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Contents

Effect of Zeolite Application and Seed Priming with Salicylic Acid on Decreasing the Cd Concentration of Inoculated Plant with Piriformospora indica Fungus under Drought Stress

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ABSTRACT

Today, urban management seems necessary to remediate soils contaminated with heavy metals, especially in industrial regions. Thus, this research was done to evaluate the effect of zeolite and seed pre-treatment with salicylic acid on reducing the Cd sorption by ornamental sunflower under drought stress. Treatments (48 treatments) consisted of applying zeolite (0 and 5 % (W/W)) in the Cd (0 and 15 mg Cd (kg soil)-1) polluted soil under cultivation of ornamental sunflower that was inoculated with P.indica in normal and intensive drought stress condition. The plants seeds were pretreated with salicylic acid (0, 0.5 and 1 mM). After 90 days, plants were harvested and atomic absorption spectroscopy (Perkin-Elmer 3030) was used for determining the Pb and Zn concentration. Application of zeolite in the soil significantly decreased and increased the plant Cd and Zn concentration by 11.3 and 14.2%, respectively. Seed priming with salicylic acid at the rate of 1 mM significantly increased the plant Zn and Cd concentration by 12.7 and 14.2%, respectively. In addition, plant inoculation with P.indica significantly decreased the catalase (CAT) and ascorbate peroxidase (APX) enzyme activity which indicate the plant resistance to abiotic stress. However, drought stress had adverse effect on the Cd sorption by plants. Moreover, the CAT and APX enzymes activities were increased. The results of this study showed that applying zeolite and seed priming with salicylic acid had additive effects on decreasing the Cd sorption by ornamental sunflower that was inoculated with P.indica fungus under drought stress.

KEYWORDS : Phytoremediation; Cd; Seed priming; Zeolite; Salicylic acid

INTRODUCTION

Today, the role of urban furniture in serving citizens in cities is not hidden from anyone. However, officials and those involved in urban affairs believe that preserving the identity and beauty of the city's appearance has a special priority, as can be seen with the irregular expansion of many large cities and increasing gradually, the population of city officials to control the social situation and deal with the affairs of the city is less than before, and sometimes maintaining the beautification of cities under the guise of functions and responding to the daily needs of citizens is forgotten. The indiscriminate influx of people from the countryside to the big cities causes an inappropriate image and confusion in the identity and body of the city, so that in many cases to meet the needs of citizens, accuracy is sacrificed speed and maintaining identity and beauty to especially in urban furniture is forgotten [1].

One of the ways to beautify the urban space is to plant ornamental plants to improve the condition of urban furniture [2]. Meanwhile, ornamental sunflower is considered as one of the useful plants in this regard, but the main problem is the presence of heavy metal pollutants in the central regions of the country. It can cause pollution and absorption of elements by such plants. Given that today in the central regions of the country with the change of climate to semi-arid, there is a problem of lack of organic matter and water stress; we should find a suitable solution to reduce the absorption of heavy metal by this plant in drought stress conditions [3].

Using metals immobilization methods as a practical alternative to traditional methods to improve landscaping is increasingly being expanded and developed. In fact, remediation of soils contaminated with heavy metals in contaminated soils is laborious and costly [4]. Immobilization technique for soil remediation is less expensive relative to other methods and its long-term modification causes the formation of metal minerals in the soil [5]. These methods do not remove contaminants from the soil, but transform species into less bio-availability forms by reducing its solubility and forming more stable minerals [6]. Adding organic and inorganic amendments to contaminated soil to reduce the solubility and bioavailability of metals via the process of chemical adsorption or deposition can prevent the transfer of contaminants to deeper soil layers and groundwater that is a positive point in environmental studies. Low cost and its minimum impact on soil properties are among the most important advantages of this method and compared to other soil remediation methods, it has a positive and long-term effect [7].Accordingly, some researches pointed to the role of zeolite in reducing the availability of heavy metals in sewage sludge and concluded that zeolite can prevent the heavy metal entering in the soil and groundwater. However, the effect of the interaction of heavy metals and their role in heavy metals uptake by plants was not mentioned [8]. In addition, it has been reported that using natural clays such as zeolite and bentonite has a positive role on increasing the soil sorption properties and thereby decreasing the Pb uptake by plants [9]. The important point of this research is that despite the positive effect of chemical Immobilization of heavy metals in reducing the availability of heavy metals in the soil, it is necessary to provide a suitable solution to increase the growth of non contaminated plants in heavy metal contaminated environment.Seed priming and foliar application of organic amendments are suitable ways to increase the plants growth [10, 11]. However, their performance efficiency has depended to the plants growth condition that should be considered separately. According this, in a study conducted the effects of seed priming and foliar application of salicylic acid on yield and essence of fennel (Foeniculum vulgare Mill.) under drought stress condition and concluded that seed priming and foliar application of salicylic acid had significant effects on increasing the plant growth. However, they have mentioned that the amount of applied salicylic acid had significant effect on the plant growth yield. However, they didn't consider the role of soil physic-chemical properties such as type of soil pollutant in their studies [12]. Accordingly, it seems that it is necessary to calibrate these organic amendments for different regions, separately. Generally, salicylic acid, commonly occurring in vascular plants, plays essential roles in regulation of plant growth, development and in plant response to environmental. However, plant physiological characteristics and plant growth conditions can have different effects on the performance of these organic additives [13]. In addition, plant inoculation with Piriformospora indica (P.indica) fungus can help to increase the plant resistance to abiotic stress such as heavy metal toxicity [14-16]. However, the physiological characteristics of the plant, plant growth conditions and the type of contaminants in the soil can affect the growth rate of the plant, which should be considered separately in different studies. Thus, this research was conducted to investigate the effect of zeolite and seed pretreatment with salicylic acid with the aim of reducing the absorption of heavy metal by ornamental sunflower under drought stress conditions.

