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Effects of Migriheal® on Plasma Proteome of Patients with Migraine Headaches

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A B S T R A C T

Recently, based on herbal medicine of Iran, an herbal drug with medical effects named MigriHeal®, has been registered; it helps in prevention of migraine headache strokes. In this study, MigriHeal was administered for pure migraine patients and the aim of this study is proteomic analysis of plasma from patients with migraine headaches before and after treatment with MigriHeal®. Before and after administration of MigriHeal®, patient's plasma was obtained, and then 2DE (2-dimensional electrophoresis) proteomic analysis of 11 patients was done. The Progenesis Same Spots ver.4 software was used for statistical analysis of the gels; according to ANOVA test analysis, we showed that the expression of two proteins named alpha-2-HS-glycoprotein (p-value: 0.023) and alpha-1-B-glycoprotein (p-value: 0.008) was decreased. Two identified proteins have anti-inflammatory effects and based on this finding we concluded inflammation is a pathologic mechanism of migraine, presumably, MigriHeal® has anti-inflammatory effects. Although these are not specific for the migraine and are altered in other diseases, these two proteins may be migraine biomarkers if measured simultaneously with quantitative methods in an expanded population.

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Introduction

Migraine is a type of periodic headache that appears to be a vascular phenomenon induced by strong intracranial vasoconstriction and vasodilation (neurovascular theory). Recently it has been proposed that inflammation and neurogenic processes also are included in the causation of this disease [1]. However, Ebn-e-sina (*Avicenna*, 980–1037) was actually the first medical scientist who addressed neurovascular theory of migraine in *Qanoon-fel-teb* (The Canon) [2]. In the United States, about 18% of females and 6% of males are affected by migraine and the medical burden of this disease is over 1$ billion per year [3]. Migraine is ranked as one of the top 20 causes of healthy life lost and disability by World Health Organization (WHO) which is indicating the importance of this disease [4].

According to previous studies, migraine headaches apparently are associated with vascular inflammation. Different neuropeptides and cytokines such as C-reactive protein (CRP), interleukin-1 (IL-1), IL-6 and tumor necrosis factor-α (TNF-α), are included in migraine inflammatory process [5]. In addition, according to the studies of Ansari *et al.* [6, 7] and others [8], levels of some molecules such as melatonin (in plasma, serum or urine), nitric oxide (NO) (in serum or plasma) and calcitonin gene related protein (CGRP) (plasma) has been altered in migraine patients, and they have been suggested as possible biomarkers for migraine [7,8]. Increases in CGRP and NO levels and a decrease in melatonin level are directly incorporate in migraine pathology [7, 8] and presumably, changes in the level of these biomarkers are related to alteration in the expression on the gene and enzymes synthetizing them. On the other hand, there is a direct relation between migraine and other diseases such as hypertension, obesity and elevation of body mass index (BMI), insulin resistance, metabolic syndrome and cardiovascular diseases [9]; it is likely that protein biomarkers for migraine exist. Therefore, proteomic analysis can be a powerful tool for determining these biomarkers or a help for determining medical strategies [10].

Two therapeutic strategies exist for migraine: abortive (rescue) and prophylactic (preventive). According to the approaches, there are many drugs in the market for migraine such as abortive drugs (eg. Analgesics, Triptans and Ergots) and preventive drugs (eg. Beta-blockers, Antidepressants and Anticonvulsants); most of them work on pain systems in general, not just activated pain pathways involved in migraine and they have frequent unpleasant and sometimes debilitating side effects. For the reasons, natural medications for migraines often appeal to people with chronic migraine [7].

