MESMAP – 8
ABSTRACTS & PROCEEDINGS BOOK
20-22 October 2022, Izmir-Türkiye
www.mesmap.com

FULL TEXT–ORAL PRESENTATION

H. PERFORATUM L. AFFECTS LOCOMOTOR ACTIVITY, BODY WEIGHT AND BLOOD GLUCOSE

Betül Önder Uzgan[1*], Berrak Damla Yağan, Bülent Gündüz

[1] *Department of Biology, Faculty of Science, Canakkale Onsekiz Mart University, 17000, Canakkale, TURKEY*
E-mail: betulonder.uzgan@comu.edu.tr, ORCID ID: 0000-0002-6423-6704

Abstract

Hypericum perforatum is a perennial herb that is widely distributed in all Mediterranean and mainly contains hyperforin and hypericin. It is commonly used as an antidepressant. In rats fed a high-fat diet, *H. perforatum* was found to prevent weight gain. It has been shown to cause the total glucose level to remain elevated for six weeks. We investigated how different doses of *H. perforatum* extract affected Syrian hamster locomotor activity, body weight, food consumption, and blood glucose levels. The aerial parts of *H. perforatum* were subjected to maceration in ethanol using a Soxhlet apparatus. Syrian hamsters received injections of plant extract at doses of 100 mg/kg, 200 mg/kg, and 300 mg/kg. The animals' daily body weights and food intake were calculated, along with their locomotor activity for the course of the 10-day experiment. Blood glucose levels were measured twice, before and after the extracts were injected. Results showed that as doses increased, animals' locomotor activity, body weight, and food intake decreased. After being administered into hamsters, an extract at a dose of 300 mg/kg considerably raised blood glucose levels. High doses of *H. perforatum* extract can therefore be thought of as both an antidepressant and an anti-obesity medication. The decrease in daily activity rhythms, in particular, may be an indication of *H. perforatum's* sedative effect.

Key Words: *H. Perforatum,* locomotor activity, body weight, food intake, blood glucose

1. Introduction

Hypericum perforatum L., a plant used for medicine that can be found in Europe, Asia, North Africa, America, and the Mediterranean, has been utilized for healing by many ancient civilizations. *H. perforatum* (St. John's wort) is known as an herbal medicine for anxiety disorders, seasonal affective disorder, and depression.

Numerous bioactive substances, such as, flavonoids, phloroglucinols, naphthodianthrones, phenylpropanes, tannins, amino acids, essential oils, xanthones, and other substances, have been reported to be present in *H. perforatum*. These substances have a variety of biological properties and can be used as antioxidants (Li et al., 2008; Al-Fatimi et al., 2007; Arda et al., 2002, and Vorbach et al., 1997). *H. perforatum* affects serotonergic, dopaminergic, noradrenergic, and GABA receptors and reuptake of neurotransmitters, owing primarily to the presence of hyperforin and hypericin (Nathan, 2002). *H. perforatum* is a plant that increases the circadian rhythm's sensitivity to light, making it employed as an antidepressant in situations of mood disorders (Harrer, 2000; Boivin, 2000).

H. perforatum inhibited both locomotor hyperactivity at doses of 25, 50, and 100 mg/kg, especially after 2 and 6 hours of ethanol abstinence (Coskun et al., 2006). However, while repeated doses of *H. perforatum* had anxiolytic and anti-panic effects, they did not cause any change in locomotor activity (Beijamini and Andreatini, 2003). Additionally, it was claimed that *H. perforatum* could not alter the circadian system's sensitivity to light because it did not alter the activity levels of rats when it was applied, and the antidepressant effect may not be connected to this (Francis, 2005). In fact, the

observation of a significant reduction in locomotor activity at a dose of 200 mg/kg shows that the findings in the literature on locomotor activity are incompatible (Diana et al., 2007).

It was shown that *H. perforatum* reduced insulin resistance in rats fed a fructose diet and reduced weight gain in rats fed a high-fat diet (Husain et al., 2011). Contrary to these findings *H. perforatum* has been found to have significant impact on adipocyte-related disorders by including insulin resistance in white adipose tissue cells (Amini et al., 2009). In studies with humans, however, *H. perforatum* increased the total glucose level in male individuals and even caused it to remain elevated for 6 weeks. Due to the fact that long-term *H. perforatum* therapy decreased insulin secretion and worsened glucose tolerance in this investigation, it was assumed that this impact was likely mediated by beta cells (Stage et al., 2011).

With all of this information mentioned above, the study's objective is to evaluate the effects of various doses of this plant, which has been used extensively in the health industry, particularly on the circadian rhythm, as well as changes in blood glucose, food intake, and body weight in Syrian hamsters with strong photoperiodic properties.

