

We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

6,900

Open access books available

186,000

International authors and editors

200M

Downloads

Our authors are among the

154

Countries delivered to

TOP 1%

most cited scientists

12.2%

Contributors from top 500 universities



WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com



Melatonin as a Food Supplement for Sleep Disorders

Ioulia K. Tseti

Abstract

N-acetyl-5-methoxytryptamine commonly known as melatonin is a neurohormone produced in the pineal gland as a response to decrease in ambient light and regulates the sleep-wake cycle. Melatonin is a derivative of the amino acid tryptophan and is produced in humans and other mammals. Melatonin supplements are used to treat insomnia and sleep disorders and to adjust sleep schedules due to jet lag. Synthetic melatonin is available as a food supplement in various dosage forms such as pills, granules for oral solution, orodispersible granules, and syrups in order to address patients' needs. Melatonin is often combined with water-soluble vitamins such as B complex vitamins and minerals like zinc in order to be more effective.

Keywords: melatonin, food supplements, jet lag, sleeping disorder, dosage forms

1. Introduction

In 1960, the chemical structure of melatonin was defined [1] since significant attention was attracted towards its use a few years earlier when the dermatologist Dr. Lerner and his colleagues observed that melatonin could cause the lightening of frog skin [2]. Melatonin has been found to affect a wide range of physiological processes such as sleep-wake cycles [3], circadian rhythms [4], sexual maturation [5], and aging [6].

Since then, exogenous melatonin has demonstrated a series of clinical effects [7, 8], and numerous clinical studies have been conducted, where improved sleep quality was documented following exogenous melatonin administration [9]. Recent studies demonstrated analgesic [10], anxiolytic [11], anti-inflammatory, and anti-oxidative effects [12] following administration of melatonin.

2. Melatonin as a food supplement and its uses in modern life

Melatonin is synthesized from tryptophan via 5-hydroxytryptophan and 5-hydroxytryptamine (serotonin). This is followed by *N*-acetylation of serotonin by *N*-acetyltransferase (arylalkylamine *N*-acetyltransferase, AA-NAT) to *N*-acetylserotonin (NAT) and *O*-methylation by acetylserotonin *O*-methyltransferase (ASMT) [previously known as hydroxyindole-*O*-methyltransferase (HIOMT)] to melatonin (*N*-acetyl-5-methoxytryptamine). The rhythm of melatonin production is endogenous, being generated by clock genes in the suprachiasmatic nuclei (SCN), the major central rhythm-generating system or "clock" in mammals. The rhythm, as for

the circadian system in general, is synchronized to 24 h primarily by the light-dark cycle acting via the retina and the retinohypothalamic projection to the SCN [13].

In humans melatonin is metabolized, 70% to 6-sulphatoxy melatonin (aMT6s), primarily within the liver, by 6-hydroxylation, followed by sulfate conjugation; this mechanism varies through species. A number of minor metabolites are also formed, including the glucuronide conjugate. *N1*-acetyl-*N2*-formyl-5-methoxykynuramine and *N1*-acetyl-5-methoxykynuramine were initially reported as brain metabolites [14, 15], but were proved difficult to detect in plasma or urine except after administration of exogenous melatonin [16]. Exogenous oral fast release or intravenous melatonin has a short metabolic half-life, i.e., 20–60 min, depending on the species—with a large hepatic first-pass effect and a biphasic elimination pattern [17]. Slow release, prolonged release, and surge sustained preparations are designed to extend the time of high circulating melatonin [18]. Melatonin has low bioavailability, in general, although it has been found that transmucosal administration increases bioavailability [19]. A critical feature of exogenous melatonin with regard to its clinical uses is its very low toxicity and lack of addictive properties [20, 21].

A T_{\max} of approximately 50 min has been reported following oral immediate-release formulation of melatonin. $T_{1/2}$ of both oral and intravenous melatonin was about 45 min [22]. Over 80% of melatonin dose is excreted exclusively in the urine, as 6-sulfatoxymelatonin (6-SMT) following first-pass hepatic metabolism [23, 24]. Melatonin is short-lived in humans with a half-life in plasma of only 40–50 min [23]. Following oral administration, it is rapidly absorbed with peak plasma levels occurring between 20 min and 2 h depending on dose [14].

