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Research Article

Quantitative Determination of Amlodipine Besylate without Derivatized in Pure Form and Tablet Dosage Forms with UV Spectrophotometric Method

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ABSTRACT:
The present work describes the development and validation of UV Spectrophotometric method for direct determination of underivatized amlodipine besylate (ADB) in pure and tablet dosage forms. The validation parameters of linearity, precision, accuracy, recovery, specificity, limit of detection and limit of quantification were studied. The range of quantification for proposed method was 2-17 µg/mL. The precision of method was calculated as the relative standard deviation (RSD) and less than 2 %, and accuracy (relative error) was better than 6 % (n = 6). The developed method was successfully applied for the assay of pharmaceutical dosage forms which do not require any preliminary separation or treatment of the samples. The RSD values for Norlopin® tablet (5 mg) and Norvasc® tablet (5 mg) was found to be less than 2 %. The results obtained from this method were compared with two reference method reported in literature and no significant difference was found statistically ($p>0.05$).

Keywords : Amlodipine besylate, tablets, UV spectrophotometric method

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1. INTRODUCTION

Amlodipine besylate (ADB), (4R,S)-3-ethyl 5-methyl 2-(2-amino-ethoxy-methyl)-4-(2-chlorophenyl)-1,4-dihydro-6-methylpyridine-3,5-dicarboxylatemonobenzene sulphonate (its empirical formula is: $C_{20}H_{25}ClN_2O_5.C_6H_6O_3S$, Fig.1), is a dihydropyridine type long acting channel blocker with slow onset of vasodilatory action [1-3].

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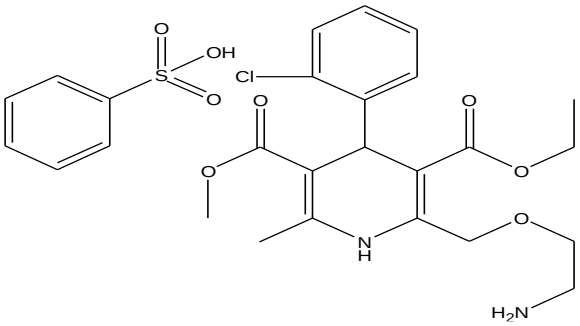

Figure 1. Chemical structure of amlodipine besylate (ADB)

It may also be used for dilated cardiomyopathy and exhibits ameliorating effects on plasma and myocardial catecholamines with a significant reduction of calcium deposition [4,5]. In addition to calcium channel blocking ability, amlodipine also inhibits vascular smooth muscle cell growth through interactions with targets other than L-type calcium channels [3-5]. Several analytical procedures are available in the literature for the analysis of ADB in pharmaceutical dosage, such as High performance liquid chromatography [6-12], high performance thin layer chromatography [13-15], electrochemical analysis [16,17] and titrimetric and spectrophotometric method using bromate-bromide mixture [18]. As far as sensitivity and economical methods of assay are concerned, the determination of ADB by spectrophotometric methods have been proposed based on the extractable ion-pair complex formation followed by extraction and charge-transfer complexes formation [19-26], complex reaction with 2,3-dichloro 5,6-dicyano 1,4-benzoquinone and ascorbic acid in N,N-dimethylformamide medium [27], oxidative coupling with 3-methyl 2-benzothiazoli- none hydrazone hydrochloride [28], complex formation with sodium hydroxide and ninhydrine [29,30], derivative spectroscopy [31-32], the reduction of iron (III) in acid medium [33] and application of oxidants [34]. However, many of these methods are limited in their applications because ADB was derivatizated with different substances. These procedures suffer from such disadvantages as poor selectivity, heating or extraction step and are expensive and time consuming. Therefore there is a need for a simple spectrophotometric method for the assay of ADB without derivatization in pharmaceutical preparations.

Therefore, in this study we are aim at developing simple and sensitive method, which would overcome the difficulties encountered in most spectrophotometric method. The UV Spectrophotometric method was developed for determination without derivatizated of ADB in tablet dosage forms and completely validated (by using linearity, precision, accuracy and sensitivity parameters). We also indicated that determination of ADB without derivatization in tablet dosage forms was possible. The proposed method is recommended for the routine analysis since it is rapid, simple, accurate and also sensitive and specific by no

derivatized. The results obtained this developed and validated method was statistically compared with two reference method [12,18].

2. MATERIALS AND METHODS

2.1. Equipments

A Thermospectronic (HEλIOSβ) double-beam UV-Visible spectrophotometer with a fixed slid width 2 nm and its data processing system was used. The curves of the UV-Visible spectra (N=6. Δλ=18.0 nm) of standard and tablet solutions were recorded in a 1 cm quartz cells between in wavelength ranges of 250-450 nm at scan speed of 600 nm/min.

2.2. Reagents and Solutions

ADB standard was kindly supplied by Novartis Pharmaceutical Industry (Ankara-Turkey). Purity of this substance checked by standard methods (melting point, NMR and IR spectra) and no impurities were found. Ethanol, acetonitrile and other analytical chemicals were purchased from Merck (Germany). Pharmaceutical dosage forms (Norlopin®, Norvasc®) containing ADB were obtained commercially in pharmacy and were claimed to contain 5 mg of the drug and other substance as excipien.

The stock solution of ADB was prepared in ethanol-acetonitrile (30/70, v/v) solvent mixture to a final concentration 100 μg/mL. For calibration, standard solutions containing 2, 4.5, 7, 9.5, 12 and 17 μg/mL were daily prepared by diluting the stock solution to a constant volume with ethanol-acetonitrile mixture. The quality control samples (QC) were prepared at 5.75, 10.75 and 14.50 μg/mL concentrations from stock solution of ADB. These samples were used in analysis of standard samples as quality controls for the purpose of checking recovery of analyte in the daily analyses of standard samples.

2.3. Tablets

Twenty tablets each of Norvasc and Norlopin tablet were carefully weighed and ground to finely divided powders. Accurate weights equivalent to 5 mg ADB was dissolved in 100 mL ethanol-acetonitrile (30/70, v/v) solvent mixture, mixed well. Solution was stand for about 10-15 min and filtered up using 12 mm filter paper and then the solution volume was made up to the 250 mL with same solvent mixture**.** The final concentration of these solutions was 20 μg/mL.

2.4. Method Validation

The validation of method was carried out by establishing specifity, linearity, recovery values, limits of detection (LOD), limit of quantification, within- and between-day precision and accuracy according to International Conference on Harmonization guidelines (ICH) [35] for validation of analytical procedures.

3. RESULTS AND DISCUSSION

3.1. Method Development

To develop a sensitive the UV spectrophotometric method, various solvent systems were tried, such as water, methanol, ethanol and acetonitrile individually or in combinations of different proportions. The final decision of

using ethanol-acetonitrile (30/70, v/v) mixture was based on sensitivity, interference, easy of preparation, suitability for drugs, content estimation and cost in that order. Methanol-asetonitrile mixture can be used. But, methanol was abandoned because of its toxicity.

The standard solutions were scanned wavelength range of 250-450 nm in the 2 nm band width against a similarly prepared blank in spectrophotometer. The λ_{max} was found to be 360 nm. This wavelength was used for all measurements. The UV absorption spectrums of ADB were monitored: the one broad shouldered peak with maximum wavelengths at 360 nm in ethanol-acetonitrile (30/70, v/v) solvent medium was observed as shown in Fig. 2 and 3. However, these shouldered peaks were not appeared when the bandwidth was chosen as 5 nm and it was monitored a single well-defined maximum peak in UV spectrums. These maximum wavelengths were broader at low concentrations so that analysis couldn't be performed; at higher concentrations the peaks were sharper but analysis couldn't be carried out because of the shoulder.

