Clinical Evaluation of Melatonin Alone and in Combination with Pizotifen in the Prophylaxis of Migraine

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Abstract :

The treatment of migraine headache targets the neurovascular mechanism and involves the use of serotonin receptor antagonists. Some of these drugs are used for the treatment of acute attacks; while others are effective as prophylactic measures to decrease the duration and frequency of attacks. Pizotifen, a 5-HT_A antagonist, is one of the prophylactic drugs for which the clinical use resulted in low outcomes in reducing migraine symptoms. Melatonin, a serotonin derived neurohormone, was reported to exert many functions like sleep induction, anti-inflammatory, neurovascular regulation, cytoprotection and modulation of neurotransmitter release. In the view of the involvement of serotonin in the pathophysiology of migraine and the properties of melatonin, the present study has been conducted to evaluate the effectiveness of melatonin alone or in combination with pizotifen for the improvement of migraine symptoms. The study was conducted on 72 patients, which were under neurologist supervision during the entire period of study. The patients were instructed to avoid any precipitating diet (chocolate, cheese,… etc) and where randomly divided into 4 groups each of 18. The first group was treated with melatonin (3mg, 30 minutes before bed time); the second with pizotifen (0.5mg twice daily); the third with melatonin (at night) and pizotifen (twice daily); and the fourth with placebo (at night). The treatment was continued for 42 days and was followed up and monitored each week. After a month of treatment, the severity, duration and frequency of migraine attacks were recorded using special migraine scoring system.The results revealed that melatonin alone significantly decreased the severity, duration and frequency of migraine attack by 48%, 53% and 45.75%, respectively; while these produced by pizotifen were significantly reduced by 25%, 45.3% and 27.5%, respectively. The effect of pizotifen was generally enhanced by the addition of melatonin and the improvement in migraine symptoms were, severity (59%), duration (62.7%) and frequency (58%). These effects were generally low in placebo treated group and the reduction in severity, duration and symptoms were (12.2%, 20% and 16.2%, respectively). The distribution of patients according to their response as complete or partial was significantly different among treated groups; and within the same group is differ according to the measured parameter suggesting involvement of factors other than treatment in the improvement of migraine symptoms such as psychological state, stress and others.The study has concluded the effectiveness of melatonin alone and in combination with pizotifen as a prophylactic measure in term of reducing the severity, duration and symptoms of migraine headache.

Key words: migraine headache, pizotifen, melatonin.