MATERIALS AND METHODS

To investigate the effect of zeolite and seed priming with salicylic acid on decreasing the Cd concentration of the plants that was inoculated with P.indica under drought stress, a nonsaline soil with the low organic carbon was selected. Some of the soil physic-chemical properties in this study have been shown in Table 1.

Table 1. Selected soil physic-chemical properties of soil used in this experiment

Soil	Parameter
pH	7.5
EC (dS m^{-1})	1.2
Organic Carbon (%)	0.2
Soil Texture	Loam
$CaCO_3$ (%)	18
Pb availability(mg kg^{-1})	ND*
Cd availability (mg kg^{-1})	ND
CEC (C mol (kg soil)$^{-1}$)	14.2

* ND: Not detectable by atomic absorption spectroscopy (AAS)

This research was done (48 samples) as a factorial experiment in the layout of randomized completely block design in three replications. Treatments included applying zeolite at the rate of 0 and 5% (W/W), seed priming with salicylic acid at the rate of 0, 0.5 and 1mM, inoculation of plants with P.indica under normal (full irrigation (D0)) and intensive (70% water depletion of field capacity) drought stress (D1). Plants in this experiment were ornamental sunflower that was cultivated in the soil polluted with the Cd at the rate of 0 and 15 mg Cd (kg soil)-1. The Cd was spiked in the soil at the mentioned rates and for reach to equilibrium one month incubated. The plant seeds of ornamental sunflower were prepared from Pakan Bazr Company in the Esfahan, center of Iran. First, the seeds were soaked in water for a few minutes and then immersed in 96% alcohol for 15 seconds in laminar and then put in sodium hypochlorite solution (1:10 (v/v)) for one minute. Then Seeds were primed in salicylic acid solution at the rates of 0, 0.5 and 1 mM. After 12 h, seeds were germinated on moisturized filter paper for 3 days. After that, seedlings were grown in pots filled with sand and perlite in the ratio of 2:1, and transferred to growth chamber under controlled conditions. When the plants were germinated, the most vigorously growing seedlings were selected for the experiment. After that, half of the seeds were inoculated with P. indica by immersion in inoculums (adjusted nearly to 2×106) under gentle shaking for 3 hours. The non inoculated seedlings were also dipped in sterilized distilled water containing Tween 0.02%. Thereafter, both types of inoculated and non-inoculated seedlings (10 seedlings) were planted in the uncontaminated top soil layer in the center of each pot at a depth of 1cm. After 20 days, the plants were exposed to drought stress and then after 90 days the plants were harvested. The concentration of Pb and Zn in plant biomass was measured using AAS. Soil microbial respiration was measured based on the standard method [17]. Catalase (CAT) and ascorbate peroxidase (APX) enzyme activity was also determined [18, 19].

Statistical analysis

Statistical analyses were done based on the ANOVA procedure via SAS V.9.1 software. Accordingly, the mean differences were calculated according to the least significant difference (LSD) test. The $P<0.05$ value was considered to determine the significant difference.

RESULTS AND DISCUSSION

Plant Cd concentration (Table 2) was also affected by the treatments. The highest plant Cd concentration was belonged to the plants which cultivated in the soil without receiving any organic amendment, while the lowest was measured in the plant that grown in the soil that treated with 5 % (W/W) zeolite.

Increasing the application rate of zeolite from 0 to 5 % (W/W) significantly decreased the plant Cd concentration by 11.6% which was cultivated in the Cd-polluted soil that can be related to the role of zeolite on increasing the soil sorption properties and thereby decreased the soil Cd availability. Increasing soilCEC due to addition of zeolite confirms our results. Accordingly, decreasing the soil Cd availability can increase the soil Zn availability (data was not shown) and consequently increase the plant Zn concentration (Table 3).

Table 2. The effect of zeolite, seed priming with salicylic acid, drought stress on the plant Cd concentration (mg (kg)-1) in the presence of P.indica

Drought stress	Cd concentration (mg (kg)$^{-1}$)	Zeolite % (W/W)											
		0			5			0			5		
		+P.indica						*-P.indica*					
		Seed priming with salicylic acid (mM)											
		0	0.5	1	0	0.5	1	0	0.5	1	0	0.5	1
D0	0	ND**	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	15	14.5h*	14.2j	13.9m	14.1k	13.8n	13.4q	15.1b	14.6g	14.0l	14.9d	14.2j	14.1k
D1	0	14.5h	14.2j	13.8n	14.2j	13.7o	13.3r	14.9d	14.6g	14.0l	14.5h	14.0l	13.6p
	15	14.8e	14.7f	14.5h	14.6g	14.2j	13.9m	15.3a	15.0c	14.7f	15.0c	14.5h	14.3i

*Similar letters show no significant differences, **ND: Not detectable by Atomic absorption spectroscopy, +P.indica and –P.indica are the presence and absence of P.indica.