Administration of melatonin 45 min before intended clinical effect may therefore be recommended. However, external factors, such as caffeine [25], smoking [26], and other medications [27, 28], which may potentially affect the pharmacokinetics of melatonin, should be considered prior to exogenous melatonin administration.

Long-term use of sedative-hypnotics for insomnia lacks evidence of treatment and has traditionally been discouraged for reasons that include concerns about potential adverse drug effects, such as cognitive impairment, daytime sedation, motor incoordination, and risk of motor vehicle accidents and slips and falls. In addition, the effectiveness and safety of long-term use of these agents remain to be determined [29]. Moreover, several studies have been conducted to assess the effects of sleep hygiene interventions and various non-pharmacological interventions, such as physical activity, bright light exposure, and noise abatement, but no definite effect on nighttime sleep has been reported [29]. Many people seek treatment for insomnia using alternative and complementary medicine [30]. Generally, the main goal of non-pharmacological remedies in the treatment of primary insomnia is to correct behavior patterns that are not conducive to a good quality sleep, and nutrients might play a significant role in this setting, but no evidence is available as to the preferred alternative treatment of insomnia.

In addition to melatonin, other micronutrients, such as zinc and magnesium, may play a role in facilitating sleep. Zinc exhibits an antidepressant-like activity, as observed in a preclinical model of depression [31–34]. Significant clinical correlates were shown [35] related to its action as an antagonist of the glutamate/*N*-methyl-*D*-aspartate receptor. Magnesium has beneficial effects on mood and is crucial, together with zinc, in the endogenous synthesis of melatonin [36]. Various food supplements contain combinations of melatonin with either magnesium or zinc.

Since melatonin production declines with age and is lower in middle-aged and elderly adults with insomnia than in good sleepers [37], supplementation with exogenous melatonin is very common. Exogenous melatonin can effectively treat

insomnia by mimicking the natural endogenous melatonin, binding to the same receptors, and activating the same downstream pathways. The effect of melatonin on sleep is believed to be a consequence of mechanisms that involve an increase in sleep propensity by enhancing the amplitude of circadian clock oscillations via melatonin type 1 (MT1) receptors and the synchronization of the circadian clock via melatonin type 2 (MT2) receptors [39]. By activating MT1 (melatonin type 1 receptor) and MT2 (melatonin type 2 receptor) receptors, melatonin and nonselective MT1/MT2 receptor agonists have shown to improve sleep quality, increase total sleep time, improve sleep efficiency, and decrease sleep onset latency in insomnia patients [38].

The mammalian circadian clock covers a wide range of physiological processes and plays a pivotal role in reproduction [40, 41]. It is currently accepted that dysregulation of the circadian rhythm caused by night shifts, jet lag, and sleep deprivation has a detrimental effect on the reproductive system [42, 43]. Melatonin is produced not only by the pineal gland but also in glial cells, meningeal cells, and in other peripheral tissues, and its cyclical pattern of secretion is responsive to zeitgebers [44]. Melatonin permeability into the central nervous system was described decades ago [45], and its efficient transport through the blood-brain barrier promotes accumulation in the brain at levels higher than the ones existing in the blood. Melatonin also possesses neuroprotective and antioxidant properties [42]. Modulation of redox signaling systems influences the reproductive system in both animals and humans [43, 46], and it is known that insufficient endogenous production of melatonin has been associated with disturbances in the reproductive system due to increased levels of reactive oxygen species (ROS), which are harmful to the male and female gametes [47]. Unhealthy lifestyles and psychosocial stress are aspects of modern life that have a negative impact on gynecological health and reproduction [48]. Epidemiological studies show that night shifts may negatively influence fetal development and may exacerbate gynecological and metabolic disorders, including endometriosis, diabetes, and obesity [49]. Consequently, melatonin supplementation has been considered as a therapeutic approach in gynecological practice owing to its antioxidant properties and its action as hormone modulator.

The neurohormone melatonin is not stored in the pineal gland, but rather is released into the bloodstream and can penetrate all body tissues [50]. It is important to note that “darkness” stimulates the pineal gland to secrete melatonin, whereas exposure to light inhibits this mechanism [51].

Regarding the actual administration of melatonin, it has been shown that the timing of melatonin administration is more crucial in producing the best results than the actual dose; this is secondary to the normal physiologic function of the circadian rhythm [51]. It has been reported that when melatonin was administered at bedtime as a “sleeping pill,” it was not effective unless high doses were used [52]; however, when small doses of melatonin were administered to patients about 2–4 h before bedtime, it was shown to be effective in decreasing sleep latency [53].

