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Research Article

Sleep Sleep potentiating effect of Pleurotus squarrusolus ethanolic extract on diazepam-induced sleeping time in male wistar rats

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Abstract

This study examines the sleep-promoting properties of an ethanolic extract derived from Pleurotus squarrosulus in male Wistar rats, particularly in conjunction with the sleep-inducing effects of diazepam. The extract was subjected to analysis using High-Performance Liquid Chromatography (HPLC), which revealed the presence of gallic acid, p-coumaric acid, ferulic acid, catechin, rutin, apigenin, quercetin, and kaempferol. Among these compounds, gallic acid and quercetin exhibited the highest concentrations. Diazepam, a traditional sedative, was administered alone as well as in combination with two different doses of the extract, namely 250mg and 500mg. The results demonstrated a significant increase in sleeping time in both the Diazepam + Sample 250mg and Diazepam + Sample 500mg groups compared to the control group, indicating an enhancement in sleep quality. These findings are consistent with previous research, which highlighted the presence of bioactive compounds known for their diverse biological effects. This study introduces Pleurotus squarrosulus as a potential natural sleep aid, showcasing a promising synergy when combined with diazepam. Further investigation into the mechanisms and safety considerations of this combination offers an optimistic path for the development of integrated approaches to sleep management.

Keywords: anxiolytic; sedative activity; sleep; insomnia; oyster mushroom; pleurotus squarrosulus; diazepam

Introduction

Insomnia and depressive symptoms appear to possess a mutually influential connection, as indicated by existing literature. Prior investigations have highlighted a bidirectional association between depression and insomnia: individuals suffering from depression often display exacerbated symptoms of insomnia, while those afflicted by insomnia are more prone to the development of depression (1,2).

In a community-based inquiry, (3) delved into the interplay between sleeplessness and depression. They obtained responses from a sample of the population (N = 1007) utilizing the Insomnia Severity Index, Patient Health Questionnaire-9 (PHQ-9), and average sleep duration. Subsequently, regression analysis was employed to assess the prevalence risks (PR) of depressive symptoms based on the intensity of insomnia and sleep duration. The findings underscore a robust correlation between sleeplessness and the severity of depression, with this association contingent upon regular sleep duration. A subsequent examination of data from 1,922 women, utilizing Pearson Correlation and meta-analytic techniques, unveiled a significant, medium-sized connection between symptoms of prenatal depression and sleeplessness.

The correlation between rapid eye movement (REM) sleep disorders and depression is another aspect to consider. The amygdala, a pivotal region in regulating REM sleep, possesses neuronal extensions into the brainstem. In individuals afflicted by depression, the amygdala may exhibit abnormalities such as enlargement, hyperactivity, or malfunction, as it is intricately linked to the processing of negative thoughts. While these relationships have opened new avenues for research into the neurobiology and treatment of depression, their precise significance remains to be fully elucidated (4).

Methodology

HPLC-DAD quantification of phytocompounds in *Pleurotus squarrosulus* ethanolic extract

The analysis was performed on a Shimadzu (NexeraMX) HPLC system with uBONDAPAK C18 column (length 100 mm, thickness 7 µm, diameter 4.6 mm). The mobile phase was aqueous methanol and the detector, UV diode array detector (DAD) at 254 nm.

Procedure

Exactly, 10g of the test sample was measured into an amber bottle and 20 ml of acetonitrile and methanol mixture was added to it and the mixture was shaken vigorously for 30 mins. Afterwards, the aqueous end was run off while the organic solvent end was collected into 25 mL standard flask, made up to mark and ready for analysis. Standard form of analyte was first injected into the HPLC, and this generated a chromatogram with a given peak area and peak profile. These were used to create a window in the HPLC in preparation for the test sample analysis. An aliquot of the extracted extract was injected into the HPLC, to obtain a corresponding peak area and peak profile in a chromatogram. Then the peak area of the sample was compared with that of the standard, relative to the concentration of the standard to obtain the concentration of the sample (6).

Concentration of sample = (peak area of sample × standard concentration)/Peak area of standard

Sedative Property of *Pleurotus squarrosulus* ethanolic extract

The animals were divided into three groups of five animals each.

The sleep-potentiating effect of the extract was studied in animals that received diazepam (50mg/kg) one hour after extract or vehicle administration. The time from loss of righting reflex till its recovery was taken as sleeping time (7).

1. Group 1: Control group; received diazepam 50mg/kg intraperitoneally only.
2. Group 2: received *Pleurotus squarrosulus* ethanolic extract 250 mg/kg b.w orally, then diazepam 50mg/kg intraperitoneally, 1 hour after.
3. Group 3: received *Pleurotus squarrosulus* ethanolic extract 500 mg/kg b.w orally, then diazepam 50mg/kg intraperitoneally, 1 hour after.

Results

HPLC determination of phyto-compounds present in *Pleurotus squarrusolus* ethanolic extract shows the extract contain gallic acid (3,4,5-trihydroxybenzoic acid) a phenolic compound, as its most abundant phyto-compound, with a concentration of 8.01mg/g followed by quercetin with a concentration of 4.76 mg/g.

