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Investigating the Biological Impact of Melatonin on Male Albino Mice: A Comprehensive Characterization

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Melatonin (Mel) is a chemical that has been found in bacterial, higher plants, invertebrates, and vertebrates. The plant extract was analyzed using high-performance liquid chromatography (HPLC) to find Mel. Mel concentrations differed greatly, according to the data. The aim of this study was to see how Mel generated from *Ziziphus spina-christi* leaves affected thyroid, liver enzymes, and lipid profiles in male albino mice. Twenty male mice were divided into four groups, each containing five mice, and were administered intraperitoneally (i.p) injections of Mel in three doses (4, 7 and 10 mg/ml), except for the control group, which received only a dose of normal saline solution. In comparison to the control therapy, the results showed that the Mel dose of 10 mg/ml had the greatest impact on lowering the levels of thyroid hormones (T3 and T4), lowering the levels of the liver enzymes Alanine transaminase (ALT), Aspartate aminotransferase (AST), and Total serum bilirubin (TSB) and lowering lipid profile (cholesterol (CHO), low-density lipoprotein (LDL), very low-density lipoprotein (VLDL), and triglycerides (TG)). In addition to raising the level of high-density lipoprotein (HDL) in mice's blood serum with substantial variations from the control therapy. The review summarizes, this evidence implicates Mel in a broad range of effects with a significant regulatory influence over many of the body's physiological functions.
Keywords: Blood serum, Medicinal plants, tryptophan, Rhamnaceae family, methanol, TLC and pineal gland.

INTRODUCTION

Medicinal plants are gaining importance in these fields of research (Al-Halbosiy, *et al*., 2020). *Ziziphus spina-christi* is a member of the Rhamnaceae family. The world's tropical and subtropical climates are home to it's around 100 species of deciduous or evergreen trees and plants (Dweck, 2005). According to Said *et al*. (2006), the genus *Ziziphus* is well-known for its therapeutic benefits as a liver protector, anti-inflammatory, antimicrobial, hypotensive, hypoglycemia, anti-inflammatory, antioxidant, antitumor, and stimulant of the immune system. The leaves are excellent for treating fever, asthma and liver issues when applied topically(Miche , 2002). According to earlier research, *Z.spina-christi* can be particularly effective in the management of hepatic and nephritic disorders. Conducted a study to give *Z.spina-christia's* anecdotal use as an antibacterial agent scientific justification (Adzu *et al*., 2003).An indolamine, melatonin (Mel) (N-acetyl-5-methoxytryptamine) is derived from the necessary amino acid tryptophan. Mel, which was first discovered in the pineal gland, is a hormone that regulates the circadian rhythm and affects the immune system, metabolism, and sleep (Motlhalamme, 2020).

There is evidence that Mel affects and enhances lipoprotein metabolism (Dominguez-Rodriguezet *et al*., 2005).

Because plant extracts are widely used, strategies for using plant extracts in treatment have been proposed (Al -Haidari *et al*., 2016). A rise in (HDL) and a decrease in cholesterol, (LDL), and (TG) in plasma have been used to demonstrate the hypolipidemic effects of Mel (Al-Mahbashy *et al*., 2006). The role of thyroid hormones (Triiodothyronine (T3) and thyroxine (T4)), in fat oxidation and thermoregulation is well recognized (Gullu *et al*., 2004).To avoid hepatic impairment, Mel suppresses myeloperoxidase (MPO), (ASO), and (ALS) (Shokrzadeh *et al*., 2014).This study's aim was to evaluate the physiological effects of Mel made from leave extract plant on the parameters of mouse blood serum.

MATERIALS AND METHODS

***Plant materials*:** Plant specimens was collocated from Iraq-waist. The specimen was pulverized into fine particulates

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employing a Blender after desiccation for a six-hour interval within a hot air oven maintained at a temperature of 60 °C. Roughly 10% of the original weight of the plant's segment represented its dehydrated mass.

***Sample preparation and extraction*:** The previously stated process was adjusted for extraction and analysis (Debnath *et al*., 2011). 250 mL rotating containers were filled with 80g of herbal powders, 250 mL of methanol, and left at room temp for six hours after the process of centrifugation, the resulting supernatants were subjected to separation and subsequently stored at a temperature of 4°C, awaiting further analysis through techniques such as TLC and HPLC as found in these sources (Muszyńska *et al*., 2011, Ansari *et al*., 2010)

***Animals*:** White albino mice with a weight range of 20–35 g were kept in the animal home of the Ministry of Technological Sciences in a laboratory setting at a temperature of (22±2 °C) with a 12-hour light–dark cycle, and access to food and water always. The tests were conducted on healthy animals without any prior oxidative stress induction therapy. The Animal Care Committee approved every method of handling and every set of circumstances for experiments (Schaffazick, 2008).