Using the curative herbs for the treatment of headache in Persia can be come from the 6th century BC. Some Persian physicians such as Ebn-e-Sina recognized the healing actions of plants to a specific analgesic, sedative or prophylactic drug property [11]. Recently based on anecdotal evidence found in traditional Iranian medicine a novel herbal remedy named MigriHeal® with prophylactic effect against migraine headache attacks was introduced by Ansari et al. This drug has been patented by the Invention and Patent Registration Office of I.R. of Iran (IRC: 1228143083). Evidences of clinical trials are suggestive of effectiveness of this drug in rapid recovery of migraine headaches. In longtime, One or two round of consumption of this drug, leads to decrease in intensity of headache, decrease in the durance of headache, increase in interval between headaches and finally, full cessation of migraine attack in migraine patients [7,12]. MigriHeal® consists of high amounts of melatonin [6] which can reduce the pathological NO and thus toxicity of this small molecule in body [13-15]. Nevertheless, mechanisms of action of MigriHeal® have not been understood and more studies are required to elucidate its therapeutic function.

We hypothesized that MigriHeal® acts via alteration in the level of the proteins included in the synthesis of these biomarkers and other unknown proteins incorporating in migraine. In this study, changes in the plasma proteome of migraine patients, before and after 4 months consumption of MigriHeal® was analyzed using 2-dimensional electrophoresis proteomic technique.

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Materials and methods

Patients and phlebotomy

In this study, blood samples of patients with migraine who were receiving MigriHeal® drug, was collected before and after consumption of the drug. Written consent was obtained before phlebotomy. Migraine was diagnosed by a neurologist using computed tomography (CT) scan, magnetic resonance imaging (MRI) and classic symptoms. Diagnosis of neurologist was the criteria for selection of patients. Then, headache signs and questions like lifestyle, nutrition of the patients, intra-family relationships, stress, job conditions and social activities were asked. Among 11 selected patients, 8 cases were female. Age range of the incorporating patients was 25-42. All of the patients were married and have children. The mean age of female patients was 38±2 and the mean age of male patients was 30±4.2. All were living in Tehran. All female patients were in the follicular phase of menstrual cycle during phlebotomy. Also all of them were diagnosed with pure migraine and were normotensive.

Before beginning of the treatment, phlebotomy was done. Then MigriHeal® was administrated for patients 20 g/day. MigriHeal® herbal powder was boiled in water and inhaled for 10 min. This program was continued for 20 sessions. Intensity, durance and time of headache and also signs of headache such as nausea, vomiting, avoidance from light and sound was recorded by patients in the daily report form. At the end of 20 sessions, based on daily report analysis, decision making for recalling treatment was done. Recalling treatment period included administration of drug at least 2-4 sessions per month by inhalation rout. After completing the treatment course, again phlebotomy was done. In the 2 steps of venipuncture sampling, 4 mL EDTA blood was drawn. Phlebotomy was done in Araamesh clinic.

Having migraine headache based on The International Classification of Headache Disorders (ICHD) [4] was the inclusion criteria and having inflammatory and immunologic disease, having a hypertension and cardiovascular disease history and withdraw from treatment was exclusion criteria of this study.

Preparation of plasma samples

Blood collection was done by venipuncture rout and using needle with gauge 21. Samples were collected in EDTA tubes and in less than 2 h were centrifuged in 4ºC with 3000 rpm for 8 min. The separated plasma was again centrifuged with above conditions. Five 150 µL aliquots were prepared from each sample and the remnant was divided in 500 µL aliquots. Microtubes immediately placed in -80ºC freezer. The entire process, from phlebotomy to placing plasma samples in freezer, was done in 4 h.

Using Proteominer Kit and according to its instructions, abundant proteins were depleted from plasma samples. Then using 2D-CleanUp Kit, remaining proteins were purified and precipitated; this precipitate was solved in rehydration solution. Next using 2D Quant Kit, protein concentration of rehydration solution was determined. Finally 2-dimensional electrophoresis (2-DE) was done.

2-DE technique

For all samples, 2-DE analysis was done with triple repeat. First dimension was done using BIORAD PROTEAN IEF Cell; 7 cm IPG with pH range 3-10. Isoelectric Focusing (IEF) was done in-gel and 30 µg protein was loaded for each gel. For second dimension, Bio Rad Electrophoresis Tank was used. For this step, 12% SDS-PAGE gel was prepared. After electrophoresis completed, gels were stained using silver nitrate and scanned using GS-800 Calibrated Densitometer and documented.