Garfinkel et al. [54] investigated 12 elderly subjects (mean age 76 ± 8 years) with chronic illness and insomnia in a crossover study using wrist actigraphy comparing administration of PR-melatonin for 3 weeks with placebo. PR-melatonin 2 mg produced a statistically significant improvement in sleep efficiency and wake time after sleep onset was shorter. Sleep latency decreased, but this was not statistically significant, while total sleep time was not affected.

The side effect profile of melatonin therapy is quite reassuring and is largely superior to other sleep-inducing agents. For example, melatonin therapy does not cause withdrawal or dependence symptoms, unlike benzodiazepines (BZDs) and z-drugs such as zolpidem [55].

Potential harmful effects of exogenous melatonin therapy might result in amenorrhea when used in large doses, which is likely due to suppression of gonadotropin-releasing hormone (GnRH) [56]. However, this effect is readily reversible with cessation of the medication.

Sleep disorders, regardless of the etiology, are frequently encountered by physicians and other health-care providers. According to data from the Centers for Disease Control and Prevention (CDC), up to about 70 million Americans suffer from chronic sleep problems [57], while according to the American Psychiatric Association (APA), approximately 30% of all adults suffer from sleep disorders [58]. Considering that, supplements containing melatonin are widely used. Over the counter (OTC) melatonin-containing supplements can be easily found either online or at pharmacy stores, with beneficial claims on jet lag [59], as well as occasional sleepiness, sleep problems caused by stress, overall mood, and overall health.

Melatonin is often combined with vitamins, such as B complex vitamins and micronutrients, i.e., zinc or magnesium. Clinical studies have been conducted demonstrating the synergistic effect of these combinations [60]. Magnesium supplementation improves sleep efficiency, sleep time and sleep onset latency, early morning awakening, and insomnia objective measures such as the concentration of serum renin, melatonin, and serum cortisol, in older adults [61]. Meanwhile, there is clear evidence on the antidepressant effect of vitamin B12 [62] and vitamin B6 for therapy of hormone-related depression in women [8].

OTC melatonin food supplements are supplied in various pharmaceutical dosage forms in order to accommodate all patients' needs. Usually these food supplements contain 1 mg of melatonin in order to be able to bear the EFSA claim of "Melatonin contributing to the reduction of time taken to fall asleep and to the alleviation of subjective feelings of jet lag" [34].

Pharmaceutical dosage forms of melatonin-containing supplements include tablets and granules either for direct administration or for oral solution preparations, while recently the push and drink form is becoming popular. In this dosage form, the solid mixture containing melatonin and other ingredients is airtightly separated from the solution used for dissolution of the solid. This is achieved by using a container based on a closing storage cap-vial system, in which the closing storage cap contains the solid composition, while the vial contains the solution composition. The nutritional supplement is prepared just prior to use by an immediate procedure, and it can be consumed directly from the vial.

3. Conclusions

Since melatonin has a very low side effect profile and limited evidence of habituation and tolerance, it is widely used among people that suffer from sleep disorders. Various clinical trials have been conducted proving the efficacy of melatonin in treating sleep disorders regardless of the etiology. A plethora of OTC melatonin-containing food supplements, displayed in various pharmaceutical dosage forms, is nowadays available covering the needs of the patients.

Conflict of interest

The authors declare no conflict of interest.

IntechOpen

IntechOpen

Author details

Ioulia K. Tseti
Uni-Pharma Pharmaceutical Laboratories SA, Athens, Greece

*Address all correspondence to: meristoudi@uni-pharma.gr

IntechOpen

© 2020 The Author(s). Licensee IntechOpen. This chapter is distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/3.0>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited. 