Table 3. The effect of zeolite, seed priming with salicylic acid, drought stress on the plant Zn concentration (mg (kg)-1) in the presence of P.indica

Drought stress	Cd concentration (mg (kg)$^{-1}$)	Zeolite % (W/W)											
		0			5			0			5		
		+P.indica						*-P.indica*					
		Seed priming with salicylic acid (mM)											
		0	0.5	1	0	0.5	1	0	0.5	1	0	0.5	1
D0	0	11.9a*	19.2e	19.6c	12.4z	20.9o	21.4a	11.0d	17.8j	18.4f	11.8b	18.3g	19.3d
	15	10.8e	17.1o	18.3g	11.9a'	18.3g	19.6c	10.5f	16.5q	17.3n	11.3c	17.9i	18.3g
D1	0	10.2h'	17.6l	17.9i	13.9x	18.1h	18.3g	9.6i'	15.4v	15.9t	10.4g'	16.4r	16.9p
	15	9.1j'	16.4r	17.1o	13.5y	17.7k	17.5m	8.2l'	15.3w	16.5q	8.7k'	15.7u	16.1s

*Similar letters show no significant differences, +P.indica and –P.indica are the presence and absence of P.indica.

The antagonistic effect of nutrient elements such as Zn with heavy metals was reported by researchers [20, 21]. However, drought stress condition had negative effect on plant Zn concentration which can be related to the role of drought stress on the plant growth (date was not shown). In a study, the researches investigated the interaction effect of Cd and Zn in Matthiola flavida plant and concluded that using Zn sources can prevent the heavy metal sorption and increase the heavy metal uptake by plant which they related to the antagonistic role of Zn and Cd [22]. The important point in this research is that by using zeolite we have been able to reduce the availability of heavy metal in the soil and in other words to help its chemical immobilization in the soil, which can be an important point in reducing the absorption of heavy metal by plants. . Considering that ornamental sunflower is one of the plants used in beautifying the urban space, using this method can greatly help to clean the urban space, in other words, to plant non contaminated plants in contaminated land. In addition, seeds priming with salicylic acid significantly ($P<0.05$) decreased the plant Cd concentration, as the results of our study showed that seed priming with salicylic acid at the rate of 1 mM significantly decreased the plant cd concentration by 14.9%. In addition, the additive effect of plant inoculation with P.indica and seed priming with salicylic acid on reducing the Cd uptake by plant was significant. It can be concluded that inoculation of the plant with P.indica has been able to help increase the plant's resistance to environmental stresses such as heavy metals toxicity by increasing the availability of nutrients elements such as Zn in the plant. Meanwhile, seed pretreatment with salicylic acid has also been able to help increase plant resistance to heavy metal stress. Our results showed that plant inoculation with P.indica which grown in the soil that received 5 % (W/W) zeolite significantly decreased the plant Cd concentration in the normal and intensive drought stress by 19.8 and 25.3%, respectively. It can be concluded that plant inoculation with P.indica had significantly increased and decreased the plant Zn and Cd, respectively that can be related to the antagonistic effect of Cd and Zn. In addition, regardless of soil pollution to heavy metal, increasing plant nutrient uptake due to the P.indica inoculation can be ignored.

The effect of P.indica fungus on nutrient uptake by anise plant (Pimpinella anisum) under water deficit stress conditions was investigated and concluded that plant inoculation had significant effect on increasing the plant nutrient uptake [23]. However, they did not consider the soil physic-chemical conditions such as soil pollution. The P.indica significantly increases the number of roots which enable the plant to absorb water and nutrients, and thereby increases root growth and photosynthetic levels. The positive effects of fungi symbiosis on survival and growth of host plants in arid and semi-arid regions, that had two major problems of drought and salinity, has attracted the attention of many researchers [24, 25]. According to the results of our study, drought stress had negative effect on plant Zn concentration and among this, plant inoculation could help to increase the plant resistance to abiotic stresses such as heavy metals that is in the line with the results other studies [26]. It has been also reported that P. indicaconfers drought tolerance on Zea mays L. through enhancement of antioxidant activity and expression of drought-related genes that is similar to our results [27]. Accordingly, our results showed that with the change of conditions from normal to intensive drought stress, the APX (Table 4) and CAT (Table 5) enzyme activity has increased. Drought stress can stimulate the production of reactive oxygen species (ROS) that damage lipids, carbohydrates, proteins and DNA [28].