الخلاصة

ان العلاجات التقليدية لصداع الشقيقة تتجه نحو الآلية الوعائية العصبية المسببة لداء الشقيقة وتتضمن استخدام مغلقات مستقبلات السيروتونين. بعض هذه الادوية تستخدم لعلاج النوبات الحادة للصداع بينما البعض الاخر يستخدم كعلاج وقائي للتقليل من فترات وتردد النوبات. أن عقار البيزوتفين هو احد الادوية الوقائية، الا ان استخدامه السريري قليل الفعالية في خفض علامات داء الشقيقة. وهذا العلاج معروف باغلاقه لمستقبلات السيروتونين نوع (5-HT_A). ان عقار الميلاتونين، وهو هرمون عصبي مشتق من السيروتونين، قد ثبت ان له وظائف متعددة مثل تحفيز النوم، كمضاد للالتهابات، تنظيم الاوعية الدموية العصبية، وقاية الخلايا، وتحوير في افراز النواقل العصبية. في ضوء اشتراك السيروتونين في سببية داء الشقيقة والخواص المعروفة للميلاتونين فان الدراسة الحالية قد اجريت لتقييم الاستخدام السريري للميلاتونين لوحده او مع البيزوتفين لعلاج علامات الشقيقة. اجريت الدراسة على ٧٢ مريضاً وقد كانوا تحت الاشراف الطبي لذوي الاختصاص العصبي خلال فترة الدراسة، وقد ارشدوا لتجنب الاغذية المسببة للشقيقة (الشكولاتة، والجبن، وغيرها) وقد صنفوا عشوائياً الى ٤ مجاميع كل منها مكون من ١٨ مريضاً. عولجت المجموعة الاولى بالميلاتونين (٣ملغم قبل النوم بنصف ساعة)، والثانية بالبيزوتفين (٠.٥ملغم مرتين يومياً)، والثالثة بالميلاتونين والبيزوتفين معاً وبالجرع اعلاه، والرابعة بالعلاج المموّه (placebo) بشكل كبسولة ليلاً. استمر العلاج لفترة ٤٢ يوماً بمتابعة اسبوعية. بعد شهر من العلاج، تم تسجيل حدة وفترة وتردد نوبات صداع الشقيقة باستخدام نظام تنقيط خاص. اثبتت الدراسة ان الميلاتونين لوحده قد ادى الى تقليل منطقي في حدة وفترة وتردد نوبات الصداع بنسبة ٤٨%، ٥٣%،٤٥.٧٥% على التوالي، بينما كان التقليل بالبيزوتفين بنسبة ٢٥%، ٤٥.٣% و٢٧.٥% على التوالي. وعلى العموم فان تأثير البيزوتفين قد ازداد باضافة الميلاتونين وان التقليل في علامات الصداع كانت، الحدة (٥٩%)، الفترة (٦٢.٧%) والتردد (٥٨%). ان هذه التاثيرات كانت قليلة جدا عن المجوعة المعالجة بالعلاج المموّه وكانت نسبة الانخفاض في الحدة، الفترة، والتردد هي ١٢.٢%، ٢٠%، و ١٦.٢% على التوالي. ان توزيع المرضى بحسب استجابتهم للعلاج، ان كان جزئياً او كلياً، كان مختلفاً بصورة منطقية بين المجاميع، وضمن المجموعة الواحدة فان التوزيع كان مختلفاً ايضاً بحسب المعيار المقاس مما يدل على تدخل عوامل اخرى غير العلاج في هذا التباين مثل الحالة النفسية، الاجهاد وغيرها. يمكن الاستنتاج من هذه الدراسة بان الميلاتونين لوحده او مع البيزوتفين قد أظهر فعالية وقائية في التقليل من حدة، فترة، وتردد نوبات صداع الشقيقة.

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Introduction

Migraine headache is a primary disorder, resulting from dysfunctions of the trigeminovascular system. The disorder manifests as recurrent attacks of sever pain that varies in frequency from one attack a year to two or more a week [(1)]. Migraine headaches are classified according to the accompanied aura into classical (with aura) and common (without aura) [(2)]. Aura means visual scotomas or even hemianopia and speech abnormalities followed by sever throbbing unilateral headache that lasts for a few hours to 1-2 days [(3)]. The variation in the severities of pain among migrainous patients has encourage vigorous initiation and prophylactic therapies [(4)]. The vasomotor changes are greatly suggested to underlie the pathophysiology of migraine [(5)]. The marked increase in the amplitude of temporal artery pulsation and relief of pain by ergotamine may support this hypothesis [(6)]. In addition, serotonin released from platelets and serotonergic nerve endings in the meningeal blood vessels has been implicated in the initiation of migraine headache [(5, 7)]. In this regard, serotonin receptor antagonists are typical antimigraine drugs of current use for the acute management and prophylaxis of migraine [(6, 4)]. Pizotifen, now, is one of the prophylactic drugs for migraine headache to reduce the severity, frequency and duration of attacks. The drug act by blocking 5-HT_A receptors. However, the clinical use of this drug has reported to be accompanied by weight gain and antimuscarinic effects [(3)]. Melatonin, N-acetyl-5-methoxytryptamine, is a serotonin derivative produced and released by the pineal gland and some other tissues and is believed to participate in the regulation of sleep-wake cycle[(8)]. The release is coincides with darkness (begin around 9PM and lasts until about 4AM) and suppressed by day-light [(9)]. Several studies have conducted to investigate the properties and effects of melatonin; and these revealed that this neurohormone processes free radical scavenging[(10)], nitric oxide synthase inhibiting[(11)], dopamine release inhibiting [(12,13)], GABAergic stimulating [(14,15,16)], glutamate neurotoxic protecting [(17)], opioid analgesic [(18)], neurovascular regulating [(19)] and serotonine modulating actions[(20)]. In addition, this agent has been studied for a number of other functions including contraception; antioxidant action; prevention of aging; and treatment of depression, human immunodeficiency virus (HIV) infection and a variety of cancers [(9, 21)].In the view of available data about melatonin properties and functions and the pathophysiology of migraine, this study has been conducted to evaluate the clinical effectiveness of melatonin alone and in combination with pizotifen as prophylactic therapy in migraine patients.