References

- [1] Lerner AB, Case JD, Takahashi Y. Isolation of melatonin and 5-methoxyindole-3-acetic acid from bovine pineal glands. *The Journal of Biological Chemistry*. 1960;**235**:1992-1997
- [2] Lerner AB, Case JD, Takahashi Y, Lee TH, Mori W. Isolation of melatonin, the pineal gland factor that lightens melanocytes. *Journal of the American Chemical Society*. 1958;**80**(10):2587
- [3] Reiter RJ. Pineal melatonin: Cell biology of its synthesis and of its physiological interactions. *Endocrine Reviews*. 1991;**12**(2):151-180
- [4] Gillette MU, Tischkau SA. Suprachiasmatic nucleus: The brain's circadian clock. *Recent Progress in Hormone Research*. 1999;**54**:33-58. discussion 58-59
- [5] Díaz López B, Díaz Rodríguez E, Urquijo C, Fernández AC. Melatonin influences on the neuroendocrine-reproductive axis. *Annals of the New York Academy of Sciences*. 2005;**1057**:337-364
- [6] Pierpaoli W, Regelson W. Pineal control of aging: Effect of melatonin and pineal grafting on aging mice. *Proceedings of the National Academy of Sciences of the United States of America*. 1994;**91**(2):787-791
- [7] Brzezinski A. Melatonin in humans. *N Engl J Med*. 1997;**336**:186-195
- [8] Andersen LPH, Werner MU, Rosenberg J, Gögenur I. A systematic review of peri-operative melatonin. *Anaesthesia*. 2014;**69**:1163-1171
- [9] Bourne RS, Mills GH, Minelli C. Melatonin therapy to improve nocturnal sleep in critically ill patients: Encouraging results from a small randomized controlled trail. *Critical Care*. 2008;**12**:R52
- [10] Borazan H, Tuncer S, Yalcin N, Erol A, Otelcioglu S. Effects of preoperative oral melatonin medication on postoperative analgesia, sleep quality, and sedation in patients undergoing elective prostatectomy: A randomized clinical trial. *Journal of Anesthesia*. 2010;**24**:155-160
- [11] Caumo W, Torres F, Moreira NL, Auzani JAS, Monteiro CA, Londero G, et al. The clinical impact of preoperative melatonin on postoperative outcomes in patients undergoing abdominal hysterectomy. *Anesthesia and Analgesia*. 2007;**105**:1263-1271
- [12] Küçükakin B, Lykkesfeldt J, Nielsen HJ, Reiter RJ, Rosenberg J, Gögenur I. Utility of melatonin to treat surgical stress after major vascular surgery—A safety study. *Journal of Pineal Research*. 2008;**44**:426-431
- [13] Klein DC. Photoneural regulation of the mammalian pineal gland. *Ciba Foundation Symposium*. 1985;**117**: 38-56
- [14] Arendt J. Melatonin and the Mammalian Pineal Gland. London: Chapman Hall; 1995
- [15] Hirata F, Hayaishi O, Tokuyama T, Seno S. In vitro and in vivo formation of two new metabolites of melatonin. *The Journal of Biological Chemistry*. 1974;**249**:1311-1313
- [16] Ma X, Idle JR, Krausz KW, Tan DX, Ceraulo L, Gonzalez FJ. Urinary metabolites and antioxidant products of exogenous melatonin in the mouse. *Journal of Pineal Research*. 2006;**40**:343-349
- [17] Lane EA, Moss HB. Pharmacokinetics of melatonin in man: First pass hepatic metabolism. *The Journal of Clinical Endocrinology and Metabolism*. 1985;**61**:1214-1216