Table 4. The effect of zeolite, seed priming with salicylic acid, drought stress and the presence of P.indica on APX enzyme activity (Unit/mg protein)

Drought stress	Cd concentration (mg (kg)$^{-1}$)	Zeolite % (W/W)											
		0			5			0			5		
		+P.indica						*-P.indica*					
		Seed priming with salicylic acid (mM)											
		0	0.5	1	0	0.5	1	0	0.5	1	0	0.5	1
D0	0	14.0n*	13.6p	13.3s	13.4r	13.1u	12.6w	14.4j	14.1m	14.0n	13.7o	13.2t	12.8v
	15	14.5i	14.1m	13.7o	14.1m	13.5q	13.2t	14.9f	14.5i	14.2l	14.5i	14.1m	13.6p
D1	0	15.1e	14.8g	14.3k	14.7h	14.4j	14.0n	15.5b	15.2d	14.8g	15.1e	14.3k	14.0n
	15	15.4c	15.2d	14.8g	15.2d	15.1e	14.5i	15.9a	15.5b	15.1e	15.4c	15.1e	14.7h

*Similar letters show no significant differences, +P.indica and –P.indica are the presence and absence of P.indica.

Table 5. The effect of zeolite, seed priming with salicylic acid, drought stress and the presence of P.indica on CAT enzyme activity (Unit/mg protein)

Drought stress	Cd concentration (mg (kg)$^{-1}$)	Zeolite % (W/W)											
		0			5			0			5		
		+P.indica						-P.indica					
		Seed priming with salicylic acid (mM)											
		0	0.5	1	0	0.5	1	0	0.5	1	0	0.5	1
D0	0	11.5x*	11.2z	10.9	11.0b'	10.7e'	10.3f'	11.9v	11.5x	11.2z	11.3y	11.0b'	10.8d'
	15	11.9v	11.6w	11.0b'	11.6w	11.2z	10.7e'	12.5s	12.3t	11.9v	12.0u	11.5x	11.1a'
D1	0	15.9g	14.3p	14.8o	15.5k	14.1q	13.8r	16.4b	16.0f	15.4l	16.1e	15.7i	15.4l
	15	16.1e	15.7i	15.3m	15.6j	15.2n	14.8o	16.6a	16.3c	15.9g	16.4b	16.2d	15.8h

*Similar letters show no significant differences, +P.indica and –P.indica are the presence and absence of P.indica.

Using zeolite had significant effect on increasing the soil microbial respiration (Table 6) in Cd-polluted soil. Accordingly, the highest soil microbial respiration was belonged to the soil that amended with the highest rate of zeolite used in this soil. Inoculation of plants with P.indica improved the microbial respiration in Cd (15 mg Cd (kg soil) -1)polluted soil by 13.9% that maybe related to the role of P.indicaon plant root stimulation on increasing the plant's root exudate and provided suitable conditions for the growth of soil microorganisms especially in the drought conditions [29].

Table 6. The effect of zeolite, seed priming with salicylic acid, drought stress and the presence of P.indica on soil microbial respiration (mg C-CO2 (kg soil)-1)

Drought stress	Cd concentration (mg (kg)$^{-1}$)	Zeolite % (W/W)											
		0			5			0			5		
		+P.indica						-P.indica					
		Seed priming with salicylic acid (mM)											
		0	0.5	1	0	0.5	1	0	0.5	1	0	0.5	1
D0	0	17.2d*	18.1y	17.6b'	17.2d'	17.2d'	16.5g'	17.1e	19.1t	18.4w	17.0f	18.4w	17.8a'
	15	18.9u	22.5i	22.9f	18.0z	22.8g	23.4e	19.6r	21.3m	21.7k	19.1t	21.9j	22.6h
D1	0	18.4w	18.3x	18.0z	18.5v	17.4c'	17.1e'	20.6p	21.0n	21.6l	20.1q	19.4s	18.5v
	15	21.0n	23.5d	24.1b	21.0n	24.1b	24.3a	20.9b	22.9f	23.4e	21.0n	23.7c	24.1b

*Similar letters show no significant differences, +P.indica and –P.indica are the presence and absence of P.indica.

Regardless of salicylic concentration, plant priming with salicylic acid significantly improved the microbial respiration of microorganism in the soil by 14.4%. Drought stress had adverse effect on soil microbial respiration. Our results showed that plant under drought stress condition reduced the soil microbial respiration, as, our study showed that microbial respiration of the soil under drought stress was significantly lower relative to normal stress by 15.3%. However, plant inoculation with P.indica and seed priming with salicylic acid can alleviate the negative effects of Cd toxicity in heavy metal polluted soil.. Increasing the soil microbial respiration and decreasing the APX and CAT enzyme activity confirms our results clearly, as, the results of our study showed that increasing soil pollution with Cd significantly increased the plant catalase enzyme activity. The highest CAT enzyme activity was belonged to the plants that grown in the soil with the greatest soil pollution to Cd. At this time, plant inoculation with P.indica significantly reduced the plant Cd and CAT enzyme activity. Accordingly, inoculation of plant cultivated in the Cd-polluted soil (15 mg Cd (kg soil) -1) with P.indicasignificantly decreased the CAT enzyme activity under normal and intensive drought stress by 16.3 and 18.4%, respectively. Using 5% (W/W) zeolite in Cd-polluted soil (15 mg Cd (kg soil)-1 significantly decreased the CAT enzyme activity by 13.5%. Using zeolite along with inoculation of sunflower plant with P. indica and seed pretreatment with salicylic acid can have an additive effect on reducing the ability of the plant to absorb heavy metals. The important point of this research is that today we should look for a suitable solution to chemical immobilization of heavy metals in the soil, although many studies have shown that the use of organic additives such as organic fertilizers has to some extent helped reduce the availability of heavy metals in the soil [30-32], but the use of those compounds due to their decomposition effects may causes to re-distribution of heavy metals in the soil and can return heavy metals to their environment which can affect the beautification of urban spaces and endanger the health of the community.