Densitometry analysis of images, statistic evaluation of spots, and bioinformatics analysis were performed by Progenesis Same Spots ver.4 software. For representing the proteome pattern, a reference gel (figure 1) was selected. Then using this software, changes in expression of proteins before and after administration of MigriHeal® was analyzed.

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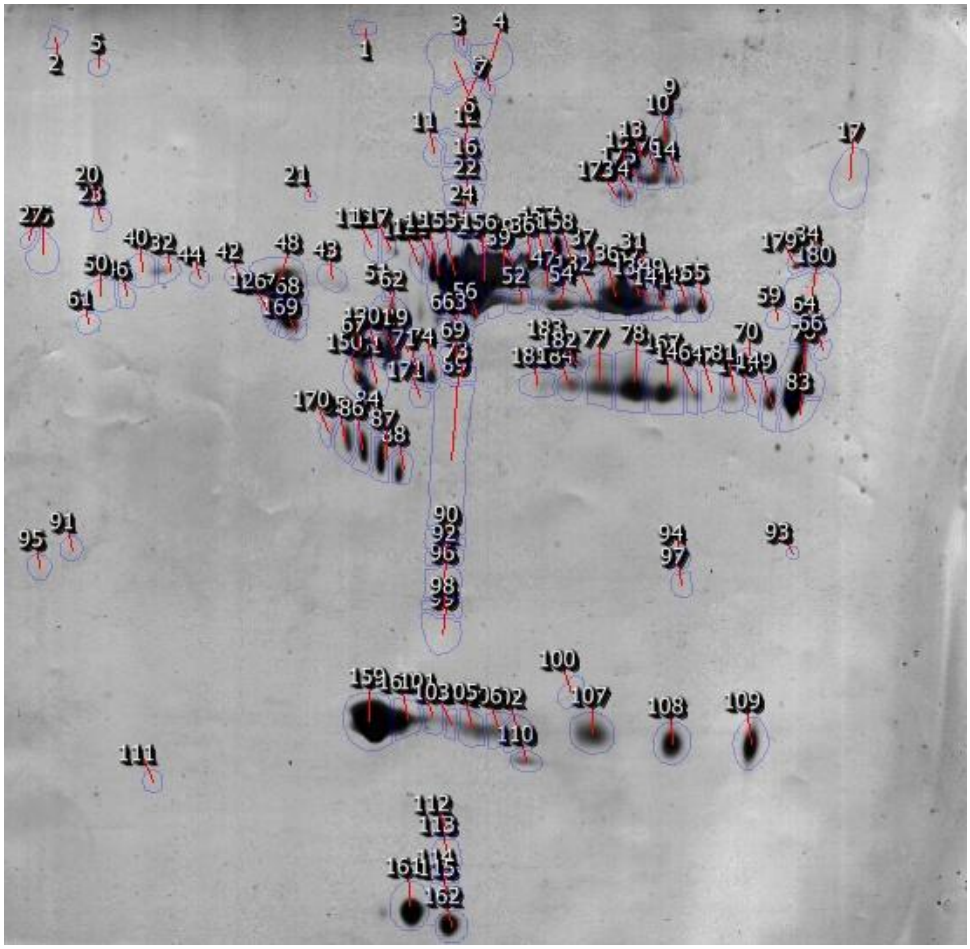

Fig. 1. Reference 2-DE gel of a patient with migraine headache. 183 spots were detected. Location of spots No. 40 and No. 116 are shown with arrows.

Matrix Assisted Laser Desorption/Ionization Time of Flight Mass Spectrometry (MALDI-TOF MS) analysis

Using spot picker, protein spots were picked up and transferred to York University in England. Protein bands were destained using acetonitrile, and using Dithiothreitol (DTT) and iodoacetamide were reduced and alkylated respectively and were trypsinized in 37ºC for 24 h. Finally, the extracted peptides were sequenced and identified using MALDI-TOF MS.

Statistical analysis

The entire data was presented as mean fold change ± 2 standard deviation (SD). Statistic significance was analyzed using ANOVA test and p-values below 0.05 were considered statistically significant.