3.2. Linearity

Linearity of the assay was demonstrated over concentration range of 2 to 17 µg/mL ADB in six replicate at separate concentrations. The linearity was evaluated by linear regression analysis, which was calculated by least square regression method. Linear range was determined by plotting the absorbance at its λ_{max} versus sample concentration. The regression equation of calibration curve and regression coefficient (r) for standard samples were found as A=0.0122x+0.0298 and 0.999, respectively (Fig.2.).

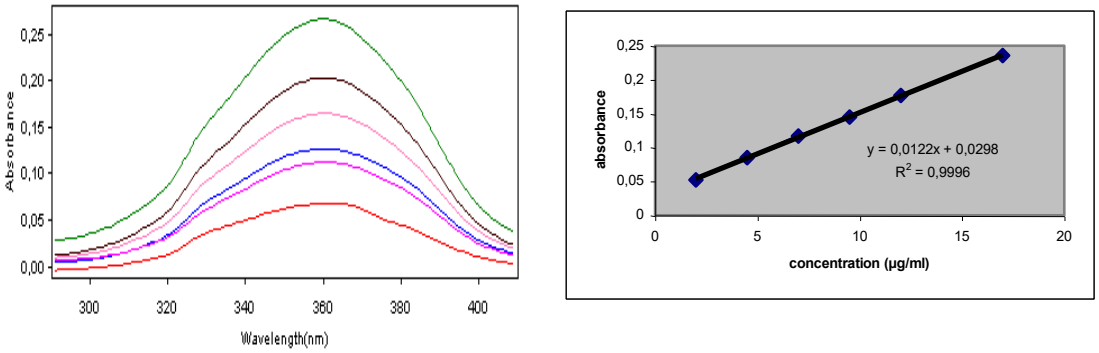

Figure 2. UV Spectrums and calibration curve of standard solutions of ADB

3.3. Limit of Detection and Quantification

Spectrophotometrically, LOD and LOQ were determined by an empirical method that consisted of analyzing a series of standard solutions containing decreasing amounts of ADB. This method, although not applicable for complex matrices, is useful for simple samples. The LOQ was defined as the lowest concentration on the calibration curve that presented a RSD that did not exceed 10% and the LOD was defined as the lowest concentration that presented a RSD that did not exceed 20%. LOQ and LOD values for standard samples were approximately deemed to be 2 µg/mL and 1.5 µg/mL, respectively.

3.4. Precision and Accuracy

Repeatability was evaluated by assaying samples, at same concentration and during the same day. Assay precision and accuracy were assessed by assaying three quality control samples (5.75, 10.75 and 14.50 µg/mL) in six replicate on one day for within-day precision and once daily for six days for between-day precision. Concentrations of ADB in quality control samples were determined by application of the appropriate standard curve obtained on that occasion. The within-day and between-day relative standard deviation (RSD) were <2% for standard samples (n=6). Precision studies of UV spectrophotometric method showed acceptable RSD values. The accuracy of this analytic method for assay determination was checked for quality control samples and the relative errors for accuracy were < 6 % (Table 1).

Table 1. Precision and accuracy of the method for determination of ADB (n=6)

Added (µg/mL)	Within-day			Between-day		
	Found±SD[a] (µg/mL)	Precision RSD %	Accuracy[c] RE%	Found±SD[a] (µg/mL)	Precision RSD%	Accuracy RE%
5.75	5.91±0.15	1.96	2.85	6.08±0.14	1.82	5.65
10.75	10.68±0.13	1.04	-0.65	10.80±0.20	1.57	0.45
14.50	14.17±0.21	1.30	-2.24	14.41±0.14	0.88	-0.63

SD[a]: Standard deviation of six replicate determinations, RSD: % Relative standard deviation
Accuracy[c]: %Relative Error

3.5. Recovery

The accuracy was determined by recovery of known amounts of ADB reference standard added the tablet samples at the beginning of the process. For recovery study, the tablet solutions according to the procedure described at Section *2.4.* was prepared. The tablet solutions to 2.5, 5.0 and 7.5 µg/mL concentrations were transferred in ADB standard solution to 7.5 µg/mL concentration. The final concentrations of these solutions were 10, 12.5 and 15 µg/mL. The absorbance of solutions prepared was measured with spectrophotometer. The percentage recovery of added ADB standard was calculated by comparing the found and added concentrations (C_{found}/C_{added}x100) in each case. The mean recoveries and the RSD values for these recovery values were found ranged 99.0- 110.0% and 1.0-2.0%, respectively. No interference from the common excipients was observed. The results showed in Table 2.

Table 2. Recovery values of ADB in pharmaceutical preparations (n=6)

Formulation name	Taken (μg/mL)	Added (μg/mL)	Found±SD (μg/mL)	Recovery±RSD (%)
Norlopin-5 mg	2.50	7.5	9.93±0.10	99.3±1.00
	5.00	7.5	12.54±0.15	100.3±1.19
	7.50	7.5	16.27±0.28	108.5±1.72
Norvasc-5 mg	2.50	7.5	9.97±0.18	99.7±1.80
	5.00	7.5	12.61±0.14	100.9±1.11
	7.50	7.5	15.04±0.18	100.3±1.19

SD: Standard deviation of six replicate determinations, RSD: Relative standard

3.6. Stability

To determine of the stability of ADB standard solutions at ambient and refrigeration temperature, ADB solutions to 4.5, 12 and 16 μg/mL concentrations and stock solution were stored at ambient, 4°C and deepfreeze for 24 h and 1 week. Then the stability measurements were carried out. The results were evaluated comparing these measurements with those of standards and expressed as percentage deviation. The results indicated that the stock solution and solutions to 4.5, 12 and 16 μg/mL concentrations were found to be stable after one week with no significant change in concentration.

3.7. Interferences study

The effects of common excipients and additives were tested for their possible interferences in the assay of ADB. In addition to the active ingredient, amlodipine besylate, each tablet contains the following inactive ingredients: microcristaline cellulose, dibasic calcium phosphate anhydrous, sodium starch glycolate and magnesium stearate. It has been determined any interference of these substances at the levels found in dosage forms.

3.8. Comparison of Proposed Method with Two Reference Methods

The suggested UV spectrophotometric method was applied to the quantitative determination of ADB in tablet dosage forms. Also the developed and validated method was statistically compared with two reference methods in literature [12, 18]. One of reference methods is HPLC method (I reference method) and other is indirect spectrophotometric method (II reference method). In the indirect spectrophotometric method, ADB had been reacted with bromate-bromide mixture in HCl medium and then the unreacted bromine was measured absorbance with spectrophotometer.

The proposed method was linear over the concentration range 2-17 μg/mL. The I and II reference method had been found as linear over the concentration range 7.55-241.6 μg/mL and 1.25-12.5 μg/mL, respectively. The average recovery value for ADB in 5 mg tablet composites ranged to 98.0- 100.0% and the RSD (Table 3) values obtained within- and between-day assay of quality control samples ranged from 0.5 to 2.0% for proposed method, which indicated high accuracy and precision.