CONCLUSIONS

Using zeolite at the rate of 5% (W/W) significantly decreased and increased the plant Cd availability, respectively. Among this, seeds priming with salicylic acid significantly help to increasing and decreasing the plan Zn and Pb availability, respectively. Although, increasing the rate of salicylic acid from 0 to 1mM had more significant effect on increasing the plant resistance to abiotic stresses.

Decreasing the APX and CAT enzyme activity with decreasing the plant Cd concentration confirms our results clearly. However, the amount of salicylic acid used for seed priming and soil physic chemical properties such as type of pollutant are important factors that should be considered. In addition, this research should be examined in the field study in the future researches.

Conflicts of interest

The author declares that there are no conflicts of interest.

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The Substituent Effects on Chemical Reactivity and Aromaticity Current of Ritalin Drug

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ABSTRACT

In this study, the effects of four substitutions in two different positions of Methylphenidate (MPH, Ritalin) structure on chemical reactivity indices and aromaticity current of benzene ring were investigated at the density functional theory (DFT) level. The results were interpreted using natural bond orbital (NBO) analysis. The findings indicated that by increasing the participation of the studied substitutions in intramolecular interactions, their effect on the chemical reactivity indices and aromaticity current increased. Therefore, the substituents NO2 and Cl on the benzene ring, with the highest participation in intramolecular interactions, caused the highest increase in the resonance interactions of the benzene ring. As a result, they increased the values of the Nuclear Independent Chemical Shift (NICS) in the geometric center of the ring. Also, the above substitutions decreased the energy gap between HOMO (highest occupied molecular orbitals) orbitals and LUMO (lowest unoccupied molecular orbitals) and increased chemical reactivity indices. On the other hand, The NBO results represented that electron-withdrawing substituents at positions R7 and R9reduced the accumulation of negative charge on adjacent atoms and the benzene ring.

KEYWORDS : Ritalin; NICS;Chemical hardness;NBO analysis

INTRODUCTION

Methylphenidate (MPH), under the brand name of Ritalin, is a similar compound to amphetamines and stimulates the central nervous system. It is used to treat depression, narcolepsy and the Attention Deficit Hyperactivity Disorder (ADHD) in children [1]. Methylphenidate has two chiral centers and is found in the form of four optical isomers D and L-threo and D and L-erythro. However, Ritalin is marketed as a racemic mixture of the optical isomers D-threomethylphenidate and L-threomethylphenidate [2]. Numerous theoretical and experimental studies have been devoted to investigate crystal structures, MPH conformal analysis and its analogues in solid and soluble states[3–6] and NMR and IR spectroscopy techniques have been used in this field [7,8]. Conformational analysis of neutral and protonated forms of methylphenidate has also been performed by Gilbert et al. using molecular and quantum mechanics methods [9]. Ritalin structure consists of two rings (benzene ring and the hexagonal ring of piperidine), both of which are attached to a carbon atom (C8)(Figure1). Substitution at different two-ring positions produces a large number of MPH analogues, many of which have been synthesized and studied [10]. Misra et al. studied 80 methylphenidate analogues using quantitative structure-activity relationships (QSAR) to obtain a preliminary model for the binding affinity of those compounds to dopamine carriers [11]. In addition, Gatley et al. investigated the affinities of methylphenidate derivatives with respect to dopamine norepinephrine and serotonin carriers [12]. As mentioned above, the substituent effects in different positions of Ritalin structure on its biological activity have been investigated.

Many studies have been done on conformational analysis and the investigation of crystallographic structures of MPH and its derivatives. However, the substituent effectsat different positions of MPH on effective structural parameters such as aromaticity current of benzene ringand chemical reactivity indices have not been studied so far. In this study, the effects of substituent changes in MPH on the reactivity indices and the benzene aromaticity current were investigated using DFT methods. The results were interpreted using NBO analysis based on molecular structure.

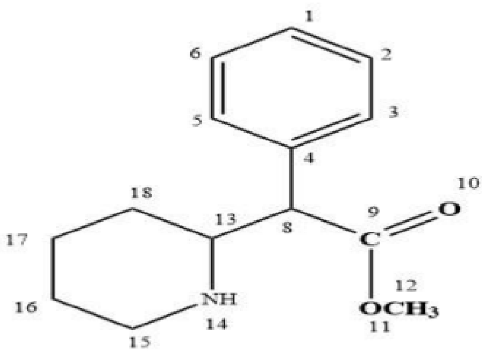


Figure1. The chemical structure of Ritalin and the atomic numbering used in this study.