Results and discussion

Eleven selected patients were satisfied from treatment progress and disappearance of migraine headaches including intensity, durance and interval between headaches and almost reached lifestyle without migraine headache. 8 of these patients were female. Statistical analysis for 30 gels of 11 patients with migraine headache was done. After selection of reference gel (figure 1) and statistical analysis, 183 spots were seen.

Among these 183 spots, density of 2 spots was statistically different (significant) before and after administration of MigriHeal®. Density of these spots or expression of these proteins was

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decreased after administration of MigriHeal®. These 2 spots, 116 and 40, were determined using MALDI-TOF MS. These proteins were alpha-1- B-glycoprotein and alpha-2-HS-glycoprotein, respectively (table 1).

Table 1. Identified spots characteristics.

Spot No.	Before MigriHeal® consumption to after consumption ratio	Protein	Molecular weight on gel (kDa)	pI on gel	p-value
40	3.4	Alpha-2HS-glycoprotein	53	4.3	0.023
116	7.2	Alpha-1-B-glycoprotein	70	5.2	0.008

The location of these 2 spots and change in their expression level is shown in figures 2 (spot No. 116) and 3 (spot No. 40). In part A of figures 2 and 3, a section of final gel for before and after administration of MigriHeal® is shown. In part B of figures 2 and 3, numbers on the plot are representing amount of spot density or protein expression. For normalization and deleting high variation data, the vertical axis is based on log. For normalization, spot density is divided by total density of the gel spots, so it has no unit. As shown in both figures 2 & 3, the density of protein spots is decreased after treatment with MigriHeal® (p-value: < 0.05).

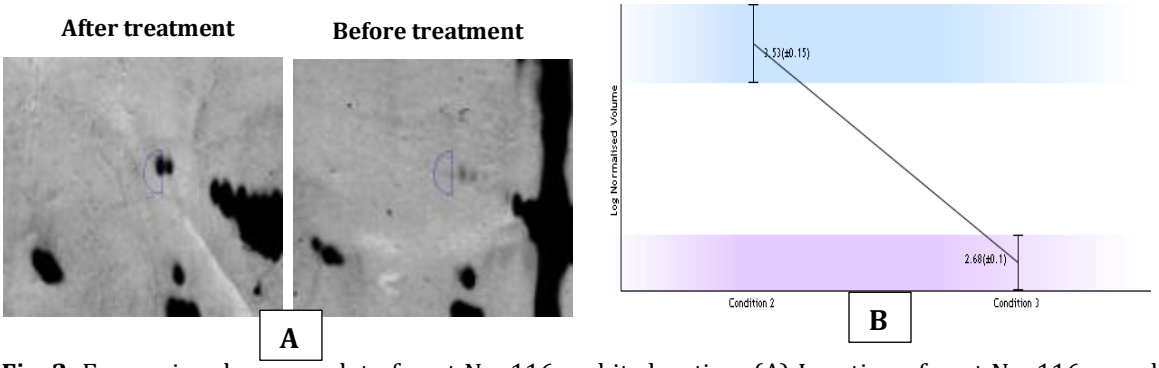

Fig. 2. Expression decrease plot of spot No. 116 and its location. (A) Location of spot No. 116 on gels from before and after treatment with MigriHeal® drug. (B) Expression difference of alpha-1-B-glycoprotein before (blue, 3.53±0.15) and after (purple, 2.68±0.1) treatment with MigriHeal®. Results are represented as expression difference ± 2SD.