Table 3: Statistical comparison (Student *t*-test and F-test) of two reference methods and proposed method

	Method	n	X	RSD (%)	Std. Error Mean	*t*-values	P-Values
First comparision	Proposed method	10	101.36	2.2498	1.2989	-2.149	0.098
	HPLC	10	97.90	1.6573	0.9568		
Second comparision	Proposed method	10	101.37	2.2498	1.2989	0.016	0.988
	Metod C	10	101.39	1.7128	0.9888		

n: Number of determination, [a]X: mean, RSD: Relative Standard Deviation, t_c: Calculated t values
H_o: Hypothesis: no statically significant difference exists between two methods $t_t > t_c$; H_o hypothesis in accepted (α=0.05)

In I and II reference method**,** the average recovery value had been ranged to 97.58-99.76% and 100.0-101.0% for ADB in 5 mg tablet composites, respectively, and also the RSD values had been ranged from 0.85 to 1.04% and from 0.5 to 1.26%, respectively. The results obtained were compared statistically by Student's *t*-test (for accuracy) and variance *F*-test (for precision) with reference methods [12, 18] at 95 % confidence level with five degrees of freedom**.** The results showed that the P-values were higher than P=0.05 indicating that there was no significant difference between the proposed and reference methods (Table 4).

Table 4: Comparision of the proposed method with existing spectrophotometric methods for the determination of ADB in pharmaceutical formulations

Reagent	λ_{max} nm	Range μg mL^{-1}	RSD %	Reference
Bromothymol Blue[a]	405.0	5-40	0.99	28
MBTH[b]	630.0	5-40	0.87	28
Eriochrome Black T[a]	495.0	5-50	0.88	22
Indigocamine[a]	590.0	25-150	0.87	22
Rhodizonic acid[a]	450.0	100-1500	-	20
p-Chloranilic acid	540.0	100-600	0.52	26
Sodium hydroxide	465.0	20-100	1.90	29
Underivatized ADB	360.0	2-17	0.5-2.0	This work

[a]Extractive method, [b] 3-Methyl-2-benzothiazolinone hydrazone hydrochloride

Also, the newly proposed method for the determination of ADB in pharmaceutical preparations compared favorably with other spectrophotometric methods used derivatized (Table 4). It was found that method has advantages of high performance (RSD <2 % for pure samples and dosage forms) and fast response. Therefore can be used applied as an alternative to the existing methods.

4. CONCLUSIONS

Today, HPLC method with different detection and spectrophotometric methods are widely used as analytical techniques for determination of ADP in tablet dosage forms. However, the determination of ADP reacted with different

substances in the most of these methods has been made. For this, the UV spectrophotometric method were developed and completely validated for quantitative determination of underivatized ADB in tablet dosage forms in this study and also the results show good recoveries of ADB from the spiked tablet samples, without any interference from the excipients. The proposed method are rapid, simple, accurate, reproducibility and convenient since it do not require any special working conditions. For this reasons, it can be used for determination from pharmaceutical preparations of ADB in routine quality control measurement, where economy and time are essential.

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Conflict of Interest

Author has no personal financial or non-financial interests.

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Research Article

Genotoxic Effects of Ceramic Materials and the DNA Damage in Ceramic Workers

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ABSTRACT:
The ceramic sector is an important industrial activity around the world. Ceramic workers are potentially exposed to a wide range of chemical mixtures mainly silica. Occupational exposure to silica dust is still considered to be a health problem in the ceramic industry, especially in developing countries. On the other hand, apart from silica, ceramic workers can expose to many hazardous and genotoxic chemicals such as metals and metal oxides, polycyclic aromatic hydrocarbons (PAHs), and nanoparticles. This review aims to summarize data retrieved from studies about genotoxic effects in ceramic workers and other studies about the genotoxic effects of ceramic materials mainly silica. Overall, the data in this review confirm that increased DNA damage in the different cells of ceramic workers demonstrates the possibility of health risks in the individuals exposed to ceramic materials.

Keywords : Ceramic, DNA damage, genotoxicity, silica, workers

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1. INTRODUCTION

The ceramic sector that consists of two types of main processes, one the manufacture of tiles and the other, the supply of raw materials that offers a wide range of materials with great impact on our daily lives, is an important industrial activity around the world. Briefly, a ceramic material can be described as an inorganic, heat-resistant material composed of both metallic and non-metallic

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compounds. Due to their durability, strength, non-corrosive properties, and ability to stand at very high temperatures, ceramics are also employed for specific uses which are required in metallurgical processes, glass production, and many other key processes across all industries [1].

Ceramic workers are potentially exposed to a wide range of chemical mixtures including silica, lime, heavy metals, and polycyclic aromatic hydrocarbons (PAHs) [2]. Silicon (Si) is the second most common element after oxygen which is the main constituent of silica (i.e. silicon dioxide), therefore apart from the ceramic industry, exposure to silica dust can occur in a large variety of occupations, such as miners, brick, pottery, sand, foundry, and construction workers [3, 4]. Especially in the production of heat-resistant bricks, materials with a high content of crystalline silica (quartz) are used. The stone, consisting of 100% quartz, is produced by adding clay to the production of pottery and bathroom materials. Sand can also be used for dusting, which increases the crystalline silica concentration in the air. High mixing of 0.1 to 3 mg/m^3 of crystalline silica is involved in mixing, molding, and glazing. Occupational exposure to crystalline silica dust is still considered to be a health problem, especially in developing countries. It is estimated that at least two to three million workers worldwide are occupationally exposed to crystalline silica annually [5, 6]. Inhalation of crystalline silica can lead to silicosis and cancers. Respirable crystalline silica inhaled from occupational sources has been classified as a human carcinogen (Group 1) in 1997 by the International Agency for Research on Cancer (IARC) [7] which was confirmed in a later review in 2012 [8].

Metals and metal oxides are also commonly used in the ceramic industry which has been associated with carcinogenicity. In southeastern China, increased cadmium (Cd) levels in the soil were reported in a town that is quite popular for its ceramics [9]. Another study showed higher concentrations of Cd and chromium (Cr) in *Tradescantia pallida* (T. pallida) leaves in Monte Carmelo, Brazil that has been considered a national reference source for roof tile production. In that area, the micronucleus (MN) frequency was significantly higher in *T. pallida* leaves exposed to the ceramic industry emissions [10]. Beryllium (Be), barium (Ba), zinc (Zn), aluminum (Al), potassium (K), calcium (Ca), sodium (Na), and iron (Fe) were the most abundant elements found in the emissions from the ceramic industries [11]. Also, the use of glazed ceramic dishes can be a risk for lead (Pb) toxicity. IARC has classified Cd, Be, Cr, and Ni as carcinogenic to humans (Group 1) [12, 13].

Nanoparticles (NPs) such as graphene, carbon nanotubes, and carbon black are also used in the ceramic industry for their reinforcing ability [14]. Titanium (TiO_2) NPs are used for the ceramic glaze, in tiles, or as stiffening fillers due to their high stability, anticorrosive and photocatalytic properties [15]. Still, IARC has classified bulk TiO_2 as possibly carcinogenic to humans (group 2B) which raised concerns about the genotoxic potential of TiO_2 in the nanoform [16].

Alumina (Al_2O_3) NPs are used for making cutting tools and are often included as polishing agents [17]. Copper (CuO) and nickel (NiO) oxide NPs can also be used in the ceramic industry incorporated in inks for surface coating treatments. CuO NPs increased DNA damage in lung cell lines, most of them showing a decrease in cell viability, and cause oxidative stress [18, 19]. Special attention should be given to NiO NPs considering that Ni compounds are classified as carcinogenic to humans (Group 1) [13]. In addition to these, the use of nanosized silica in the ceramic industry has the potential to increase in recent years [20].