Computational details

Geometrical optimizations of Ritalin and its derivatives (compounds 1-5, Figure 2) were performed at B3LYP / 6-311 ++ G (d, p) level of theory [13,14]. The nature of stationary points for the interested structures was determined by calculating the harmonic frequencies at the same level of theory. For minimum state structures, only the real frequency values were accepted. The energy gap values of HOMO and LUMO orbitals (HLG) were obtained using the results of molecular orbitals calculations. The reactivity indices of compounds 1-5 such as chemical hardness (η), electrophilicity (ω) and electronegativity (χ) were calculated using the following formulas [15–17]:

$$\eta = \frac{E_{LUMO} - E_{HOMO}}{2}$$

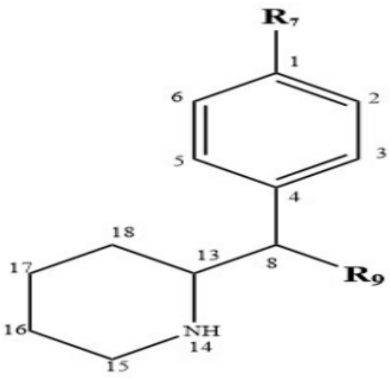
$$\omega = \frac{\mu^2}{2\eta}$$

$$\chi = \frac{-(E_{HOMO} + E_{LUMO})}{2}$$

Also, the negative of the electronegativity was defined as chemical potential (μ) [18] and chemical softness (S) was just the inverse of chemical hardness (S=1 /η).

To investigate the effect of substituent change on aromaticity current of benzene ring and to evaluate the intensity of diamagnetic currents, the Nuclear Independent Chemical Shift (NICS) technique was used on optimized structures of compounds 1-5 at B3LYP / 6-311 ++ G (d, p) level of theory. NICS was defined by Schleyer et al. as the negative value of absolute magnetic shielding computed in centers of rings or 1 Å above the molecular plane [19]. NICS at an empty point in space equals zero and in principle did not require reference molecules and calibrating (homodesmotic) equations for evaluation of aromaticity. Negative values of NICS indicated the shielding presence of induced diatropic ring currents understood as aromaticity at the specific point.

Finally, NBO analysis was performed on the optimized structures at B3LYP / 6-311 ++ G (d, p) level of theory[20,21]. All calculations were performed in the gas phase using Gaussian 09 software [22].



1.R9=CO2CH3, R7=H, 2.R9=CHNH, R7=H
3. R9=H, R7=H, 4.R9=CO2CH3, R7=Cl, 5. R9=CO2CH3, R7=NO2

Figure2. The chemical structure of Ritalin and its studied derivatives (compounds 1-5). The investigated substitutions in positions R7 and

RESULTS AND DISCUSSION

Chemical reactivity indices

The results of chemical reactivity calculations showed that compound 5 had the highest electronegativity, electrophilicity and dipole moment, as well as the lowest chemical hardness among compounds 1-5 (Table 1). With the substituent change in positions R7 and R9 of MPHstructure, the order of electrophilicity and electronegativity values in the studied compounds wassimilar and it was 5>4>2>1>3. The order of chemical hardness changes was exactly the opposite of the ordermentioned for electrophilicity and electronegativity (Figure3) and it was identical to the order of HLG values. High HLG value means that the molecule was hard. This difference could relate the stability of the molecule to its chemical hardness, meaning that a molecule with a minimum HLG was a more reactive molecule. With thesubstituent change in compounds 1-5, the order of dipole moment (d) values was 5>4>2>3>1. Therefore, the calculated reactivity indices demonstrated that theelectron-withdrawing substituents (NO2 and Cl) on theMPH structure increased its chemical reactivity.

Table 1. The calculated values of HLG (in eV), chemical hardness (η) , chemical softness(S), electronegativity(χ) , chemical potential (μ) , electrophilicity and dipole moments (d in Debye) of compounds 1-5 studied at B3LYP/6-311++G(d, p) level of theory.

Compound	HLG	η	S	□	μ	ω	d
	eV						Debye
1	-5.500	2.7511	9.8912	3.3307	-3.3307	2.019	1.1347
2	-5.433	2.7162	10.0180	3.4232	-3.4232	2.155	2.1956
3	-5.511	2.7554	9.8756	3.2186	-3.2186	1.880	1.7165
4	-5.415	2.7048	10.0502	3.5130	-3.5130	2.278	3.4028
5	-3.773	1.8858	14.4248	4.5960	-4.5960	5.597	7.2539

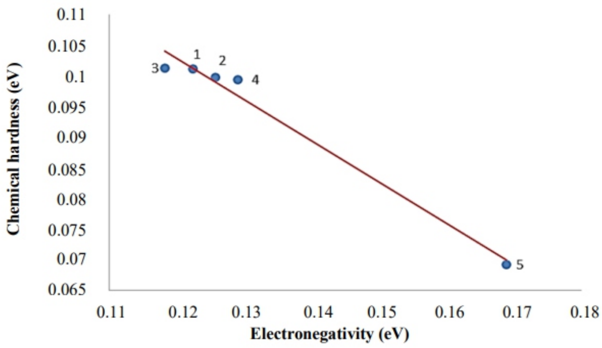


Figure 3. Chemical hardness values as a function of electronegativity values in compounds 1-5.