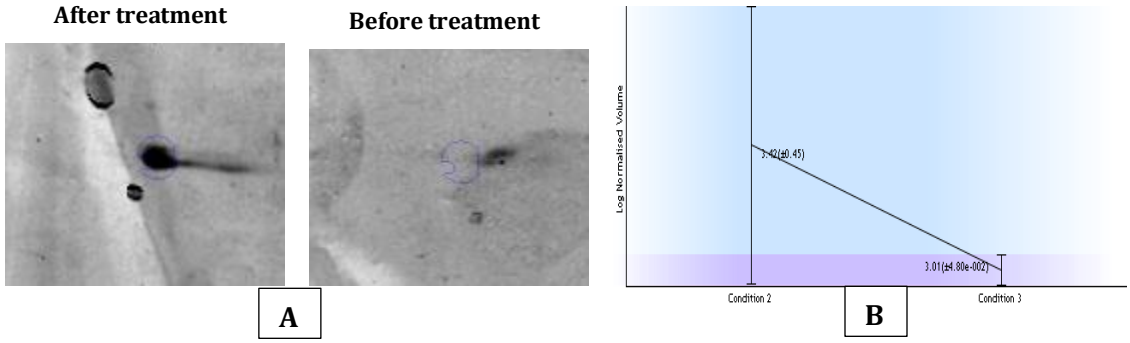

Fig. 3. Expression decrease plot of spot No. 40 and its location. (A) Location of spot No. 40 on gels from before and after treatment with MigriHeal® drug. (B) Expression difference of alpha-2-HS-glycoprotein before (blue, 3.42±0.45) and after (purple, 3.01±0.48) treatment with MigriHeal®. Results are represented as expression difference ± 2SD.

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Discussion

This study has been done for the first time in Iran for migraine patients. In this study, plasma proteins profile of migraine patients, before and after treatment with MigriHeal® was analyzed using 2-DE in conjunction with MALDI-TOF MS. According to our results, the protein expression of alpha-2-HS-glycoprotein and alpha-1-B-glycoprotein was decreased following 4-month MigriHeal® therapy.

Alpha-2-HS-glycoprotein, also called fetuin-A, is synthesized in liver and composed of two subunits: heavy chain (A) and light chain (B) [16, 17]. This protein as one of the most abundant proteins in fetal circulation is also expressed in other tissues such as kidneys, skin and brain [18]. Increase in serum transforming growth factor-β (TGF-β) levels in migraine patients is reported. One possible explanation could be its anti-inflammatory function as well as inhibitory role of this protein in platelet TGF-β1. There is a similarity between amino acid sequence of Fetuin-A and TGF-β receptor; it seems likely that Fetuin-A might inhibit the cytokine–mediated inflammatory response. In addition, it has been demonstrated that Fetuin-A induced delay in the inflammatory response in central nervous system, could decrease cerebral ischemic injury [18].

The result of our study from the view of alpha-2-glycoprotein as a possible biomarker for migraine is partly in agreement with two recent studies by Bellei et al on medication overuse headache (MOH) patients. These studies were performed in order to identify emerging biomarkers in renal dysfunctions following excessive consumption of antimigraine drugs [19,20]. The investigation of urinary proteome analysis illustrated that there was a significant difference in protein expression of controls compared to patients, especially users of non-steroid anti-inflammatory drugs (NSAIDs). According to their results, urinary levels of uromodulin, alpha-1-miroglobulin, Zn-alpha-2-glycoprotein, cystatin C, immunoglobulin κ chain and alpha trypsin H4 heavy chain might be associated with renal dysfunctions [19, 20].

This inconsistency between directions of changes in alpha-2-glycoprotein levels can be interpreted in light of possible function of this protein on decrease in expression of proinflammatory cytokines such as TNF-α, IL-1 and IL-6 as well as macrophage inactivation [18]. It seems likely that the serum level of alpha-2-glycoprotein at begin of MigriHeal® therapy increases, thereby suppressing inflammation in migraine patients. Since the intensity and frequency of migraine attacks alleviates following 4-month course of treatment, the patients has no need for counteract deleterious inflammation and in that way its serum levels decreases.

Besides, it is partly evident that elevation in Fetuin-A could lead to elevation of NO production in macrophages induced with lipopolysaccharide (LPS) [18]. The decrease in Fetuin-A levels following the end of treatment can be resulted from primary and favorable influence of MigriHeal® on decrease in NO production. In accordance with this explanation, Rafiee et al showed that aqueous extract and essence of MigriHeal® inhibit inducible nitric oxide synthase (iNOS) expression and scavenge NO from cell line RAW 264.7 stimulated with LPS as a possible in vitro model of migraine [15]. Therefore, the decrease in Fetuin-A expression after 4-month MigriHeal therapy might be due to reduced level of NO.