Molecular epidemiology is an approach commonly used in the evaluation of associations between exposure to hazardous substances and the development of diseases. Genotoxicity biomarkers can allow the detection of early effects that result from the interaction between the individual and the environment; therefore they are important tools in cancer epidemiology and are extensively used in human biomonitoring studies [21, 22].

This review aims to provide current knowledge about the genotoxic effects in ceramic workers.

2. Data Collection

Information about genotoxic effects in ceramic workers was obtained from a literature search of electronic databases such as ScienceDirect, Google Scholar, Pubmed, and Scopus. "ceramic workers", "DNA damage", ''ceramic materials'', "silica", "quartz", ''pottery workers'' and ''tile factory workers'' were used as keywords to identify epidemiological studies published in the literature. The reference list of the studies is also screened for potentially eligible studies. These were then filtered depending on whether the abstract contained any genotoxicity data in workers related to ceramic materials or silica. In many papers silica has been used as a positive control in the evaluation of the genotoxicity of other particles which were considered irrelevant.

3. Evaluation of Genotoxicity

Research about the genotoxic effects of ceramic materials is mostly focused on crystalline silica genotoxicity in animals and cell lines [23]. Epidemiological studies with ceramic workers are insufficient. In these studies, the genotoxic effects of ceramic materials were generally evaluated by the comet, micronucleus (MN), and sister chromatid exchange (SCE) assays. In a study with 45 non-smoking female workers working in mines and quarries in India and 20 age-matched controls, genotoxic effects were assessed in blood and buccal epithelial cells by comet and MN assays respectively. DNA damage in both cells was significantly increased in workers compared to the control group. It has also been reported that many of these workers suffer from cough, diarrhea, cold, headache, and fever [24]. In another study with 50 male pottery workers in India, it was shown that chromosomal abnormalities, frequency of MN, DNA damage were found to be higher in the workers compared to the controls [25].

In the studies conducted with pottery and foundry workers in Turkey, significantly increased DNA damage was shown by comet assay [26]. In another study from Turkey, genotoxic effects due to occupational silica exposure in workers working in different ceramic industries, such as glass, sandblasting, and stone crushing, were evaluated by MN assay in blood and nasal epithelium cells. The frequency of MN in blood was shown to be 2 times higher in workers than in the control group and the frequency of MN in nasal epithelial cells was 3 times higher in the workers than in the control group. Moreover, 24% of these workers were found to have early radiographical changes (profusion category of 1) [27]. In another study with Turkish ceramic workers (n=99), genotoxic damage was shown in isolated lymphocytes and whole blood by alkaline comet and in buccal cells by MN assays. Plasma 8-oxo-7,8-dihydro-2′-deoxyguanosine (8-oxodG) levels, as an indicator of oxidative DNA damage, and binucleated (BN), pyknotic (PYC), condensed chromatin (CC), karyolytic (KYL), karyorrhectic (KHC) and nuclear bud (NBUD) frequencies in buccal epithelial cells were evaluated. MN, CC + KHC, PYC frequencies in buccal epithelial cells, the tail intensity in blood cells, and 8-oxodG levels in plasma were increased in workers compared to their controls [28].

Malondialdehyde (MDA), a natural product of lipid peroxidation, is an aldehyde capable of interacting with DNA to form exocyclic DNA adducts, including 3-(2-deoxy-β-D-erythro-pentafuranosyl)pyrimido[1,2-α]purin-10(3H)-one deoxyguanosine (M1dG) adduct. M1dG adduct causes base pair and frameshift mutations in reiterated sequences which may be associated with increased cancer risk. Peluso et al. examined the levels of M1dG adducts by mass spectrometry in nasal epithelial cells of 135 workers working in the quarry, pottery, ceramic, and marble production and 118 controls in Tuscany, Italy. The results of this study demonstrated that M1dG adduct levels were significantly higher in smokers than non-smokers. It was also observed that the level of M1dG adducts was increased in the quarry and marble workers, but no significant increase was seen in the ceramic and pottery workers [29].

In another study with industrial granite workers, who were either stonecutters, carvers or were in other professions with significant silica exposure i.e. miners, stonemasons, and foundry workers but not diagnosed with silicosis, and 42 retired workers with silicosis, 8-oxodG levels were determined in the blood and urine samples by high-performance liquid chromatography (HPLC). There was no significant difference in 8-oxodG levels between the groups. Besides, no relationship was found between 8-OHdG levels and duration of exposure, smoking, or age [30].

Kasuba et al. have investigated the genotoxic effects of occupational exposure to lead acetate in pottery-glaze ceramic workers (n=30) using alkaline comet, DNA diffusion, and MN assays in peripheral blood lymphocytes. They showed

increased values of tail intensity (TI), frequency of apoptotic and necrotic cells, and frequency of MN in workers compared to controls [31].

Genotoxic effects of ceramic materials in the environment of ceramic factories are also reported. A recent study by Karla da Silva et al. demonstrated the genotoxic effects of particulate materials emitted from the ceramic industry in the meiotic pollen cells of *T. pallida* in Italy *although* the concentrations of particulate materials were lower than the established limits of the World Health Organization (WHO) [32]. Similarly, Campos et al. showed that MN frequency was significantly higher in *T. pallida* plants exposed to the ceramic industry emissions in Brasil [10].

4. Cancer Incidence

In addition to respiratory diseases, silica exposure in ceramic industries can increase the risk of cancer development. Crystalline silica is classified as a human carcinogen (Group 1) by IARC [8]. On the other hand, amorphous silica is in the group that cannot be classified in terms of its carcinogenic effect in humans (Group 3) [7].

To demonstrate the relationship between crystalline silica exposure and lung cancer, a multicentre study in 7 European countries between 1998-2002 with 2952 newly diagnosed lung cancer patients and 3104 control groups was conducted. As a result of the study, it was demonstrated that exposure to silica is an important risk factor for lung cancer. This risk cannot be explained by exposure to other occupational carcinogens or smoking, and also this risk exists in all major histological types of lung cancer [33]. A recent meta-analysis study of Poinen-Rughooputh et al. which combines the results of 85 different studies, supported the carcinogenic role of silica on the lungs, which was more pronounced at higher levels of exposure, in the presence of silicosis [23]. The increased risk for lung cancer and mortality from respiratory diseases was also reported in the Swedish porcelain factory [34].

In a retrospective cohort study, Zhang et al. investigated the health impacts of crystalline silica mixed dust and other potential occupational hazards on workers in ceramic factories. They identified the employment records of 4851 workers registered in three ceramic factories in Jingdezhen city of China between 1972 and 1974. The findings indicated that silica mixed dust in ceramic factories has detrimental effects on health and life span in ceramic factory workers [35].

5. Conclusion

Overall, it was observed that exposure to ceramic materials can increase the DNA damage both in ceramic workers and other experimental systems. MN test was the most used assay in these studies and DNA damage was observed even if the silica concentrations below the limits. There is no known safe level of exposure to carcinogens therefore ceramic workers and other workers exposed to silica should be examined in detail to avoid the development of a carcinogenic process. Specific ventilation practices, lowering the limit values as well as using suitable

protective equipment should be recommended to reduce the exposure and to prevent toxic effects.

Conflict of Interest

Author has no personal financial or non-financial interests.