Patients and Methods

Patients:

The study group comprised of a total of 72 patients with migraine in age range of (13-55 years old). The patients included in this study were under neurologist supervision during the entire period of treatment, where the vital signs and positive finding of routine physical examination were evaluated and recorded. All patients were diagnosed having migraine headache of many types (e.g. Common, classical, ophthalmoplegic and retinal migraine). The duration of disease ranged from (3 months to 10 years). Patients involved in this study were instructed to avoid diet that triggers migraine such as chocolate, cheese, etc. The patients were randomly divided into 4 groups (A, B, C and D) each of 18. The groups were treated for 42 days, followed up and monitored each week. Group A was given melatonin (3mg) 30 minutes before bed time, group B was treated with pizotifen (0.5mg twice daily), group C was treated with combination of melatonin and pizotifen (same doses in group A and B) and group D was treated with placebo (glucose filled capsules at night).

Method:

The migraine severity (MIGSEV) score was used to identify items that serve to assess the severity of migraine with a high level of clinical and psychometric relevance (figure 1) [(22)]. This is because the severity of migraine is an important determinant of patient quality of life and of health care resource utilization. Only seven items reflecting severity were identified by expert consensus. These were intensity of pain, tolerability, disability in daily activity, nausea or vomiting (or both), duration of attack, resistance to treatment and frequency of attack. Principal components analysis performed on the seven items of the severity questionnaire identified three dimensions while correlation analysis showed that the first dimension covered 4 items relating to the intensity of attacks (intensity of pain, tolerability, disability in activity and presence of nausea or vomiting). The second dimension related to resistance to treatment (resistance to treatment and duration of attack) and the third dimension to frequency of attacks. A rating system was devised. Firstly, the number of items for which the lowest possible and the highest possible rating was determined. Secondly a ternary (low, intermediate and high) overall rating of severity was attributed using the following decision tree:

A-first dimension parameters (main parameters)		
Score	Intensity of pain	Nausea
Minimum Medium Medium Maximum	Mild ☐ Moderate ☐ Intense ☐ Sever intense ☐	No nausea ☐ Mild ☐ Intense ☐ Vomiting ☐
Score	Disability in daily activity	Tolerability
Minimum Medium Medium Maximum	No disability ☐ Mild ☐ Marked ☐ Confined to bed ☐	Tolerable ☐ Barely tolerable ☐ Intolerable ☐

*Grade one----at least one minimum score without maximum score
Grade two----any other
Grade three—at least one maximum score without minimum score or at least two maxi. Score

B-2nd dimension parameters	
Resistance to treat.	Duration of attack (hr.)
No ☐ Yes ☐	< 4 hr. ☐ 12-24 hr. ☐ 4-12 hr. ☐ > 24 hr. ☐

C-3rd dimension parameters	
Frequency of attack	
< 5/year ☐ 5-10/year ☐ 1-2/month ☐	1/weak ☐ >1/weak ☐

Fig. (1) First, second and third dimension parameters of migraine [15].

low (grade 1): at least 1of the 4 items with a minimum score, and no item with a maximum score; high (grade 3): at least 1 of the 4 items with a maximum score, and no item with a minimum score or at least 2 items with a maximum score; intermediate (grade 2): all other cases. Data were expressed as mean ± SD and as percent change from baseline regarding those obtained from MIGSEV score. Response of patients was expressed as percentage from total number in each group. Analyses were done using unpaired student's *t*-test and Chi-square method when appropriate.

Results :

Effect of Different Treatment on First Dimension Parameter (Severity of Migraine)

In this study, the difference between the baseline values of migraine severity score among all groups was not significant (P>0.05) as shown in table (1) and figure (2). After 1 month, the severity score following treatment with melatonin (3mg/night), pizotifen (0.5mg twice daily), melatonin-pizotifen combination and placebo was significantly reduced by 48%, 25%, 59% and 12.2%, respectively (P<0.05). The effect exerted by melatonin was significantly differ from that exerted by pizotifen, P<0.05. In addition, melatonin was shown to enhance the effect of pizotifen when added to the treatment, P<0.05; however, this effect was not significantly differ from that exerted by melatonin alone, P>0.05. The reduction in the severity score following treatment with melatonin, pizotifen and their combination was significantly higher than that produced by placebo, P<0.05. Table (2) clearly showed that the improvement in severity score in all treated groups was complete, P<0.01 (analyzed by Chi-square method); this means that the distribution of patient's response was greatly depend on treatment regimen used with great effect achieved by melatonin and its combination with pizotifen.