2. Materials and Methods

2.1. Collection of Plant Material

Plant materials were collected in Ida Mountain (Kazdagları-Canakkale) from 342 meters (geographical coordinates; N 39º 45' 08", E 026 º 48' 54") in the area of Northwest Turkey in May 2022. Voucher specimens have been deposited.

2.2. Preparation of Crude Extract

Aerial parts were dried for 10 days at room temperature in sterile condition. A total of 20 g of each dried material was extracted for 24 hours in 300 ml 70 % ethanol using a Soxhlet apparatus. The solvent was evaporated under a vacuum and then dissolved DMSO (Dimethyl Sulfoxide).

2.3. Animals

Twenty adult male Syrian hamsters (*Mesocricetus auratus*) were obtained from the Hamster and Gerbil unit of Çanakkale Onsekiz Mart University. Hamsters were subjected to 16 hours of light and 8 hours of darkness in ventilated rooms at a temperature of 22 ± 2 ºC. Weighing 100-120 g animals were housed in plastic cages (16 x 31 x 42 cm). Animals had free access to food pellets and tap water. The procedures used in this study were approved by the Institutional Animal Ethics Committee of Canakkale Onsekiz Mart University (2019/10-09). A cool-white, fluorescent lighting system controlled by automatic programmable timers was provided.

2.4. Experimental Groups

The study was carried out under four groups. The first group (n=5) was given only DMSO as the control group. In the second group, 100 mg/kg *H. perforatum* extract was administered intraperitoneally. In the third group, 200 mg/kg *H. perforatum* extract was administered intraperitoneally and in the fourth group 300 mg/kg *H. perforatum* extract was administered. Injections were administered every day at the same time (12:00 - 13:00 h) during the 10-day experiment.

2.5. Locomotor Activity

Before starting the experiment, animals were placed into the activity wheels for 5 days. Animals that showed distinct circadian rhythms were selected for the experiment. Animals with normal locomotor activities were placed on running wheels and included in the 10-day experiment. The number of wheel revolutions was automatically recorded per 15 min interval. Vital View Data Acquisition Software (Mini Mitter Company, Inc. Bend, OR USA) was used to analyze stored results. The activity was represented as a double-plotted actogram. Since the last measurement values (excluding locomotor activity) were taken on the 10th day, the 10th day locomotor activity is not available in the graphs.

2.6. Measurement of Food Intake, Body Weight, and Blood Glucose

To estimate the food consumption a weighed amount of food pellets was placed into cages. After 24 hours, the food pellets and fragments that remained in the cages were weighed. Food intake was calculated every day and 10-day average consumptions were given. To measure their body weight, each

animal was weighed daily prior to injections throughout the experiment. Accu-Check blood test strips were used to determine the blood glucose level. On the first and tenth days of the experiment, blood (0.1 – 0.2 ml) was drawn from the orbital sinus of the eye using a hematocrit capillary tube before (11:00 - 12:00 h) and after (13:00 - 14:00 h) injections.

2.7. Statistical Analysis

Statistical analysis was performed by using SPSS 22.0 and Sigma plot 14.0. Data were examined by the Kruskal-Wallis's test and comparisons between groups were analyzed with t-tests. $p < 0.05$ was considered statistically significant.

3. Results

The locomotor activity of the control and 100 mg/kg, 200 mg/kg, and 300 mg/kg dose-injected hamsters over the course of 10 days are shown in Figure 1. As the dose increases, the activity of the animals also decreases. This reduction occurs as soon as the injections start. In particular, the efficacy of 300 mg/kg dose was the highest in reducing locomotor activity. Comparing the 100 mg/kg dose to the control group and other doses, the activity of the animals was higher.