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Review

Combination of Alginate and Chitosan Polymers in the Preparation of Nanoparticles-A Mini Review

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ABSTRACT:
Natural polymers, commonly include polysaccharides (chitin/chitosan, hyaluronic acid derivatives, alginate, etc.) and proteins (albumin, collagen, etc.), are non-toxic and biocompatible biomaterials widely used in various biomedical applications (such as drug and/or imaging agent delivery system, tissue regeneration scaffolds, as excipients in pharmaceutical formulations). Especially, alginate and chitosan are widely used for the preparation of drug delivery systems. Unfortunately, the primary drawback of both alginate and chitosan is the lack of strong mechanical properties. However, using the combination of these polymers can enhance their mechanical properties. Besides, the alginate-chitosan nanoparticles had higher drug encapsulation and a slower drug release due to the polyelectrolyte complex structure formed by the interaction between chitosan and alginate.

Keywords: Alginate, chitosan, nanoparticles, polyelectrolyte complex

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1. INTRODUCTION

Polymers can be classified according to their origin (natural or synthetic), function, source (from plants, animals, and microbial sources), polymerization mechanism, polymer structure, preparation techniques, or thermal behavior [1,2]. Natural polymers, commonly include polysaccharides (chitin/chitosan, hyaluronic acid derivatives, alginate, etc.) and proteins (albumin, collagen, etc.), are non-toxic and biocompatible biomaterials widely used in various biomedical applications (such as drug and/or imaging agent delivery system, tissue regeneration scaffolds, as excipients in pharmaceutical formulations) [3,4]. Chitosan is a cationic polysaccharide polymer while alginate is an anionic polysaccharide polymer. Chitosan is obtained by the alkaline deacetylation of chitin that is found in the cells walls of fungi, the exoskeletons of crustacean and insects [5-8].

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Alginates are the cell-wall constituents of marine brown seaweed or algae, and extracellularly in several bacteria. Alginates are produced mainly from *Macrocystis pyrifera, Laminaria hyperborea, Laminaria digitata, Ascophyllum nodosum* spp. etc. [6-9]. Alginate is a copolymer composed of mannuronic acid and guluronic acid, and it forms gel in the presence of divalent cations (such as Mg2+, Ca2+) by cross-linking the carboxylate groups of the guluronate groups at room temperature [8, 10,11]. The molecular weight of alginates ranges from 20 to 600 kDa. Alginate, which is non-toxic and biocompatible, is also widely used in the preparation of different drug delivery systems (nanoparticles, beads, microparticles, etc.) for various drug administration routes [6, 8, 12]. Sodium alginate, which is the salt of alginic acid, is the major form now widely used [7]. Due to the lack of an enzyme (alginase), alginate is not degraded inherently in mammals. Therefore, partial oxidation of alginate chains is seen as an attractive approach to render alginate degradable under physiological conditions. Slightly oxidized alginate can be degradable in aqueous media and the alginates have potential for the preparation of drug delivery systems. Also, partial oxidation of the alginate does not significantly affect its gel-forming ability in the presence of divalent cations. The rate of degradation of the gels depends extremely on the degree of oxidation as well as the temperature and pH of the medium [13].

Chitosan is composed of N-acetyl glucosamine and D-glucosamine (deacetylated units) linked by beta-(1,4) glycosidic bonds [14]. The degree of deacetylation and molecular weight of chitosan, which are very important in terms of its properties, depend on its source and production process [6]. Chitosan has a large molecular weight range and thus, it shows different properties (blood thinning, cholesterol-lowering, anti-oxidant, and antibacterial properties, drug delivery efficiency, permeation resistance, pollutant removal properties etc.) [15,16]. The biodegradability of chitin and chitosan is attributed to their sensitivity to enzymatic hydrolysis by colonic bacterial enzyme and lysozyme in human body. Lysozyme found in human tissues break the linkage between acetylated units and reduce chitosan/chitin to oligosaccharides [17-19]. Due to its biodegradable, non-immunogenic and non-toxic properties, chitosan and its derivatives have been widely used for the preparation of the different drug delivery systems (microparticles, nanoparticles, oral prolonged-release drugs, beads etc.) for the different application routes such as oral, nasal, parenteral, ocular, transdermal [14,19].

The primary drawback of both alginate and chitosan is the lack of strong mechanical properties. However, using the combination of these polymers can enhance their mechanical properties. Also, the alginate can be combined with chitosan to improve cargo protection and absorption in oral drug delivery [6,8].

Nanotechnology is an encouraging approach in the development of drug delivery systems that contain drugs with poor solubility, low absorption/permeability, low

bioavailability and other poor biopharmaceutical properties. Drug design at nanoscale has been studied extensively and nanoparticle applications are the most advanced technology in this field. In the diagnosis and/or treatment of various diseases, nanoparticulate delivery systems provide many benefits such as improving the solubility and bioavailability of drug that is poor-soluble in water, providing controlled/sustained drug release, fewer side effects, lower toxicity, site-specific delivery (targeting) of drug and/or imaging agents. Polymeric nanoparticles made from synthetic (biodegradable and non-biodegradable) and natural polymers [20-22]. Chitosan is used in the encapsulation of drug or coating of different types of nanoparticles. It has mucoadhesive properties and also is thought to disrupt intercellular tight junctions. Besides, alginate, which has carboxyl groups, is also classified as an anionic mucoadhesive polymer used for the preparation of nanoparticles [21]. The ability of alginate to form hydrogel by chelating with divalent cations enables the alginate to be widely used in the pharmaceutical field. Easy-gelling property is very useful to prepare polyelectrolyte complexes. The polyelectrolyte complex of alginate and chitosan is formed by the ionic interactions through ionic gelation between the carboxylic group of alginate and the amine group of chitosan. The interactions between these two polymers reduce the porosity of the complex. Thus, the resulting complex preserves the encapsulated drug, prevents the premature leakage/release of the drug, and effectively slows the drug release compared to drug release observed when using alginate or chitosan. The alginate network is stabilized at high pH by chitosan (it is less soluble at high pH), while the high solubility of chitosan at acidic pH is reduced by the poor solubility of the alginate network at acidic pH [23].

1.1. The alginate-chitosan nanoparticles/polyelectrolyte complex in literature

Alginate nanoparticles are usually prepared by ionotropic gelation using cationic polymers (such as chitosan) or divalent cations. Mujtaba et al. [24] prepared rosuvastatin calcium (antilipidemic drug)-containing alginate-chitosan nanoparticles to improve the solubility, dissolution, and therapeutic efficacy of the drug. Because rosuvastatin calcium has low solubility in water and poor oral bioavailability. For the preparation of nanoparticles, ionotropic pre-gelation of alginate with calcium chloride as a cross-linking agent and then polyelectrolyte complexation with chitosan was performed. The mean particle size of rosuvastatin calcium-containing alginate-chitosan nanoparticles was 349.3 nm, and the nanoparticles had positive zeta potential of 29.1 mV, and high drug encapsulation efficiency (83.65%). While a rapid drug release was obtained from the nanoparticles within the first 2 hours, a more gradual and continuous drug release was observed for the following 24 hours in phosphate buffered saline solution (PBS)-pH 7.4. Furthermore, they performed an FT-IR analysis to investigate potential interactions and reported that the carboxylic groups of the alginate interacted with the amine groups of chitosan via electrostatic interactions to form

the polyelectrolyte complex. It has been stated that the addition of chitosan for structural support to the dispersion containing calcium chloride, alginate, and drug may be beneficial in terms of achieving high encapsulation efficiency and sustained drug release [24].