Effect of Different Treatment on Second Dimension Parameter (Duration of Migraine Attack)

Table (1) and figure (2) also showed the effect of the four treatments on the duration score of migraine attack. It was shown that all patients were not significantly differ in their baseline score (P>0.05). The reduction in duration of attacks following treatment with melatonin, pizotifen, melatonin-pizotifen combination and placebo was significantly reduced by 53%, 45.3%, 62.7% and 20%, respectively (P<0.05). The effect of melatonin-pizotifen combination was significantly higher than that produced by pizotifen alone, P<0.05; however, this effect did not differ significantly from that produced by melatonin alone (P>0.05). Moreover, the reduction in duration score following melatonin, pizotifen and their combination was significantly higher that that produced by placebo treatment (P<0.05).

Table (1): Effect of treatment with melatonin, pizotifen and their combination on migraine parameters

Parameter	Time	Group A (Melatonin 3mg/night)	Group B (Pizotifen 0.5mg twice daily)	Group C (Pizotifen + Melatonin)	Placebo
1st dimension response (intensity of attack)	Baseline	2.78 ± 0.43	2.67 ± 0.49	2.72 ± 0.46	2.72 ± 0.46
	After 1 month	1.44 ± 0.78* [a]	2.00 ± 0.91* [b]	1.11 ± 0.47 * [a]	2.39 ± 0.85 * [b]
	% reduction	***48%***	***25%***	***59%***	***12.2%***
2nd dimension response (duration of attack)	Baseline	16.56 ± 5.97	16.89 ± 6.22	17.00 ± 7.00	16.67 ± 6.79
	After 1 month	7.67 ± 5.32* [a,b]	9.22 ± 5.87* [a]	6.33 ± 3.51* [b]	13.33 ± 7.29 * [c]
	% reduction	***53%***	***45.3%***	***62.7%***	***20%***
3rd dimension response (frequency of attack)	Baseline	9.83 ± 3.28	10.11 ± 2.76	9.78 ± 3.04	10.27 ± 2.96
	After 1 month	5.33 ± 2.91* [a]	7.33 ± 3.56* [b]	4.11 ± 2.95 * [a]	8.61 ± 2.85 * [b]
	% reduction	***45.75%***	***27.5%***	***58%***	***16.2%***

Data are expressed as Mean ± SD.
n=18 per group.
*P<0.05 with respect to baseline value.
Non-identical superscripts (a, b) for the same parameter within the same period considered significantly different (P<0.05).

Table (2): Distribution of patient's response to different medications according to the 1st, 2nd and 3rd dimension parameters of migraine.

Migraine Parameter \ Treatment Group		Group A (Melatonin3mg/night)	Group B (Pizotifen 0.5mg twice daily)	Group C (Pizotifen + melatonin)	Group D (Placebo)
1st dimension parameters (Severity of attack)	Complete response	13 (72.20%)	7 (38.80%)	17 (94.40%)	4 (22.20%)
	Partial response	1 (5.50%)	1 (5.50%)	0 (0.00%)	0 (0.00%)
	P (χ^2test)		***0.0002***		
2nd dimension parameters (duration of attack)	Complete response	8 (44.40%)	7 (38.80%)	10 (55.50%)	3 (16.60%)
	Partial response	6 (33.30%)	6 (33.30%)	7 (38.80%)	4 (22.20%)
	P (χ^2test)		***0.015***		
3rd dimension parameters (Frequency of attack)	Complete response	4 (22.20%)	2 (11.10%)	8 (44.40%)	0 (0.00%)
	Partial response	11 (61.10%)	9 (50.00%)	10 (55.50%)	9 (50.00%)
	P (χ^2test)		***0.002***		

Data are expressed as number and percentage of total (n=18 per treatment group).
Data are analyzed by Chi-square (χ^2) test.