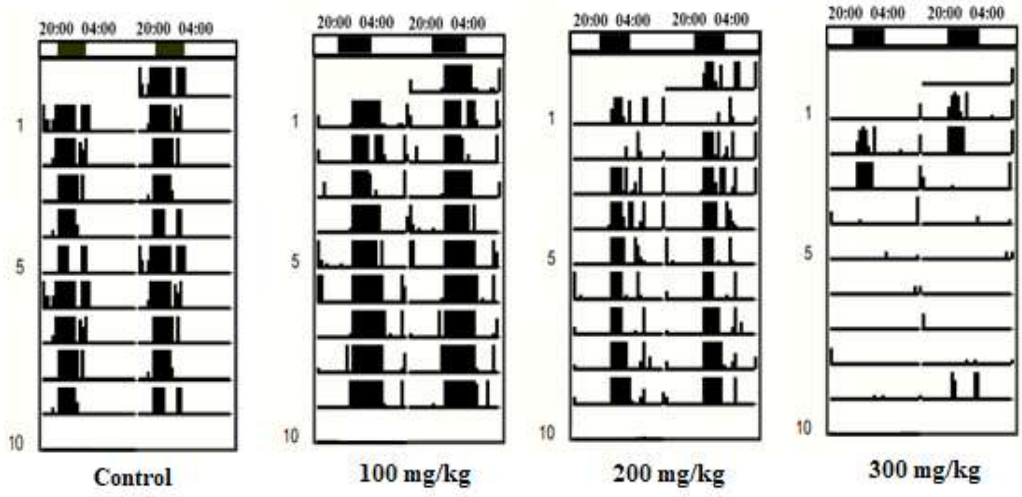

Figure 1. A representative actogram from the groups of control, 100 mg/kg, 200 mg/kg, and 300 mg/kg plant extracts. Lights are on at 04:00 h and light off at 20:00 h. The black bars at the top indicate the dark phase of the day. Each actogram shows two consecutive days.

Figure 2 illustrates the variations in the animals' body weights. The body weight of animals was significantly affected by different doses of plant extracts ($p < 0.001$). Pairwise comparison analysis showed that the body weight of animals injected with 200 mg/kg and 300 mg/kg of the extracts was significantly lower than the control group ($p < 0.001$). The group receiving 300 mg/kg had the lowest body weight ($p = 0.003$).

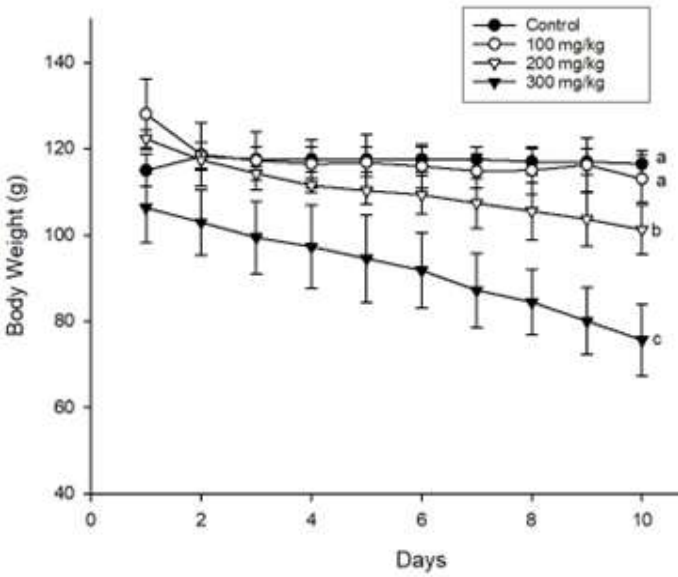

Figure 2. Hamster body weight after injections of *H. perforatum* plant extracts at doses of 100 mg/kg, 200 mg/kg, and 300 mg/kg.

Figure 3 displays the average food consumption values during the course of the 10-day experiment. The results shows that the hamsters consume statistically less food at doses of 200 mg/kg and 300 mg/kg than at the control and 100 mg/kg levels ($p < 0.001$).

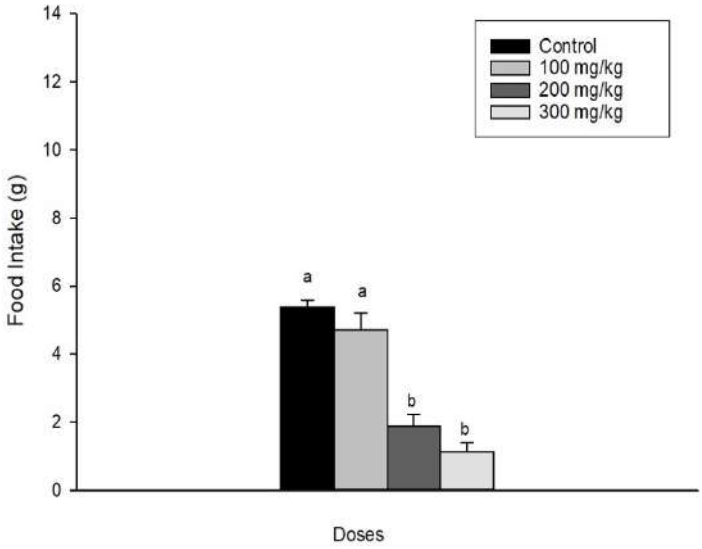

Figure 3. Average food intake following injections of 100 mg/kg, 200 mg/kg, and 300 mg/kg *H. perforatum* plant extracts.