- [18] Rajaratnam SM, Dijk DJ, Middleton B, Stone BM, Arendt J. Melatonin phase-shifts human circadian rhythms with no evidence of changes in the duration of endogenous melatonin secretion or the 24-hour production of reproductive hormones. *The Journal of Clinical Endocrinology and Metabolism*. 2003;**88**:4303-4309
- [19] Zetner D, Andersen LP, Rosenberg J. Pharmacokinetics of alternative administration routes of melatonin: A systematic review. *Drug Research*. 2016;**66**:169-173
- [20] Sugden D. Psychopharmacological effects of melatonin in mouse and rat. *The Journal of Pharmacology and Experimental Therapeutics*. 1983;**227**:587-591
- [21] Guardiola-Lemaître B. Toxicology of melatonin. *Journal of Biological Rhythms*. 1997;**12**:697-706
- [22] Harpsøe NG, Andersen LPH, Gögenur I, Rosenberg J. Clinical pharmacokinetics of melatonin: A systematic review. *European Journal of Clinical Pharmacology*. 2015;**71**:901
- [23] Waldhauser F, Waldhauser M, Lieberman HR, et al. Bioavailability of oral melatonin in humans. *Neuroendocrinology*. 1984;**39**(4):307-1325
- [24] Arendt J, Skene DJ. Melatonin as a chronobiotic. *Sleep Medicine Reviews*. 2005;**9**(1):25-39
- [25] Härtter S, Nordmark A, Rose DM, Bertilsson L, Tybring G, Laine K. Effects of caffeine intake on the pharmacokinetics of melatonin, a probe drug for CYP1A2 activity. *British Journal of Clinical Pharmacology*. 2003;**56**:679-682
- [26] Ursing C, Bhar CV, Brismar K, Röjdmarm S. Influence of cigarette smoking on melatonin levels in man. *European Journal of Clinical Pharmacology*. 2005;**61**:197-201
- [27] Hilli J, Korhonen T, Turpeinen M, Hokkanen J, Mattila S, Laine K. The effect of oral contraceptives on the pharmacokinetics of melatonin in healthy subjects with CYP1A2 g.-163C>A polymorphism. *Journal of Clinical Pharmacology*. 2008;**48**:986-994
- [28] Härrter S, Grözinger M, Wiegmann H, Röschke J, Hiemke C. Increased bioavailability of oral melatonin after fluvoxamine coadministration. *Clinical Pharmacology and Therapeutics*. 2000;**67**:1-6
- [29] Bain KT. Management of chronic insomnia in elderly persons. *The American Journal of Geriatric Pharmacotherapy*. 2006;**4**:168-192
- [30] Chung KF, Lee CK. Over-the-counter sleeping pills: A survey of use in Hong Kong and a review of their constituents. *General Hospital Psychiatry*. 2002;**24**:430-435
- [31] Krocza B, Zieba A, Dudek D, et al. Zinc exhibits an antidepressant-like effect in the forced swimming test in mice. *Polish Journal of Pharmacology*. 2000;**52**:403-406
- [32] Nowak G, Siwek M, Dudek D, et al. Effect of zinc supplementation on antidepressant therapy in unipolar depression: A preliminary placebo-controlled study. *Polish Journal of Pharmacology*. 2003;**55**:1143-1147
- [33] Siwek M, Dudek D, Paul IA, et al. Zinc supplementation augments efficacy of imipramine in treatment resistant patients: A double blind, placebo-controlled study. *Journal of Affective Disorders*. 2009;**118**:187-195
- [34] Laires MJ, Monteiro CP, Bicho M. Role of cellular magnesium in health

and human disease. *Frontiers in Bioscience*. 2004;**9**:262-276

[35] Sowa-Kuc'ma M, Legutko B, Szewczyk B, et al. Antidepressant-like activity of zinc: Further behavioral and molecular evidence. *Journal of Neural Transmission*. 2008;**115**:1621-1628

[36] Touitou Y, Bogdan A. Promoting adjustment of the sleep-wake cycle by chronobiotics. *Physiology & Behavior*. 2007;**90**:294-300

[37] Haimov I, Laudon M, Zisapel N, et al. Sleep disorders and melatonin rhythms in elderly people. *BMJ*. 1994;**309**:167

[38] Reppert SM, Weaver DR. Molecular analysis of mammalian circadian rhythms. *Annual Review of Physiology*. 2001;**63**:647-676

[39] Gamble KL, Resuehr D, Johnson CH. Shift work and circadian dysregulation of reproduction. *Frontiers in Endocrinology*. 2013;**4**:1-10

[40] Goldman BD. The circadian timing system and reproduction in mammals. *Steroids*. 1999;**64**:679-685

[41] Beaver L, Gvakharia B, Vollintine T, Hege D, Stanewsky R, Giebultowicz JM. Loss of circadian clock function decreases reproductive fitness in males of *Drosophila melanogaster*. *Proceedings of the National Academy of Sciences of the United States of America*. 2002;**99**:2134-2139

[42] Genario R, Giacomini AC, Demin KA, dos Santos BE, Marchiori NI, Volgin AD, et al. The evolutionarily conserved role of melatonin in CNS disorders and behavioral regulation: Translational lessons from zebrafish. *Neuroscience and Biobehavioral Reviews*. 2019;**99**:117-127

[43] Rocha CS, Rato L, Martins AD, Alves MG, Oliveira PF. Melatonin and

male reproductive health: Relevance of darkness and antioxidant properties. *Current Molecular Medicine*. 2015;**15**:299-311