In the groups that treated with melatonin, pizotifen and their combination the distribution of patients according to their response as complete or partial was significant (P<0.05, analyzed by Chi-square method). However, the difference between the percentages of patients with complete or partial response was only small, as shown in table (2).

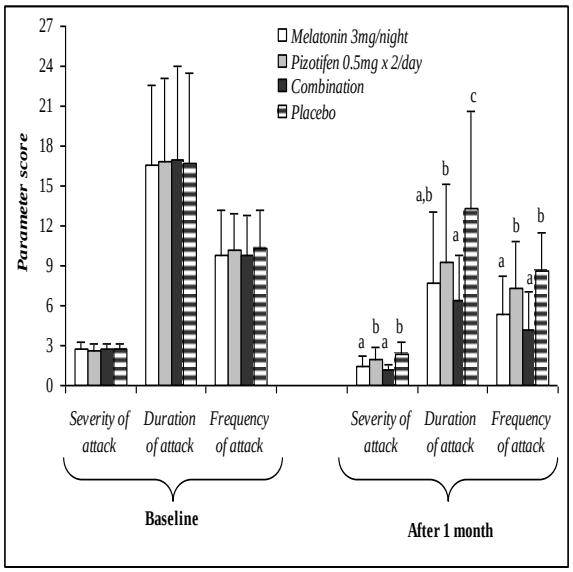

Fig (2): Migraine parameters before and after 1 month treatment with melatonin (3mg/night), pizotifen (0.5mg x2/day), their combination and placebo. Data are expressed as mean ± SD, n=18 per group. All parameters are significantly different with respect to their baseline value (P<0.05, by Paired Student's t-test). Non identical superscripts (a, b) within the same parameter considered significantly different (P<0.05, by Unpaired Student's *t*-test).

Effect of Different Treatment on Third Dimension Parameter (Frequency of Migraine Attack)

Regarding the frequency score of migraine attack, all patients were not significantly differ in their baseline score (P>0.05), table (1) and figure (2). The reduction in frequency score following treatment with melatonin, pizotifen, melatonin-pizotifen combination and placebo was significant high with percent reduction of 45.75%, 27.5%, 58% and 16.2%, respectively (P<0.05). The effect produced by melatonin and its combination with pizotifen was non-significantly different (P>0.05). On the other hand, the reduction produced by pizotifen was not significantly differ from that produced by placebo, P>0.05. However; the effect of pizotifen was significantly lower that that produced by either melatonin or melatonin-pizotifen combination, P<0.05. The response or reduction in frequency score of migraine attack in treated patients was shown to be partial and significantly (P<0.01) depend on the type of treatment used, as shown in table (2).

Discussion :

The results presented in this study demonstrated that melatonin in a dose of 3mg/day greatly and significantly reduced the severity, duration and frequency of migraine attack. The nightly administration of melatonin was to avoid sleep induction during the day; helping patients suffering from migraine with delayed sleep syndrome[16]; and as supplementation to elevate the decreased levels at night in migraine patients. In this context, Claustrate *et al.* was reported that plasma levels of melatonin in migraine patients was lower when drawn at 23.00 hr in comparison to controls [23]. In addition, Murialdo *et al.* who reported the decrease in nocturnal melatonin levels throughout the ovarian cycle of migraine patients in comparison to controls [24]. The effectiveness of melatonin in modulating the characteristics of migrainous headache may be attributed to many neurological and biochemical effects of the drug. Melatonin was reported to modulate the release of serotonin in rat hippocampus [20]. In this context, serotonergic system has been implicated in the pathogenesis of migraine and mediation of meningial vasodilation[25, 26]. Additional mechanism may be the interaction with analgesic opioids. Melatonin has been regarded as pineal opioid which may exert an analgesic action by binding its receptors in the CNS [18] and activation of GABAergic system[16]. The modulation of immune reactions and inhibition of expression of pro-inflammatory cytokines by melatonin might have a role in the observed effects in this study[10]. This suggestion is supported by findings of Bettahi *et al.* who reported that melatonin reduces nitric oxide synthase activity in rat hypothalamus [11]. Nitric oxide has been regarded as a radical neurotransmitter in the CNS [27] and many studies have indicated its participation in pain mediation at the spinal cord level [28, 29, 30]. Other properties of melatonin which might also explain the observed effects seen in this study include its structural similarity to indomethacin [31] and its anti-oxidant and membrane stabilization action during lipid peroxidation [32]. The antimigraine activity of pizotifen has been reported to be due to blockage of serotonin receptors and modulation of serotonergic system during migraine attacks[26]. The drug is clinically effective as prophylactic measure. The use of pizotifen is thus aimed to target one mechanism of migraine headache which is the neurological mechanism [34]. The effect of pizotifen is greatly enhanced by the addition of melatonin in term of the reduction in the severity, duration and frequency of migraine attacks. Since the two