Another possible explanation for our results might be related to estrogens. It is clear now that migraine headaches are obviously linked to estrogen levels in females [21]. In addition, estrogens are involved in production of vasodilators such as NO, cAMP and cGMP. Also, the estrogens in accompany with progesterone, can increase CGRP synthesis, the key pathogenic mediator in migraine. Estrogen receptor α, could lead to up-regulation of NOSs activity in endothelial cells, thereby increasing NO production which is important in migraine etiology [21]. Interestingly, it has been shown that estrogens could result in elevated levels of Fetuin-A [22]. As eight patients in current study are females, it is likely that MigriHeal® acts as one possible estrogen antagonist and thus it can decrease Fetuin-A expression.

Alpha-1-B-glycoprotein is another protein which its expression decreased following MigriHeal® therapy. This protein is a member of immunoglobulin supergene family and related to

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innate immunity. Also, it interacts through a covalent linkage to cystein rich secretory protein–3 (CRISP-3) in plasma. Although, there are limited studies pointing the probable role of this protein in cancer pathogenesis including pancreas, bladder, liver and cervix cancer, the other functions of alpha-1-B-glycoprotein remain poorly defined [23]. Based on proteomic analysis, expression of alpha-1-B-glycoprotein has elevated in patients with interstitial cystic-painful bladder syndrome [24]. This protein is also increased in pancreatic adenocarcinoma. In addition, low level of this protein was reported in patients with Alzheimer's disease [25,26]. According to our results, alpha-1-B-glycoprotein is obviously decreased in migraine patients after 4 months treatment with MigriHeal®, although further investigations need to identify the exact mechanism.

Conclusions

The main findings of this study are a significantly decrease in serum levels of alpha-2-HS-glycoprotein and alpha-1-B-glycoprotein following 4 months treatment with MigriHeal® drug in migraine patients. The alleviating effect of MigriHeal® on intensity and frequency of migraine headaches, on one hand, and its function in regulation of inflammatory pathways, on the other hand, could be regarded as a probable cause for change in levels of above-mentioned proteins after end of MigriHeal® therapy. Although, our study drawn more attention on role of these proteins as emerging biomarkers in migraine pathogenesis and discovery of novel therapeutic approaches, more studies are needed to validate our results.

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Conflict of interest

Authors certify that there is no actual or potential conflict of interest in relation to this article.

References

[1] Pietrobon D & Striessnig J. Neurobiology of migraine. Nat Rev Neurosci2003; 4:386-398.
[2] Abokrysha N. IbnSina (Avicenna) on Pathogenesis of Migraine Compared With the Recent Theories. Headache. 2009;49:923-937.
[3] Jensen R & Stovner LJ. Epidemiology and comorbidity of headache. Lancet Neurol. 2008;7:354-361.
[4] The International Classification of Headache Disorders: 2nd edition. Cephalalgia. 2004;24: 9-160.
[5] Peterlin BL, Bigal ME, Tepper SJ, Urakaze M, Sheftell FD, Rapoport AM. Migraine and adiponectin: is there a connection? Cephalalgia. 2007;27:435-446.
[6] Fooladsaz K, Ansari M, Rasaie MJ. Evaluation and Comparison of Serum Melatonin Determination in Normal Individuals and Migraine Patients. Tehran Univ Med J. 2004;62:37-43.
[7] Ansari M, Rafiee Kh, Emamgholipour S and Fallah MS. Migraine: Molecular basis and herbal medicine. In: Chen K-S. (ed.) Advanced Topics in Neurological Disorders 1st ed. InTech Press, Croatia. 2012;185-214.
[8] Loder E, Harrington MG, Cutrer M, Sandor P, De Vries B. Biomarkers in Migraine: Selected Confirmed, Probable, and Exploratory Migraine Biomarkers Headache. 2006;46:1108-27.
[9] Hamed SA. The vascular risk associations with migraine: Relation to migraine susceptibility and progression. Atherosclerosis. 2009;205:15–22.
[10] Kuakarn S, SomParn P, Tangkijvanich P, Mahachai V, Thongboonkerd V, Hirankarn N. Serum proteins in chronic hepatitis B patients treated with peginterferon alfa-2b. World J. Gastroenterol. 2013;19:5067-5075.
[11] Gorji A. Pharmacological treatment of headache using traditional Persian medicine. Trends Pharmacol Sci. 2003;24:331-334.
[12]Fallah MS, Ansari M, Roudbari SA and Rezaei F. Prophylactic treatment of migraine with a novel herbal remedy. In: 12th Congress of the International Headache Society. Kyoto, Japan. 2005. p: 945.