[44] Tan DX, Manchester LC, Fuentes-Broto L, Paredes SD, Reiter RJ. Significance and application of melatonin in the regulation of brown adipose tissue metabolism: Relation to human obesity. *Obesity Reviews*. 2011;**12**:167-188

[45] Pardridge WM, Mietus LJ. Transport of albumin-bound melatonin through the blood-brain barrier. *Journal of Neurochemistry*. 1980;**34**:1761-1763

[46] Herxheimer A, Petrie KJ. Melatonin for the prevention and treatment of jet lag. *Cochrane Database Systematic Reviews*. 2002;**2**:CD001520

[47] Kratz EM, Piwowar A, Zeman M, Stebelová K, Thalhammer T. Decreased melatonin levels and increased levels of advanced oxidation protein products in the seminal plasma are related to male infertility. *Reproduction, Fertility, and Development*. 2016;**28**:507-515

[48] Bonzini M, Palmer KT, Coggon D, Carugno M, Cromi A, Ferrario MM. Shift work and pregnancy outcomes: A systematic review with meta-analysis of currently available epidemiological studies. *International Journal of Gynaecology and Obstetrics*. 2011;**118**:1429-1437

[49] Stocker LJ, Macklon NS, Cheong YC, Bewley SJ. Influence of shift work on early reproductive outcomes: A systematic review and meta-analysis. *Obstetrics and Gynecology*. 2014;**124**:99-110

[50] Luboshizsky R, Lavie P. Sleep-inducing effects of exogenous melatonin administration. *Sleep Medicine Reviews*. 1998;**2**:191-202

[51] Hardeland R. Chronobiology of melatonin beyond the feedback to the

suprachiasmatic nucleus-consequences to melatonin dysfunction. *International Journal of Molecular Sciences*. 2013;**14**:5817-5841

[52] Cramer H, Rudolph J, Consbruch U, Kendel K. On the effects of melatonin on sleep and behavior in man. *Advances in Biochemical Psychopharmacology*. 1974;**11**:187-191

[53] Zhdanova IV, Wurtman RJ, Morabito C, Piotrovskaya VR, Lynch HJ. Effects of low oral doses of melatonin, given 2-4 hours before habitual bedtime, on sleep in normal young humans. *Sleep*. 1996;**19**:423-431

[54] Garfinkel D, Laudon M, Nof D, Zisapel N. Improvement of sleep quality in elderly people by controlled-release melatonin. *Lancet*. 1995;**346**(8974):541-544

[55] Hardeland R, Poeggeler B, Srinivasan V, Trakht I, Pandi-Perumal SR, et al. Melatonergic drugs in clinical practice. *Arzneimittel-Forschung*. 2008;**58**:1-10

[56] Pandi-Perumal SR, Srinivasan V, Maestroni GJ, Cardinali DP, Poeggeler B, et al. Melatonin: Nature's most versatile biological signal? *The FEBS Journal*. 2006;**273**:2813-2838

[57] Colten HR, Altevogt BM, editors. *Sleep Disorders and Sleep Deprivation: An Unmet Public Health Problem*. Washington, DC, US: National Academies Press; 2006

[58] Djokic G, Vojvodić P, Korcok D, Agic A, Rankovic A, Djordjevic V, et al. The effects of magnesium-melatonin-Vit B complex supplementation in treatment of insomnia. *Open Access Macedonian Journal of Medical Sciences*. 2019;**7**(18):3101-3105

[59] Abbasi B, Kimiagar M, Sadeqhniiat K, Shirazi MM, Hedavati M, Rashidkhani B. The effect of magnesium

supplementation on primary insomnia in elderly: A double-blind placebo-controlled clinical trial. *Journal of Research in Medical Sciences: The Official Journal of Isfahan University of Medical Sciences*. 2012;**17**(12):1161-1169

[60] Syed EU, Wasay M, Awan S. Vitamin B12 supplementation in treating major depressive disorder: A randomized controlled trial. *The Open Neurology Journal*. 2013;**7**:44-48

[61] Williams AI, Cotter A, Sabina A, Girard C, Goodman J, Katz DL. The role of vitamin B6 as treatment for depression: A systematic review. *Family Practice*. 2005;**22**(5):532-537

[62] COMMISSION REGULATION (EU) No. 432/2012