agents have different mechanism and targets of action in relation to migraine pathophysiology, the synergistic interaction is likely contributed to the observed results. This is very important since combination therapy may be useful for the reduction of the dosage and frequency of administration of pizotifen together with minimizing the adverse effects that include weight gain and antimuscarinic effects [(33)]. It was also reported in this study that, the percentage of responder patients within the same treated group varied according to the measured parameter (table 2). This suggests that factors other than the treatment may have a role such as life style, emotional status and stressful conditions. In conclusion, the data obtained in this study provide clinical evidences for the effectiveness of melatonin alone and in combination with pizotifen as a prophylactic measure in migraine.

Refernces :

1. Adams, R.D.: Principles of Neurology (6th ed.). McGraw-Hill, New York, 2002; 4: 172
2. Classification and diagnostic criteria for headache disorders, cranial neuralgias, and facial pain. Headache Classification Committee of the International Headache Society. *Cephalalgia* 1988; 8 (Suppl 7): 1-96.
3. Katzung, B.G. and Julius, D.J.: Histamine, serotonin and the ergot alkaloids. In: *Basic and clinical pharmacology* (8th ed.), Katzung, B.G. (ED.). McGraw-Hill, New York, 2001; 286.
4. Raskin, N.H.: Acute and prophylactic treatment of migraine: practical approaches and pharmacologic rationale. *Neurology* 1993; 43(suppl. 3): S39.
5. Moskowitz, M.A.; Macfarlane, R.: Neurovascular and molecular mechanisms in migraine headaches. *Cerebovasc. Brain Metab. Rev.* 1993; 5: 159.
6. Welch, K.M.A.: Drug therapy of migraine. *New Eng. J. Med.* 1993; 329: 1476.
7. Fozard, J.R.; Kalkman, H.O.: 5-Hydroxytryptamine (5-HT) and the initiation of migraine: New Perspectives. *Naunyn-Schmiedebergs Arch. Pharmacol.* 1994; 350: 225.
8. Cupp, M.J.: Melatonin. *Am. Fam. Physician*. 1997; 56: 1421-1425.
9. Brzezinski, A.: Melatonin in humans. *New Eng. J. Med.* 1997; 336: 186.
10. Reiter, R.J.; Calvo, J.R.; Karbownik, M.; Qi, W. and Tan, D.X.: Melatonin and its relation to the immune system and inflammation. Ann. N.Y. Acad. Sci. 2000; 917: 376-386.
11. Bettahi, I.; Pozo, D.; Osuna, C.; Reiter, R.J.; Acuna-Castroviejo, D. and Guerrero, J.M.: Melatonin reduces nitric oxide synthase activity in rat hypothalamus. *J. Pineal Res.* 1996; 20: 205-210.
12. Zisapel, N.: Melatonin-dopamine interactions: from basic neurochemistry to a clinical setting. *Cell Mol. Neurobiol.* 2001; 21: 605-616.
13. Peroutka, S.J.: Dopamine and migraine. *Neurology* 1997; 49: 650-656.
14. Ramadan, N.M.: The link between glutamate and migraine. *CNS Spectr* 2003; 8: 446-449.
15. Wang, F.; Li, J.C.; Wu, C.F.; Yang, J.Y.; Xu, F. and Peng, F.: Hypnotic activity of melatonin: involvement of semicarbazide hydrochloride, blocker of synthetic enzyme for GABA. *Acta. Pharmacol. Sin.* 2002; 23: 860-864.
16. Wu, F.S.; Yang, Y.C. and Tsai, J.J.: Melatonin potentiates the GABA(A) receptor-mediated current in cultured chick spinal cord neurons. *Neurosci. Lett.* 1999; 260: 177-180.
17. Espinar, A.; Garcia-Oliva, A.; Isorna, E.M.; Quesada, A.; Prada, F.A. and Guerrero, J.M.: Neuroprotection by melatonin from glutamate-induced excitotoxicity during development of the cerebellum in the chick embryo. *J. Pineal Res.* 2000; 28: 81-88.
18. Ebadi, M.; Govitrapong, P.; Phansuwan-Pujito, P.; Nelson, F. and Reiter, R.J.: Pineal opioid receptors and analgesic action of melatonin. *J. Pineal. Res.* 1998; 24: 193-200.
19. Savaskan, E.; Olivieri, G.; Brydon, L.; Jockers, R.; Krauchi, K. and Wirz-Justice, A.: Cerebrovascular melatonin MT1-receptor alterations in patients with Alzheimer's disease. *Neurosci Lett.* 2001; 308: 9-12.
20. Monnet, F.P.: Melatonin modulates [3H] serotonin release in the rat hippocampus: effects of circadian rhythm. *J. Neuroendocrinol.* 2002; 14: 194-199.
21. Hagan, R.M. and Oakley, N.R.: Melatonin comes age? *Trends Pharmacol. Sci.* 1995; 16: 81.
22. EL-Hasnaoui, A.; Vray, M.; Richard, A. and Nachit-Ouinekh, F.: Assessing the severity of migraine: Development of the MIGSEV Score. *Headache*. 2003; 43: 628-635.
23. Claustrat, B.; Loisy, C.; Brun, J.; Beorchia, S.; Arnaud, J.L. and Chazot, G.: Nocturnal plasma melatonin levels in migraine: a preliminary report. *Headache*. 1989; 29: 242-245.
24. Murialdo, G.; Fonzi, S.; Costelli, P.; Solinas, G.P.; Parodi, C. and Marabini, S.: Urinary melatonin excretion throughout the ovarian cycle in menstrually related migraine. *Cephalalgia* 1994; 14: 205-209.