When the glucose levels on the first day were examined, all of the animals in the group had normal (60 mg/dl) glucose levels prior to the injection (Figure 4). However, while the dose of 300 mg/kg after injection increased the glucose level (90 mg/dl), other doses did not make any difference ($p = 0.05$). When the glucose levels in the blood after injections were compared at the end of the experiment (Day

10), it was seen that the glucose levels in the 100 and 200 mg/kg doses did not differ from the control group, whereas the 300 mg/kg dose statistically increased the blood glucose more than the other groups.

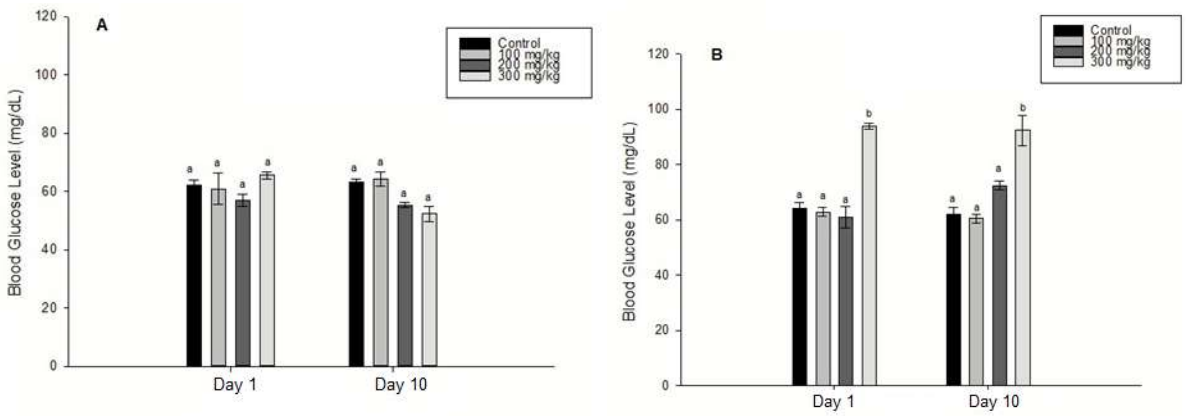

Figure 4. Blood glucose level before (A) and after (B) administration of *H. perforatum*

4. Discussion

The current study found that high doses of *H. perforatum* suppressed locomotor activity in hamsters and reduced body weight, daily food intake, and increased blood glucose levels. While *H. perforatum* doses of 200 mg/kg and 300 mg/kg decreased locomotor activity, doses of 100 mg/kg increased activity. Our findings contradict the literature's findings that high doses of *H. perforatum* increase activity, while other studies show that it decreases activity overall (Beijamini & Andreatini, 2003). This discrepancy may be explained by variations in the animal species used (Vandenbogaerde et al., 2000; Uzbay et al., 2007) and variations in the animal activity times throughout the day. Knowing the physiological characteristics of the species that will receive the extracts prepared at various concentrations is important for evaluating the impact of plant-derived extracts, particularly on behavior and locomotor activity. For instance, a low or high dose may increase activity in one species while suppressing it in another. In such locomotor activity studies, the photoperiodic feature of animals is taken into consideration. As a result, the animal we chose is the hamster, which regulates its activity based on the light-dark cycle. For example, it sleeps during the day and is active at night. Figure 1 shows that our control group is active in the dark and inactive in the light, i.e. sleeping. In comparison to the control group, *H. perforatum* 100 mg/kg given in the light phase (12:00 – 13:00) increased activity in the dark. We observe a significant reduction in activity at dosages of 200 mg/kg and, more significantly, 300 mg/kg. The 300 mg/kg dose affects animals right away after the injection begins, which is an important fact. Almost no daytime activity in the animals could be calculated after the third day. In people, particularly at large doses, we observe a comparable impact. A dose of 300 mg/kg or greater is thought to have antidepressant properties in humans, whereas doses of 100 mg/kg or less are thought to have no adverse effects on humans (Cervo et al., 2002, Hirano et al., 2004). However, these high doses imply that *H. perforatum* can be used in clinical trials for those with severe depression. The effects of these doses should be carefully evaluated due to the reducing effect of high doses on locomotor activity, as well as body weight, food intake, and blood glucose rise.