In another study, doxorubicin (anti-cancer drug)-containing chitosan-alginate nanoparticles were prepared using modified ionic gelation method [23]. In this method, alginate as a crosslinker and Tween 80 as a surfactant were used. During the preparation of doxorubicin loaded-chitosan-alginate nanoparticles, firstly, chitosan particle formation was achieved by mixing the solution of chitosan in acetic acid with Tween 80 and later, this solution was added to the dispersion of alginate-doxorubisin complex by dropping and mixed to form nanoparticles. The formation of polyelectrolyte complex was achieved by ionic cross-linking (between the amine groups of chitosan and the carboxylic groups of alginate). Doxorubicin-loaded chitosan-alginate nanoparticles had positive zeta potential (about +35 mV), a mean particle size of 100 nm, and very high encapsulation efficiency (95%). It was observed that after an initial burst doxorubicin release (up to 24 hours), chitosan-alginate nanoparticles presented a more gradual and sustained doxorubicin release for the following 72 hours in PBS-pH-7.4 [23].

Also, paclitaxel (anti-cancer drug)-containing alginate or alginate-chitosan or folate-chitosan–alginate (for targeting) nanoparticles were developed in another study [25]. They used the double emulsion cross-linking method for the preparation of the nanoparticles. In this method, CS was used for the electrostatic interaction with alginate and it was also regarded as a cross-linking agent [25]. Briefly, both alginate aqueous solution with Tween 80 and the solution of paclitaxel in dichloromethane were added into soybean oil for the formation of O/W/O emulsion by ultrasonic emulsification. On the other hand, calcium chloride and chitosan aqueous solution was added into soybean oil for the formation of W/O emulsion by ultrasonic emulsification. Later, the W/O emulsion was dropped into O/W/O emulsion and mixed for cross-linking (at 35 °C) for 4 h. The authors reported that paclitaxel-loaded-chitosan-alginate nanoparticles had a positive zeta potential value (31.1 mV), higher encapsulation efficiency (26.13%), and drug loading capacity (10.19%) compared to paclitaxel-loaded alginate nanoparticles [zeta potential: (-)26.5 mV; encapsulation efficiency: 19.66%; drug loading capacity: 8.92%]. With the addition of chitosan to the formulation, the average particle size of the nanoparticles increased from about 201 nm to about 307 nm. They also stated that the release of paclitaxel from all nanoparticle formulations took place in two stages (initial burst release followed by sustained release), however, the release of paclitaxel from chitosan-alginate nanoparticles was slightly slower than the drug release from alginate nanoparticles [25].

In another study, paclitaxel and doxorubicin-loaded alginate coated chitosan hollow nanospheres were prepared by a hard template method [26]. In this

method, the authors added the solution of chitosan in acetic acid to the suspension of sulfonated polystyrene (SPS) nanospheres used as a template. After mixing and centrifugation, the obtained product was suspended in the solution of sodium alginate and centrifuged. The final product was suspended slowly in glutaraldehyde solution and the cross-linking process was carried out for 2 h. Then, the SPS template was removed by adding of tetrahydrofuran and alginate coated chitosan hollow nanospheres were obtained. Paclitaxel and positively charged doxorubicin were loaded to nanospheres by an adsorption method and by electrostatic interaction, respectively. It was found that the drug content in the nanospheres was about 18% for paclitaxel and about 74% for doxorubicin. The authors explained that the high doxorubicin content was due to the electrostatic interaction between positively charged doxorubicin and negatively charged alginate. Also, a sustained drug release from the nanospheres was observed for both drugs [26].

On the other hand, chitosan-alginate nanoparticles loaded with nifedipine used to treat increased blood pressure were prepared by Li et al. [27]. The nanoparticles were obtained by ionotropic pre-gelation of an alginate core with calcium chloride followed by the addition of chitosan solution to form a polyelectrolyte complex. In this method, guluronic acid units on alginate react with Ca^{+2} to form "egg-box" structure (Ca^{+2}-alginate complex in pre-gel state). It has been stated that the pre-gel state is essential to allow the ionic interactions between alginate-Ca^{+2} complex and chitosan for the formation of nanoparticles. The particle sizes of the prepared nanoparticles were in the range of 20-50 nm. In various release media (phosphate buffer solution-pH 7.4, simulated intestinal fluid-pH 6.8, and simulated gastric fluid-pH 1.5), the release profile of nifedipine from nanoparticles was characterized by an initial burst release for the first two hours followed by a sustained drug release for 22 hours [27].

Thai et al. [28] prepared lovastatin (a cholesterol-lowering agent)-loaded alginate-chitosan nanoparticles by the ionic gelation method. They added calcium chloride to alginate aqueous solution and later, the obtained mixture was dropwise to chitosan solution in acetic acid, and finally, lovastatin solution in ethanol was added to the mixture and ultrasonicated to obtain lovastatin-loaded alginate-chitosan nanoparticles. They obtained the nanoparticles with the particle size in the range of 50–100 nm and observed that lovastatin release from the nanoparticles took place in two stages (initial burst release followed by slow release) [28].

In a study, the biodegradation of chitosan–alginate polyelectrolyte complexes was investigated by incubation in the solution of lysozyme, which was used for enzymatic degradation. It was reported that the polyelectrolyte complexes showed a low degradation rate due to the steric hindrance that was a result of the strong interaction between alginate and chitosan, and also that the complexes partially degraded by hydrolysis [29].

2. CONCLUSION

As a result, it has been shown in the mentioned above articles that the alginate-chitosan nanoparticles had higher drug encapsulation and a slower drug release due to the polyelectrolyte complex structure (providing strong mechanical properties) formed by the interaction between chitosan and alginate. The alginate-chitosan nanoparticles not only protect drugs but also facilitate to obtain the sustained drug release. Chitosan and alginate are natural, non-toxic, biocompatible, and oppositely charged polyelectrolyte polymers. In addition to these advantages, these polymers in single/combination are very promising biopolymers used widely in the preparation of drug delivery systems due to their low processing costs and abundance in nature.

Conflict of Interest

Author has no personal financial or non-financial interests.

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Review

Adipose Tissue As an Endocrine Organ: A Perspective From Adiponectine and Irisin

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ABSTRACT:
In recent years, adipose tissue has been defined as a new endocrine organ via its paracrine, autocrine and endocrine effects. With the secreted hormones and cytokines it plays a major role in the regulation and integration of metabolism. The metabolic pathways involving these hormones and cytokines including insulin sensitivity show a connection with the pathogenesis of obesity, type II diabetes mellitus, metabolic syndrome, inflammatory diseases, many chronic diseases and even cancer. Adipokines or adipocytokines are cytokines secreted by adipose tissue. In this mini-review, information about two of the most important adipokines, adiponectin and irisin, is given and their important effects are presented to the attention of the reader.

Keywords: Adiponectin, adipose tissue, endocrine, irisin

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1. INTRODUCTION

Adipose tissue is a tissue in which lipids are stored for use as an energy source. There are two types of adipose tissue in mammals and humans. White adipose tissue and brown adipose tissue. Fatty acids formed by lipogenesis in the presence of insulin are stored as triglycerides in the white adipose tissue, and triglycerides are broken down by lipolysis in the presence of glucagon and the released fatty acids are used as an energy source. The second type of adipose tissue, brown adipose tissue, is specialized for thermogenesis and appears brown due to the large number of mitochondria it contains. Thanks to the very high number of mitochondria they have, they provide more energy production, more calorie consumption and heat production [1]. The conversion of white adipose tissue to brown adipose tissue (browning) plays a key role in the prevention of metabolic diseases, especially obesity. The most important factor that provides browning is exercise [2].