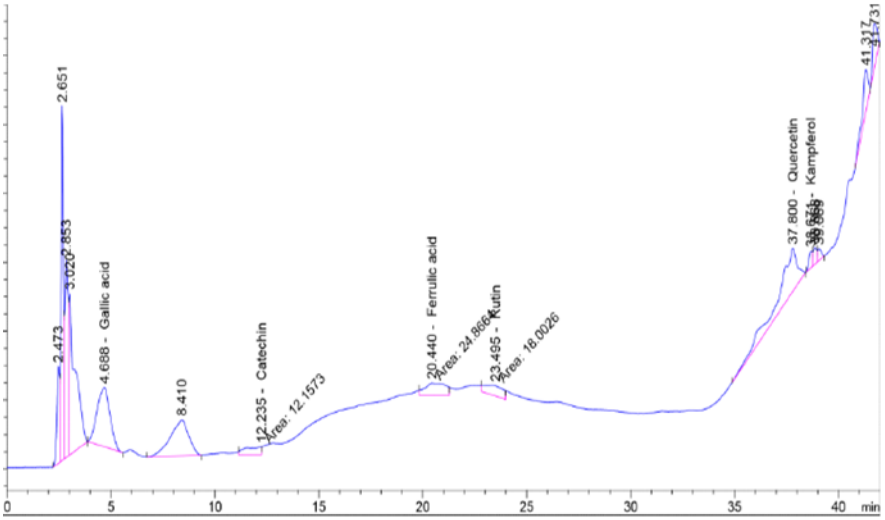

Figure 1: chromatogram showing the absorption peaks of phytocompounds present in 1mg/ml of the

ethanolic *Pleurotus squarrusolus* extract Sleep potentiating effect of *Pleurotus squarrusolus* ethanolic extract on diazepam-induced sleeping time in male wistar rats

Grouping	Sleeping time (Mins)
Control	232 ± 0.03
Diazepam + Sample 250mg	296 ± 0.12
Diazepam + Sample 500mg	298 ± 0.13

Table 1: Tabular representation of the sleeping time

Values represent mean ± standard deviation of replicate experiments (n = 3). Values are significantly different ($p< 0.05$).

Discussion

The use of HPLC analysis on the extract has brought to light a variety of bioactive compounds, encompassing gallic acid, p-coumaric acid, ferulic acid, catechin, rutin, apigenin, quercetin, and kaempferol. These compounds are renowned for their diverse biological impacts, which include antioxidant, anti-inflammatory, and neuroprotective properties. Notably, rutin, gallic acid, apigenin, and quercetin have been specifically associated with potential sleep-enhancing effects attributed to the *Pleurotus squarrosulus* extract (8,9).

The ingestion of *Pleurotus squarrosulus* may provide sleep-related advantages beyond its nutritional value due to the identified compounds. Gallic acid, classified as a phenolic compound, exhibited the highest concentration at 8.00 ± .02 mg/g, followed by quercetin at 4.75 ± 0.16 mg/g, Flavonoids, such as catechin, rutin, apigenin, and kaempferol, were also detected at concentrations ranging from 1.01 ± 0.20 to 3.08 ± 0.27 mg/g, consistent with prior studies (10). Moderate levels of phenolic compounds, specifically p-coumaric acid (2.02 ± 0.20 mg/g) and ferulic acid (2.71 ± 0.33 mg/g), were also observed in the extract.

These findings underscore the potential sleep-promoting characteristics of *Pleurotus squarrosulus*, suggesting that its consumption may contribute to improved sleep quality and overall sleep-related well-being. The prominent concentrations of rutin, gallic acid, apigenin, and quercetin further substantiate the hypothesis that this extract may possess inherent sleep-potentiating effects, offering a promising direction for further investigation in the realm of sleep research.

The observed increase in sleeping time in both the Diazepam + Sample 250mg and Diazepam + Sample 500mg groups prompts exploration into the potential synergies between diazepam and *Pleurotus squarrosulus.* Diazepam, a GABA receptor agonist, induces sedation, and the extract may potentiate its effects, possibly through modulation of neurotransmitter systems involved in sleep regulation. While the absence of a control group receiving only diazepam limits a direct comparison, the significant increase in sleeping time in extract-treated groups, when contrasted with the control, strongly suggests the extract's involvement in promoting sleep. This highlights its potential as a complementary agent to traditional sleep medications.

Conclusion

In conclusion, the study unveils a promising collaboration between *Pleurotus squarrosulus* ethanolic extract and diazepam, resulting in a significant sleep potentiating effect in male Wistar rats. This synergy suggests a novel avenue for developing integrative approaches to managing sleep disturbances, combining the benefits of traditional

medications with those of natural compounds. As research progresses, *Pleurotus squarrosulus* may emerge as a valuable player in the realm of sleep therapeutics, offering a holistic approach to addressing the complexities of sleep-related concerns.

Conflict of interest declaration

I, Akingbesote, babajide oluwaseun submit this manuscript titled Sleep potentiating effect of Pleurotus squarrusolus ethanolic extract on diazepam-induced sleeping time in male wistar rats for consideration for publication. I hereby declare that I, and any co-authors, have no conflicts of interest that could influence or be perceived to influence the research presented in this manuscript

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