The SCN (Suprachiasmatic nuclei), a structure in the brain's hypothalamus region, is at the epicenter of these effects, generating daily locomotor activity and dictating its rhythm. SCN lesions cause disruption of almost all biological rhythms. Every factor that influences the SCN also has a direct or indirect impact on locomotion. The chemical serotonin is primarily responsible for the effects of high doses of extracts obtained from the aerial parts of the *H. perforatum* plant (Cervo et al., 2002, Hirona et al., 2004). *H. perforatum* may inhibit serotonin reuptake. *H. perforatum* has been shown to increase the levels of serotonin, noradrenaline, dopamine, and gamma-aminobutyric acid (GABA) in the central

nervous system (Muller et al., 1998). *H. perforatum* inhibits the binding of serotonin to presynaptic membrane-adrenergic receptors. As a result, the level of serotonin in the synaptic cleft rises (Cui and Zheng, 2016).

SCN contains one of the densest serotonergic terminals plexes in the brain. The majority of research were conducted in typical 12-hour light/12-hour darkness settings and did not specifically examine how the serotonergic system affected circadian rhythm. The points mentioned about the serotonin system must be carefully reviewed; it is crucial when serotonin influences the SCN, particularly in terms of locomotor activity. The time period while serotonin and its associated receptors are both active plays a major role in the formation of this effect. According to studies, serotonin is a significant neurotransmitter during the day-time. The serotonergic system may have been stimulated by the daytime administration of *H. perforatum* extracts, indicating the plant's sedative properties. It is crucial to investigate if administering *H. perforatum* to animals during the dark phase as opposed to during the day has a different impact in light of the literature and our findings.

It is still unclear which bioactive substances, including phenylpropanes, flavonol derivatives, phloroglucinols, biflavones, xanthones, proanthocyanidines, some amino acids, naphthodianthrones, and essential oil constituents, could be entirely or partially responsible for the antidepressant activity of perforatum (Nahrstedt & Butterweck, 1997). *H. perforatum* species have been widely studied for the presence of specific contents, such as hypericins, to which some authors attribute antidepressant activity (Kitanov, 2001). Small concentrations of hypericin have stimulating or antidepressant effects in the brain (Hudson and Towers, 1994). Large quantities, however, would be depressant. It's interesting to note that this impact is not light-dependent. The behavioral pharmacological effect is not lost upon removing the other significant active component, hyperforin (Butterweck et al., 2003).

In addition, studies have shown that treatment with antidepressants affects food intake in both animals and humans (Gafoor et al., 2018). Serotonin administration reduces food intake (Fletcher et al., 1986). According to our findings and those of others, high serotonin levels caused by *H. perforatum* reduces food intake and suppress appetite in animals, resulting in weight loss. Thus, increased serotonin production and utilization maybe connecting molecule between the antidepressant and antiobesity effects of *H. perforatum.*

H. perforatum plant has a very variable impact on blood glucose levels. Studies have shown both a reduction in blood glucose level and an increase in its effect. The issue to be considered here is where the extract is obtained from the plant and how it is applied. Arokiyaraj et al. (2011) indicated the effect of *H. perforatum* on blood lipid and blood glucose levels in diabetic rats induced with streptozotocin. The administration of increasing doses of *H. perforatum* extract reduced the blood glucose levels of diabetic rats. On the other hand, in some studies on rats, *H perforatum* administration did not cause a decrease in blood glucose in diabetic animals (Moghadam et al., 2017). Although insufficient results in the literature make commenting on the effects of *H. perforatum* on blood glucose difficult, we can say that the results of our study have an effect on raising blood glucose. A situation we observed in our study is that especially in animals injected with a dose of 300 mg/kg, the blood glucose rises very much in the first 15 minutes after the injection and then decreases to the values we measured (90-100 mg/dl) within 1 hour (13:00 - 14:00). Stage et al. (2011) also demonstrated that using large amounts of the extract causes increased blood glucose levels. Although excess glucose in the blood is shown as the reason for this sudden increase in blood glucose, the source of this glucose should be determined. Since all experimental groups received the same diet, it seems doubtful that the excess glucose came from food. These groups also consume the least amount of food and lose the greatest weight. This extra glucose may be mostly the result of a process that promotes its quick synthesis from lipids or from glycogen stored in the liver and skeletal muscles. In general, different circumstances, such as how the extracts are obtained from various *H. perforatum* parts and how they are administered to the species (single dose, numerous doses per day, continuous dose, etc.) may produce various outcomes (Rafailovska et al., 2022).

As a result, *H perforatum*, especially at high doses, has an effect on parameters such as locomotor activity, nutrition, body weight, and glucose metabolism. More research is required to determine the active ingredient(s) of *H. perforatum* and the daily circadian mechanisms involved.

Conflict of Interest

None

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