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Adipose tissue acts as an endocrine organ in addition to its fat storage and metabolic functions. Adipose tissue is an endocrine organ that transmits soluble signals called locally and systemically acting adipokines. It also communicates with the cells of the immune system [3]. Many mediators (enzymes, cytokines, growth factors) secreted from adipose tissue play an active role in the regulation of appetite, insulin resistance, inflammation, and atherosclerosis processes. Some of these mediators are leptin, TNFα, adipsin, IL-6, plasminogen activator inhibitor, visfatin, adipsin, apelin, angiotensinogen, metallothionine, and resistin [4].

During the obesity-insulin resistance-metabolic syndrome connection process, the normal functions of adipocytes are impaired, and synthesis of proinflammatory cytokines secreted from adipocytes increases and synthesis of anti-inflammatory cytokines decreases. This increase in inflammation contributes to the formation of diabetes. Among the mediators secreted from adipose tissue, the most important hormone that increases insulin sensitivity is adiponectin. Adiponectin is a hormone that enhances fatty acid oxidation and insulin sensitivity [5]. Adiponectin reduces hepatic glucose production and potentiates the effects of insulin in the liver, thereby increasing insulin sensitivity [6]. Similarly, in animal models, adiponectin has been shown to increase hepatic insulin sensitivity [7]. It is known that adiponectin levels are decreased in obesity, type II diabetes and metabolic syndrome, and adiponectin may have a very important role in these diseases. Obese people have decreased adiponectin levels. As insulin resistance increases, adiponectin levels decrease [8]. It has been reported in the literature that the risk of developing type 2 diabetes in individuals with high adiponectin levels is lower than those with low adiponectin levels. The risk of developing type 2 diabetes in individuals with high adiponectin levels is lower than those with low adiponectin levels [9].

The relationship of TNFα and IL-6 with insulin resistance is thought to be mediated through adiponectin [10]. Adiponectin, which increases insulin sensitivity and fatty acid oxidation, also has a regulatory effect on inflammation by reducing TNFα and IL-6 levels [11]. Insufficient efficacy of adiponectin in obese individuals is one of the factors responsible for the increase in circulating TNF α and IL-6 levels. One mechanism of activating inflammatory pathways in diabetes is decreased adiponectin levels [12]. In conclusion, besides its anti-inflammatory effect, adiponectin shows antiobesity and antidiabetic effects by increasing insulin sensitivity in hepatocytes and skeletal muscle cells. Adiponectin also shows anti-atherogenic properties due to its role in preventing the formation of atherosclerotic plaques [8] and has anti-inflammatory effects on macrophages [13]. In a study conducted on 48 hypertensive and 32 normotensive individuals, it was reported that serum adiponectin levels were lower in hypertensive patients than in normotensive individuals, and low adiponectin levels were associated with risk factors for atherosclerosis, and low adiponectin levels may be a risk factor for atherosclerosis. [14]. Serum adiponectin levels have been found to be low in

ischemic heart disease [15]. One of the adiponectin gene mutations, I164T mutation has been shown to be associated with low adiponectin levels and coronary artery disease [16, 17]. This result shows that adiponectin mutations may be part of the genetic background of the metabolic syndrome. Considering all reports in the literature adiponectin can be defined as an anti-obesity, anti-diabetic and anti-atherosclerotic hormone.

Irisin, another important mediator secreted from adipose tissue, is defined as myokine or adipo-myokine. Irisin, secreted mainly from skeletal muscle and adipose tissue, is a thermogenic protein that provides energy expenditure by converting white adipose tissue to brown adipose tissue. In 2012, Boström et al. discovered a protein that is released from skeletal muscle after exercise and protects the person from metabolic diseases when systematic exercise is performed [1]. This is a membrane protein named fibronectin type III domain 5 (FNDC5) and it was understood that irisin protein is released into the circulation by proteolysis of FNDC5. FNDC5 is also referred to as protein 2 (FRCP2) and Pep containing fibronectin type III repeats. Irisin released from skeletal muscle is a hormone with autocrine, paracrine and endocrine effects [18].

Investigation of serum irisin levels in obesity, obesity-related diabetes mellitus (DM) and other obesity-related diseases is a current issue. In one of these studies, serum irisin levels of a total of 135 Type II Diabetes patients (with and without cardiovascular disease) were found to be significantly lower than the healthy control group of 70 individuals. When a comparison is made among Type II Diabetes patients, it has been reported that serum irisin levels were lower in people with cardiovascular disease than in people without cardiovascular disease. Researchers concluded that circulating irisin may be a new potential independent cardiovascular disease risk biomarker in Type II DM patients [19]. In another study that included 96 patients with Type I DM and 34 healthy individuals, the researchers concluded that higher irisin levels were associated with better glycemic control and bone health in children with Type I DM [20]. In a study investigating the relationship between single point genome mutations (SNPs) of the FNDC5 protein and obesity, it was stated that rs16835198 and rs726344 SNPs were associated with obesity [21]. Among the maternal and neonetal FNDC5 polymorphisms, rs726344 polymorphism has been reported to be associated with preterm birth [22].

According to the results of many studies revealing the relationship between irisin and glucose metabolism, it has been stated that irisin breaks insulin resistance in obese and Type II DM, and irisin can be considered as an alternative for the treatment of diseases such as obesity and Type II diabetes [23]. Although some studies with different methodologies after applying different exercise types in special patient groups have reported a few inconsistent results with the relationship of irisin to glucose metabolism, it is known that irisin stimulates thermogenesis by reducing ATP production via UCP protein by converting white

adipose tissue to brown adipose tissue [24]. Irisin expression and secretion increase in response to exercise and the mRNA expression level of UCP-1 protein, which is involved in the conversion of irisin from white adipose tissue to brown adipose tissue, increases 7-500-fold by exercise [25].

2. CONCLUSION

Since irisin and adiponectin have similar effects in terms of their antiobesity and antidiabetic effects, maintaining high adiponectin and irisin levels in diabetic patients is the basis for treating diabetes with diet/exercise. Discussions regarding the metabolic effects of irisin and adiponectin and their role in obesity still continue. The roles of these two mediators in the pathogenesis of many diseases, including diabetes-related cancer, are under investigation. Although there are very important findings about both myokines, their functions are still not fully elucidated. More studies are needed in large patient groups to use irisin and adiponectin, which are promising compounds, in the treatment of many diseases, especially cancer.

Conflict of Interest

Author has no personal financial or non-financial interests.

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Review

A Review on Epilepsy and Planned Pregnancy in Patients with Epilepsy

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ABSTRACT:
The number of patients with epilepsy varies regionally around the world, but it is one of the most common neurological diseases. The treatment and diagnosis of epilepsy is a complex condition. The term "resolved" is used in the treatment of epilepsy. When the epilepsy is resolved, it means that the person no longer has epilepsy, but it does not guarantee that it will not return. When the disease reaches certain ages, it can improve without treatment. In patients with epilepsy, pregnancy is possible, but the treatment to be applied in terms of maternal and child health should be chosen correctly. If reliable drugs are chosen in terms of teratogenicity in the treatment of epilepsy in pregnancy and pregnancy is planned, patients can become mothers with confidence. In this review, information about epilepsy disease, historical prevalence of the disease and drugs that are safe to use in patients with epilepsy are given.

Keywords: Antiepileptic drugs, epilepsy, pregnancy

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1. INTRODUCTION

Epilepsy is one of the oldest diseases with written records dating back to 4000 BC. It is a chronic disease, also known as “sara” disease among the turkish people. Epilepsy can be characterized as “the predisposition of the brain to produce epileptic seizures with neurobiological, cognitive, psychological and social consequences”. About 50 million people worldwide are thought to have epilepsy, and this makes it one of the most common neurological diseases worldwide [1]. An estimated five million people are diagnosed with epilepsy each year [1]. In the study conducted between 1990 and 2016, which calculated the burden of worldwide, global and regional neurological diseases, it was shown that epilepsy is the 5th most common disease worldwide, and It is also one of the most common (2-8) diseases in the regionally. (Figure.1) [2].