NICS results

In this study, the NICS technique at B3LYP / 6-311 ++ G (d, p) level of theory was used to evaluate the aromaticity current in the benzene ring and the intensity of the ring diamagnetic currents in MPH and its derivatives. In all compounds 1-5, the sets of points located below and above the geometric center of the ring were used. Their locations correspond to distances from -5 to +5 Å relative to the geometric center of the benzene ring with 0.5 Å steps. The NICS diagrams of compounds 1-5 were almost symmetrical along the molecule plane. Therefore, only the NICS values above the plane were presented in Figure 4. The numerical values of NICS and Figure 4 confirmed that all analogues were aromatic, and all of them exhibited a certain decrease of NICS value from the point located in the geometric center of the ring to 1 Å above or below it. The results indicated that the minima of NICS values were located at the distance of 0.5–1.0 Å below and above the plane. This result was consistent with the presence of delocalized π-electrons current above and below the molecule planes as expected for aromatic compounds. The maximum diatropic current was observed at the 0.5-1.0 Å above the geometric center of five analogues. The NICS 0 Å values in the ring center NICS (0) were affected by sigma bonds. However, the NICS values up to 1 Å from the geometric center and above ring NICS (1) were affected more by π bonds. The strongest aromaticity quality was observed in the geometric center of the benzene ring in compound 5 andcompound 2 had the least aromaticity. The order of NICS (0) values in compounds 1-5 was 5>4>1>3>2. However, this order was at NICS (0.5) as 4>5>3>1>2 and at NICS (1) at the 1 Å above the plane was 1>3>4>5>2.Therefore, the results showed that the electronwithdrawing substituents (NO2 and Cl) increased diatropic currents and enhanced aromatic quality in the geometric center of ring benzene. At NICS (0.5), substituent Cl had the greatest effect on aromaticityquality, which indicated its effect on the π-electronsclouds of the benzene ring. As the distance from the center of the ring increased, the effect of electron withdrawing substituents (NO2 and Cl) on the aromaticityof benzene ring decreased.

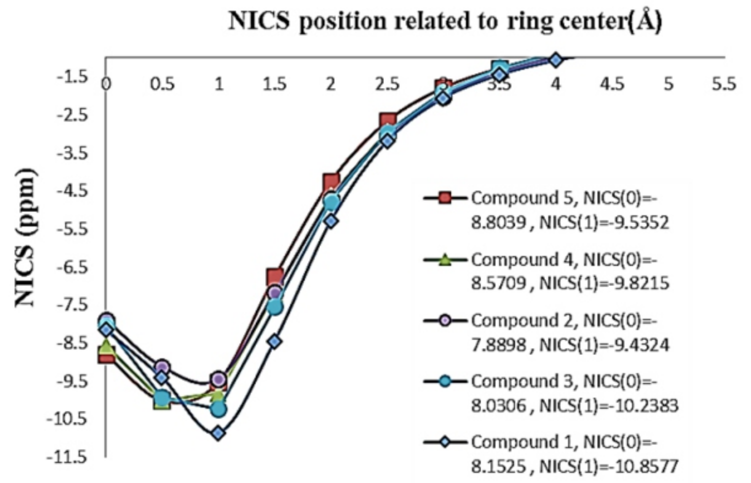


Figure 4. Aromaticity of compounds 1-5 estimated as a function of NICS (negative value of absolute magnetic shielding) versus distance from ring geometric center. NICS (0) and NICS (1) denoted values estimated at ring geometric center and 1 Å above, respectively.

NBO analysis

NBO analysis results of MPH and its analogues at the computational level of B3LYP / 6-311 ++ G (d, p) were reported in Tables 2 and 3. The results indicated that the nitrogen of piperidine ring (N14) in compound 2 had the highest value of negative charge and C9 nuclei in compound 1 (Ritalin) had the highest value of positive charge among the studied atoms. NO2 oxygens in compound 5 also had the lowest negative charge valuesamong the oxygen atoms in the investigated structures. The findings represented that the substituent change in positions R7 and R9 had the greatest effect on the values of atomic charges C1 and C8 nuclei (range of atomic charge changes was 0.28e and 0.5e for nuclei C1 and C8, respectively). The order of the values of the negative charges on atoms C1 and C8in the studied compounds was 3>2>1>4>5 and 3>5>1>4>2, respectively. As observed, the electron-withdrawing substituents at positions R7 and R9reduced the negative charge onadjacent atoms, especially C1. The order of negative charge values on the benzene ring was identical to the observed order of charge values on the C1 atom.Interestingly, both C1 and C8 nuclei had the highest negative charge in compound 3, which was free of any electron-withdrawing substituents at positions R7 and R9. The order of the negative charge values on C9 and N14atoms was 1>4>5>2 and 2>3>5>4>1, respectively.However, the least effect of substituent changing was observed in the negative charge values of O10 and O11nuclei and the order of the negative charge values on them was the same; it was 1>4>5. The NBO analysis also stated that the lone-pairs electrons of oxygens (LPOs) in compounds 1-5 were affected more in intramolecular interactions than LP Ns (lone- pair electrons of nitrogens) and LP Cls (lone-pairs electrons of chlorine) and had higher resonance energy (E (2)). The highest value of resonance interactions was related to LP O7 → σ * or π * interactions of substituent No2in compound 5 (the totalinteraction energy of LPOs in substituent NO2 was240.45 kcal/mol). The point to consider in the intramolecular interactions of NO2 and Cl substations was that all the LPOs interactions of NO2 group werewith the sigma bonds of the molecule and the benzene ring. However, the LP Cls interactions of compound 4 were with sigma bonds and also the π-electron system of the benzene ring. The NBO results obtained were in agreement with the NICS values in the geometric center of the ring NICS (0) up to one angstrom above the ring.The order of interaction energy values related to BD (1) C - C → σ * or π * and BD (2) C - C → σ * or π * interactions in the benzene ring by substituent changing was 5>4>1>2>3.

