barley's flavonoids, β-glucan, ferulic, sinapinic, and ß-hydroxy acids (BHA) contents, the major predominant polyphenols, with its potent free radical scavenging nature[27,28].

Nutrition is essential for emotional well-being and mental health. Significant epidemiological evidence[29] indicates that an unhealthy diet may be associated with depression. On the other hand, several randomized controlled trials and scientific studies[30,31] have suggested that a healthy diet may directly reduce depression. Talbinah is a traditional Arab diet that has been used to cure depression for several years. It is a food product with the high potentiality to be used as a functional food[32]. Prophetic medicine and scientific evidence[33] have accorded the claim that Talbinah gives rest to the patient's heart and relieves some of his sorrow and grief. Apart from religious and cultural applications, scientific evidence supports the use of zinc-rich Talbinah in treating depression symptoms[34]. Talbinah contains 5 mg zinc per serving, according to Badrasawi et al[25], which could help with depression symptoms. Several recent studies[35-37] have confirmed that the consumption of talbinah has an ameliorating effect on depressive symptoms. Moreover, daily oral administration of Talbinah improves the content of neurotransmitters, which makes it potentially safe for sedation[24].

Limitations

Regarding the limitations of this study, first, in this study, male rats were deliberately chosen to avoid the interference of hormonal disturbances/changes, which are particularly important in

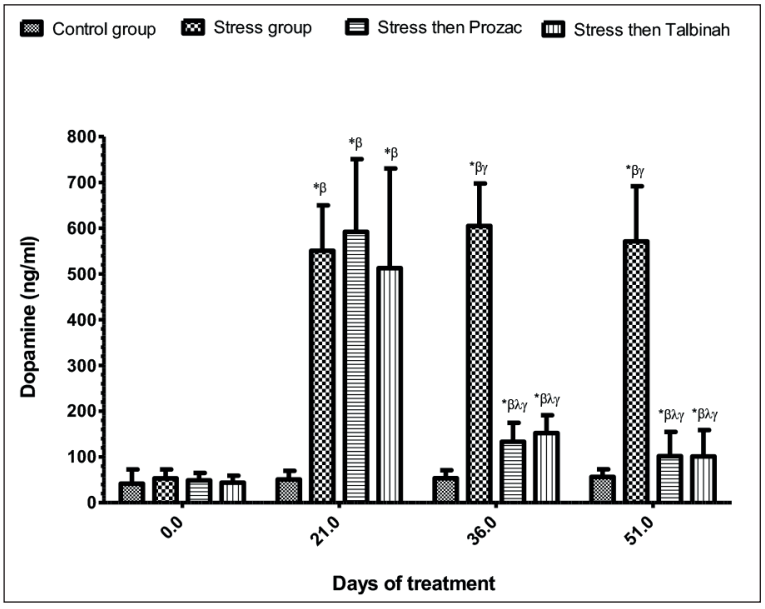

Figure 3. Effect of chronic oral administration of Talbinah on urine dopamine (DA) content in CUMS-indued depression on rats. The values are expressed mean ± SEM. Statistical analyses were performed between various groups and treatment intervals. Two-way ANOVA followed by Tukey's honestly significant difference test was used to analyze the data. *, β, λ, γ $p<0.001$ are considered statistically significant. *: significantly different from the control group of day 0.0. β: significantly different from the control group of the same day. λ: significantly different when compared to the stress group of the same day. γ: significantly different when compared to day 21.

testing the drugs for psychological and behavioral analysis. According to the literature, there is still variability in neurotransmitter levels between adult males and female rats, even for the neural response to drugs, due to the difference in neurotransmitter and hormone levels during the estrous cycle. However, in the future, similar research should be conducted to compare the effect of gender difference on the antidepressant activities of Talbinah. Another limitation is the short period (30 days) in which we monitored the impact of Talbinah and Prozac on depressive symptoms and the level of neurotransmitters in urine. It is known that the treatment of depression cases requires at least six months of using the antidepressant medications. Accordingly, it would be better to repeat the same experiment but follow the effects of Talbinah for six months to a year, which gives the study more reliability[38].

Conclusions

Based on our findings, these restored urine levels of DE, NE, and 5-HT indicate Talbinah's efficacy, which may be attributed to the modulatory effects on the central monoaminergic system. In addition, these *in vivo* findings will support the investigation of the pathophysiological mechanism of the rat's CUMS-induced depression model and the evaluation of Talbinah's efficacy in the treatment of MDD. Further, our results validate the traditional use of barley porridge for the management of depression. However, other possible mechanisms could play a role in the antidepressant-like effect, and further pharmacological experiments should be designed to confirm these findings. The current research could be an important step in developing new dietary products that can be used to treat depression.

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Conflicts of Interest

The authors declare no conflicts of interest.

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