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Considering the estimated rates around the world, this number is estimated to be around 600 thousand in our country, but no official figures have been reached on this subject. Epilepsy is a disease that affects people of all ages, races, social classes and geographical locations. The worldwide incidence and prevalence of epilepsy is slightly higher in men than in women [3]. The reason for this difference can be shown as hiding the disease of women due to socio-cultural reasons in various cultures [4]. Patients with epilepsy suffer from social problems such as stigma, exclusion, restrictions, overprotection and isolation, as well as seizures of epileptic disease.

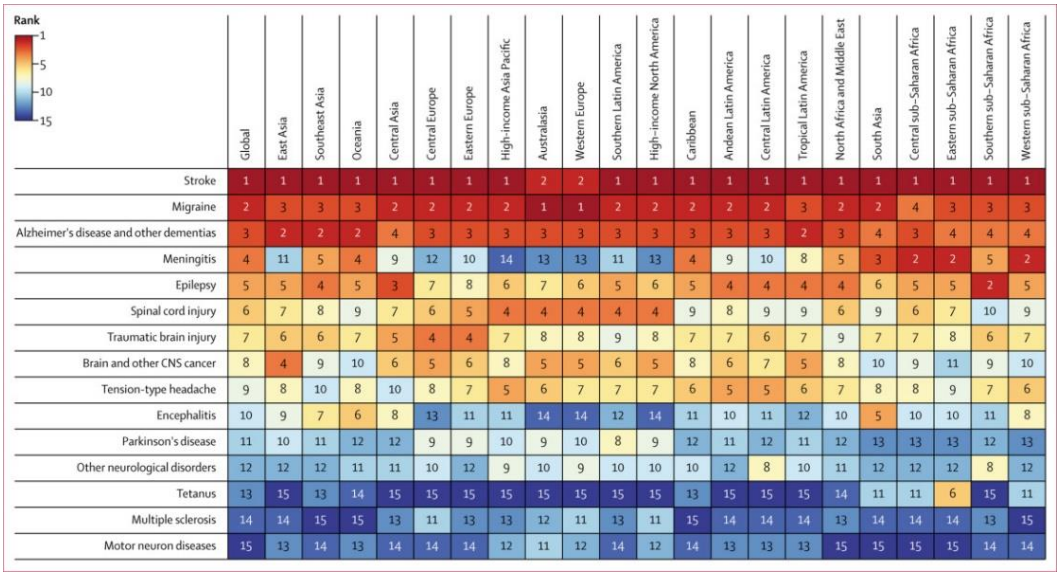

Figure 1. Ranking of epilepsy in neurologic diseases according to different regions [2]

Observations made by W. Allen Hauser et al. in the USA between 1935 and 1984 showed that the prevalence of epilepsy was 86 per 100,000 during childhood (first years of life), while it decreased to 23-31 per 100,000 at the age of 30-60, this rate increases up to 180 per 100,000 in the advancing age group, that is, in the group over the age of 85. that is, an increased incidence of epilepsy is observed in the youngest and older groups [5].

Features that define an epileptic syndrome include family history, age of onset, presumed etiology, EEG (electroencephalography) and neuroimaging findings [6]. Family history and EEG observations are considered the most important tools in the evaluation of epilepsy patients [7]. However, due to the stress in the family at the time of the seizure, the events cannot be accurately conveyed when taking the family history, problems may arise because the diagnosis is partially based on remembered family and patient information.

Apart from the difficulties in reaching clinical diagnoses, the physician's lack of knowledge and training to diagnose specific epilepsy is also an important problem. The diagnosis and treatment of the patient's epilepsy should be decided in consultation with the specialist in the management of epileptic seizures. In the

study by Leach et al. in the United Kingdom, to showed that in addition to adequate findings in the diagnosis of epilepsy, the importance of the physician who made the diagnosis is great. In a comparison between neurologists and non-specialist physicians, the rate of misdiagnosis was found to be 5.6% versus 18.9%, respectively [8]. It is not possible to avoid misdiagnosis, but with appropriately trained doctors, the probability of epilepsy being misdiagnosed can be kept lower.

Remission, a medical term for the absence of disease activity, is used to treat epilepsy. However, due to the difficulty in understanding the word by the public, experts suggested using the term "resolved" [9]. Epilepsy is not necessarily lifelong and is considered resolved if a person has not had a seizure for the past 10 years, has taken a break from seizure medication for at least the past 5 years, or is over one year old [10]. When the epilepsy is resolved, it means that the person no longer has epilepsy, but it does not guarantee that it will not return. In addition, when the patient reaches certain ages, it may improve and may not need treatment. In these cases, the physician's decision about what to do is important.

The percentage of untreated patients to the total number of patients with active epilepsy is called the "treatment gap" [11]. The 'treatment gap' ranges from 10 percent in developed countries to 75 percent in low-income countries [12]. This shows that three out of every four patients in low-income countries do not receive the necessary treatment, and this rate even goes up to 90% in some countries [1].

Epilepsy treatment should begin with monotherapy. Antiepileptic drug (AED) monotherapy remains the most appropriate approach for the treatment of most patients with epilepsy (except when the patient is experiencing more than one type of epilepsy) [13]. The main reason for recommending monotherapy is that it is treated with a single drug to reduce side effects and toxicity, as the drugs used have various side effects, ranging from minor deterioration in the CNS to liver failure and even suicide [14]. When patients fail monotherapy, initiation of a new AED monotherapy, initiation of AED polytherapy, or various non-pharmacological (epilepsy surgery, vagus nerve stimulation, ketogenic diet, reactive neurostimulation) treatments may also be administered. Apart from these, complementary and alternative therapies such as acupuncture, traditional Chinese medicine, cannabinoids, melatonin, vitamin supplement and yoga have been researched, but there is no evidence for their efficacy in treatment [15].

1.1. Epilepsy and Pregnancy

AED has many teratogenic effects, especially congenital heart defects, neural tube defects, cleft lip and cleft palate. Treatment reaching toxic levels or undesirable side effects caused by multi-drug therapy should be avoided. Although it has been shown that new generation AEDs are partially better in terms of teratogenicity, they lead to unsuccessful results in terms of seizure control, which leads to polytherapy or dose increase and an increased risk of teratogenicity [16]. As a

result of studies on the safety of AEDs used during pregnancy, levetiracetam, oxcarbazepine and lamotrigine were reported to be the least risky drugs [17-21]. Although it has low risk, drug monitoring and dose adjustment may be required during pregnancy in pregnant women taking lamotrigine and oxcarbazepine [22].

2.1. CONCLUSİON

Although pregnancy is risky in patients with epilepsy, patients who plan to have children should be informed that they can have an uneventful pregnancy and have children. The most important condition for this is to plan the pregnancy. In an epileptic patient who wants to become pregnant, it is recommended to discontinue the drug or drugs used as the first choice, if possible, and to apply monotherapy, in which drug levels are carefully controlled, as a second option. In a planned pregnancy, monotherapy should be applied before fertilization occurs when the dosage of the drug is high enough to control seizures and low enough not to harm fetal development. that is, the lowest effective dose should be treated.

Conflict of Interest

Author has no personal financial or non-financial interests.

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