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[13] Ansari M, Mahrooz A, Sharif Tabrizi A and Vardasbi S. Effect of aqueous extract of melilotousofficinalis on production of nitric oxide (no) in endotheliuma cells. Daneshvar 2006;64:15-20.
[14] Ansari M, Paknejad M and Ansari A. Effects of three medicinal herbs essontial oil on nittric oxide production in cultured vascular endothelioma cell line. In: 11th Asian Pacific Congress of Clinical Biochemistry. 2007. Beijing, China.
[15] Rafiee Kh, Ansari M, Mahdian R, Paknejad M, Fallah MS, Azizi M. In vitro Effects of a Herbal Remedy for Migraine Treatment, MigriHeal®, on Basal and LPS-induced Nitric Oxide. J. Basic. Appl. Sci. Res. 2013;3:206-211.
[16] Fiore CE, Celotta G, Politi GG, Pino LD, Castelli Z, Mangiafico RA, et al. Association of high alpha2-Heremans–Schmid glycoprotein/fetuin concentration in serum and intima-media thickness in patients with atherosclerotic vascular disease and low bone mass. Atherosclerosis. 2007;195:110–115.
[17] Singh M, Sharma PK, Garg VK, Mondal SC, Singh AK, Kumar N. Role of fetuin-A in atherosclerosis associated with diabetic patients. J Pharm Pharmacol. 2012;64:1703-1708.
[18] Wang H, Sama AE. Anti-inflammatory role of Fetuin-A in Injury and Infection. CurrMol Med. 2012;12:625-633.
[19] Bellei E, Cuoghi A, Monari E, Bergamini S, Fantoni LI, Zappaterra M, Guerzoni S, Bazzocchi A, Tomasi A, Pini LA. Proteomic analysis of urine in medication-overuse headache patients: possible relation with renal damages. J Headache Pain. 2012;13:45-52.
[20] Bellei E, Monari E, Cuoghi A, Bergamini S, Guerzoni S, Ciccarese M, et al. Discovery by a proteomic approach of possible early biomarkers of drug-induced nephrotoxicity in medication-overuse headache. J Headache Pain. 2013;14:6.
[21] Gupta S, Mehrotra S, Villalón CM, Perusquía M, Saxena PR, MaassenVanDenBrink A. Potential role of female sex hormones in the pathophysiology of migraine. PharmacolTher. 2007;113:321-340.
[22] Hashimoto S, Miwa M, Akasofu K, Nishida E. Changes in 40 serum proteins of post-menopausal women. Maturitas. 1991;13:23-33.
[23]Udby L, Johnsen AH, Borregaard N. Human CRISP-3 binds serum α1B-glycoprotein across species. BiochimBiophysActa. 2010;1800:481–485.
[24] Goo YA, Tsai YS, Liu AY, Goodlett DR, Yang CC. Urinary Proteomics Evaluation in Interstitial cystitis/Painful Bladder SySndrome: A Pilot Study. IntBraz J Urol. 2010;36:464-479.
[25] Zeng Z, Hincapie M, Pitteri SJ, Hanash S, Schalkwijk J, Hogan JM, Wang H, Hancock WS. A proteomics platform combining depletion, multi-lectin affinity chromatography (M-LAC), and isoelectric focusing to study the breast cancer proteome. Anal Chem. 2011;83:4845-4854.
[26] Da Costa LA, García-Bailo B, Borchers CH, Badawi A, El-Sohemy A. Association between the plasma proteome and plasmaα-tocopherol concentration in humans. J NutrBiochem. 2013;24:396-400.

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