25. Raskin, N.H.: Repetitive intravenous dihydroergotamine as therapy for intractable migraine. *Neurology* 1986; 36: 995-997.
26. Lance, J.W.; Lambert, G.A.; Goadsby, P.J. and Zagami, A.S.: 5-Hydroxytryptamine and its putative aetiological involvement in migraine. Cephalalgia Suppl. 1989; 9: 7-13.
27. Vincent, S.R.: Nitric oxide: A radical neurotransmitter in the central nervous system. *Prog. Neurobiol*. 1994; 42: 129-160.
28. Meller, S.T. and Gebhart, G.F.: Nitric oxide and nociceptive processing in the spinal cord. *Pain* 1993; 52: 2002-2012.
29. Meller, S.T.; Cummings, C.I.; Traub, R.J. and Gebhart, G.F.: The role of nitric oxide in the development of the hyperalgesia produced by intraplanter injection of carrageenan in the rat. *Neuroscience* 1994; 60: 367-374.
30. Gordh, T.; Sharma, H.S.; Alm, P. and Westman, J.: Spinal cord nerve lesion in the upregulation of neuronal nitric oxide synthase in the spinal cord – An immunohistochemical investigation in the rat. *Amino Acids* 1998; 14: 105-112.
31. Peres, M.E.P.: Melatonin, the pineal gland and their implications for headache disorders.*cephagia*.2005; 25(6): 403-411.
32. Garcia, J.J.; Reiter, R.J. and Guerrero, J.M.: Melatonin prevents changes in microsomal membrane fluidity during induced lipid peroxidation .*FEBS Lett.* 1997; 408: 297-300.
33. Rang, H.P.; Dale, M.M. and Ritter, J.M.: Other peripheral mediators: 5-hydroxytryptamine and purines. In: *Pharmacology* (4th ed.). Churchill Livingstone, London, 1999; p. 172.
34. Humphrey, P.P.; Feniuk, W.; Perren, M.J.; Beresford, I.J.; Skingle, M. and Whalley, E.T.: Serotonin and migraine. *Ann. N.Y. Acad. Sci.* 1990; 600: 587