***Melatonin preparation*:** Before injection, the Mel extraction solution was produced by dissolving 100 mg/ml of Mel extraction in 100 ml ethanol and dilution with normal saline. The resulting solution had a 1% final ethanol content (Moreira *et al*., 2015).

***Experimental Protocol*:** Twenty adult male white albino mice (n = 5) were placed into five groups. The mice in Group I were only given saline intraperitoneally (i.p). Mel dosages (4, 7, and 10) mg/ml, respectively, were given intravenously (i. p) into groups II, III, and IV respectively. The mice were slaughtered after 28 days. Each mouse's blood was obtained.

***Blood Collection*:** While the mice were under moderate anesthesia, blood samples were taken from the heart in serum separation tubes. The tubes were centrifuged at 15,000 rpm for 20 minutes at 4°C after being allowed to clot for 30 minutes at room temperature.

***Serum Biochemical Analyses*:** Alanine transaminase (ALT) (Young, 1990), Aspartate aminotransferase (AST) (Young, 2000),, and Total serum bilirubin (TSB),(CH.) (Burtis *et al*., 2005), (T.G) (Young, 2001), Triiodothyronine (T3) (Wayne, 2005) and thyroxin (T4)(Geneva World Health Organization, 2004) ,HDL and LDL levels were assessed using methods outlined in earlier studies (Nauck *et al*., 2002), whereas VLDL concentration was estimated using a previously known algorithm (Friedwold *et al*., 1972) .

***Statistical analyses*:** SPSS (AL. Mohammed *et al*., 1986) was used to perform statistical analysis. ANOVA was used to compare the significant differences between the means. For each test, a P value less than ($P < 0.001$) was acceptable to show statistical significance.

RESULTS

***Effect of Melatonin extract on thyroid hormones (T3 and T4)*:** The administration of *Z. spina-christi* leaves extracts improved the thyroid. Furthermore, *Z. spina-christi* considerably decreased the levels of T3 and T4 in serum mice blood, however, following (i.p) injection of the extract for four weeks compared to the control Table 1.

Table 1. Effect of Melatonin extract doses on Triiodothyronine (T3) and thyroxin (T4) levels of mice blood serum.

Treatment	T3 (ng/ml) (Mean ± SE)	T4 (ng/ml) (Mean ± SE)	P-value
Control	0.550±0.00577	70.20±0.57735	
4mg/ml of Mel	0.410±0.00577	54.10±0.57735	
7mg/ml of Mel	0.470±0.00577	44.80±0.57735	P<0.001*
10mg/ml of Mel	0.320±0.00577	20.30±0.57735	

***Effect of Melatonin extract on Alanine transaminase (ALT), Aspartate aminotransferase (AST), and Total serum bilirubin (TSB) levels*:** The liver enzyme (AST, ALT and TSB) levels were improved by the administration of *Z. spina-christi* leaf extracts. Additionally, after administering the extract intraperitoneally (i.p.) for four weeks, *Z. spina-christi* significantly reduced the levels of liver enzyme in the serum of mice's blood in contrast to the control group (Table 2).

***Effect of Melatonin extract on (CH.), (T. G), HDL, LDL and VLDL levels of mice blood serum*:** Lipid profile values (CH, T.G, HDL, LDL, and VLDL) improved with the administration of *Z. spina-christi* leaf extracts. When administered intraperitoneally (i.p.) to mice for four weeks,

Table 2. Effect of Melatonin extract doses on Aspartate aminotransferase (AST) and Alanine transaminase (ALT), Total serum bilirubin (TSB) levels of mice blood serum.

Treatment	AST (U/L)	ALT (U/L)	TSB (mg/dl)	P-value
		(Mean ± SE)		
Control	184.70±0.57735	27.80±0.57735	3.83±0.57735	
4mg/ml of Mel	47.33±0.57735	23.50±0.57735	3.50±0.57735	P<0.001*
7mg/ml of Mel	60.50±0.57735	19.00±0.57735	2.66±0.57735	
10mg/ml of Mel	54.60±0.57735	10.70±0.57735	1.88±0.57735	

Table 3. Effect of Melatonin extract doses Cholesterol (CH.), Triglycerides (T. G), and HDL, LDL and VLDL levels of mice blood serum.

Treatment	Control	4mg/ml of Mel	7mg/ml of Mel	10mg/ml of Mel
		(Mean ± SE)		
CHO(mg/dl)	144.34±0.57735	102.17±0.57735	116.84±0.57735	72.60±0.57735
T.G(mg/dl)	210.00±0.57735	176.98±0.57735	186.30±0.57735	133.15±0.57735
HDL(mg/dl)	19.00±0.57735	29.66±0.57735	32.10±0.57735	35.00±0.57735
LDL(mg/dl)	83.34±0.57735	37.12±0.57735	20.21±0.57735	10.97±0.57735
VLDL(mg/dl)	42.00±0.57735	35.39±0.57735	37.26±0.57735	26.63±0.57735
P -value	P < 0.001*			

the *Z. spina-christi* plant extract resulted in a substantial drop in lipid levels (CH, T.G, LDL VLDL) and an increase in HDL levels in the blood serum when compared to the control group (Table 3).

DISCUSSION

For thousands of years of human history, plants have been used for food and medicine. Natural chemicals from various sorts of plants have been gathered and extracted in order to develop novel treatments in modern times (Al-Mothafar and Al-Shahwany, 2022).

***Effect of Melatonin extract on thyroid hormones (T3 and T4)*:** Depending on the age and gender (male/female) of the mice, Mel has different stimulatory and suppressive effects on thyroxine hormones (Grau *et al*., 1985). It was reported that exogenous Mel administration impaired the circadian rhythm of T3 and T4 and that this effect was more pronounced if Mel was given at night (Zwirska-Korczala *et al*., 1991). As shown in Table 1, our results agree with this observation. Wright *et al*. (1997) reported that Mel administration in vitro led to a dose-dependent decrease of thyroid function, particularly at the onset of the incubation period. It has been proposed that Mel administration, especially at later hours of the day, suppresses mitotic activity rates and thus leads to a strong inhibition of thyroid gland function.

***Effect of Melatonin extract on Alanine transaminase (ALT), Aspartate aminotransferase (AST), and Total serum bilirubin (TSB) levels*:** When the liver is injured, substantial amounts of these enzymes are released into the blood (Sun *et al*., 2020). Mel treatment has been shown in several investigations to reduce oxidative stress and improve morphological performance in caused hepatocellular damage (Cao *et al*., 2017). Exogenous Mel administration has been studied as a therapy for a variety of disorders, including nervous system injury (Yang *et al*., 2020).In addition to hepatic damage (Crespo *et al*., 2020). Blood levels of the liver enzymes AST, ALT, and TSB were significantly lower in the treated group compared to the control group when it came to liver enzymes (Table 2). These findings were consistent with the prior study's findings. The activities of serum AST and ALT are reduced (Al Olayan *et al*., 2020).

***Effect of Melatonin extract on (CH.), (T. G), HDL, LDL and VLDL levels of mice blood serum*:** Mel has been shown to be safe even at high doses for brief periods of time, and randomized clinical trials show that long-term Mel treatment has few side effects that are comparable to placebo (Andersen *et al*., 2016). These results are a positive indicator, as the decrease in the level of CHO, T.G, LDL and VLDL With increased the HDL percentage reduces the risk of arterial blockages and heart attacks. In addition to high blood pressure diseases resulting from High percentage of fats inside the blood vessels and arteries, as many studies have shown that high cholesterol and low-density protein fats (LDL) in the blood are strong risk factors for coronary heart disease, while a high ratio of HDL to bad cholesterol (LDL, VLDL, and T.G) may protect against this disease (Castelli, *et al*., 1992) (Table 3).

***Conclusion*:** According to recent research (Popović *et al*., 2016), regularly using synthetic chemicals has a detrimental effect on the health of both humans and animals. Synthetic chemical feed additions have been replaced by herbs, essential oils, extracts, powders, and other phytogenic feed additives. Mel's application as a medicinal treatment for liver diseases and damage has not received much attention. Future research on Mel's impact in this area should focus more on clinical trials. Further research is required to understand the mechanisms of action. In rats given a high-cholesterol diet, Mel treatment improves both the plasma lipoprotein cholesterol profile and the quantity of lipids (CH, TG) in the liver. We can underline the importance of using medicinal plants as a main source of the active compounds used in the creation of novel therapeutic drugs, as well as the necessity of further investigation to ascertain Mel's effects on a range of ailments, including specific tumors, cardiovascular conditions and mental disorders.

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***SDG's Addressed*:** No poverty, Good health and Well-being, Decent Work and Economic Growth

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