The above order was exactly the opposite of the observed order for the electrical charge of the carbon atoms of the benzene ring and very close to NICS (0) (Figure 5). On the other hand, the findings showed that the substituent change in positions R7 and R9had the greatest effect on the LP N14 interaction energy of the piperidine ring. The range of change in resonance energy associated with the LP N14 → σ * or π * interactions by substituent change was 1.85 kcal/mol. The order of resonance energy values related to the interactions of LP O10 and LP O11 was almost the same and was the opposite of the order of their negative charge values. From the whole results, it could be stated that with increasing the participation of LPOs in the intramolecular interactions of the studied compounds, the negative charge on them decreased. Meanwhile, the chemical reactivity increased and the aromaticity of the benzene ring in its geometric center was enhanced.

Table 2. Calculated values of natural atomic charges (in atomic unit (e)) and total resonance energy (∑E (2) in kcal/mol) values related to LP →σ*or π*interactions of nitrogen, oxygen and chlorine lone- pairs electrons of compounds 1-5 at B3LYP/6-311++G** level of theory.

Compound=1	C_1	C_8	C_9	O_{10}	O_{11}	N_{10}	N_{14}
Charge	-0.2067	-0.3256	0.8344	-0.6163	-0.5484	-	-0.6896
∑E(2) LP(1) → σ* or π*	-	-	-	3.61	10.48	-	21.01
∑E(2) LP(2) → σ* or π*	-	-	-	50.49	56.02	-	-
Compound=2							
Charge	-0.2084	-0.2978	0.1702	-	-	-0.6168	-0.7046
∑E(2) LP(1) N → σ* or π*	-	-	-	-	-	13.01	19.89
Compound=3							
Charge	-0.2121	-0.3914	-	-	-	-	-0.6933
∑E(2) LP(1) N → σ* or π*	-	-	-	-	-	-	19.57
Compound=4							
Charge	-0.0376	-0.3243	0.8336	-0.6155	-0.5478	-	-0.6901
∑E(2) LP(1) → σ* or π*	-	-	-	3.62	10.47	-	21.15
∑E(2) LP(2) → σ* or π*	-	-	-	50.3	56.58	-	-
Compound=5							
Charge	0.0651	-0.3302	0.8318	-0.6115	-0.547٦	-	-0.6907
∑E(2) LP(1) → σ* or π*	-	-	-	3.63	10.51	-	21.42
∑E(2) LP(2) → σ* or π*	-	-	-	51.00	56.74	-	-

Table 3. Calculated values of natural atomic charges (in atomic unit (e)) and interaction energy (E (2) in kcal/mol) values related to LP →σ*or π*interactions of oxygen and chlorine lone- pairs electrons of compounds 4 and 5 at B3LYP/6-311++G** level of theory.

Compound	Type	Charge	Intra-molecular Interactions	E(2)
4	**Cl_7**	-0.0059		
	LP(1)Cl_7		LP(1) Cl_7 → BD*(1) C_1 - C_2	1.63
			LP(1) Cl_7 → BD*(1) C_1 - C_6	1.63
	LP(2)Cl_7		LP(2) Cl_7 → BD*(1) C_1 - C_2	4.09
			LP(2) Cl_7 → BD*(1) C_1 - C_6	4.07
	LP(3)Cl_7		LP(3) Cl_7 → BD*(2) C_1 - C_6	12.05

5				
	N_7	0.4851		
	O_{7-1}	-0.3821		
	LP(1) O_{7-1}		LP(1) O_{7-1} → BD*(1) C_1 - N_7	4.20
			LP(1) O_{7-1} → BD*(1) N_7 - O_{7-2}	2.34
	LP(2) O_{7-1}		LP(2) O_{7-1} → BD*(1) C_1 - C_2	0.68
			LP(2) O_{7-1} → BD*(1) C_1 - N_7	12.07
			LP(2) O_{7-1} → BD*(1) C_5 - C_6	0.53
			LP(2) O_{7-1} → BD*(1) N_7 - O_{7-2}	18.99
	O_{7-2}	-0.3833		
	LP(1) O_{7-2}		LP(1) O_{7-2} → BD*(1) C_1 - N_7	4.20
			LP(1) O_{7-2} → BD*(1) N_7 - O_{7-1}	2.33
	LP(2) O_{7-2}		LP(2) O_{7-2} → BD*(1) C_1 - C_6	0.68
			LP(2) O_{7-2} → BD*(1) C_1 - N_7	12.05
			LP(2) O_{7-2} → BD*(1) $C_{۲}$ – $C_{۳}$	0.54
			LP(2) O_{7-2} → BD*(1) N_7 - O_{7-1}	18.95
	LP(3) O_{7-2}		LP(3) O_{7-2} → BD*(1) N_7 - O_{7-1}	162.82

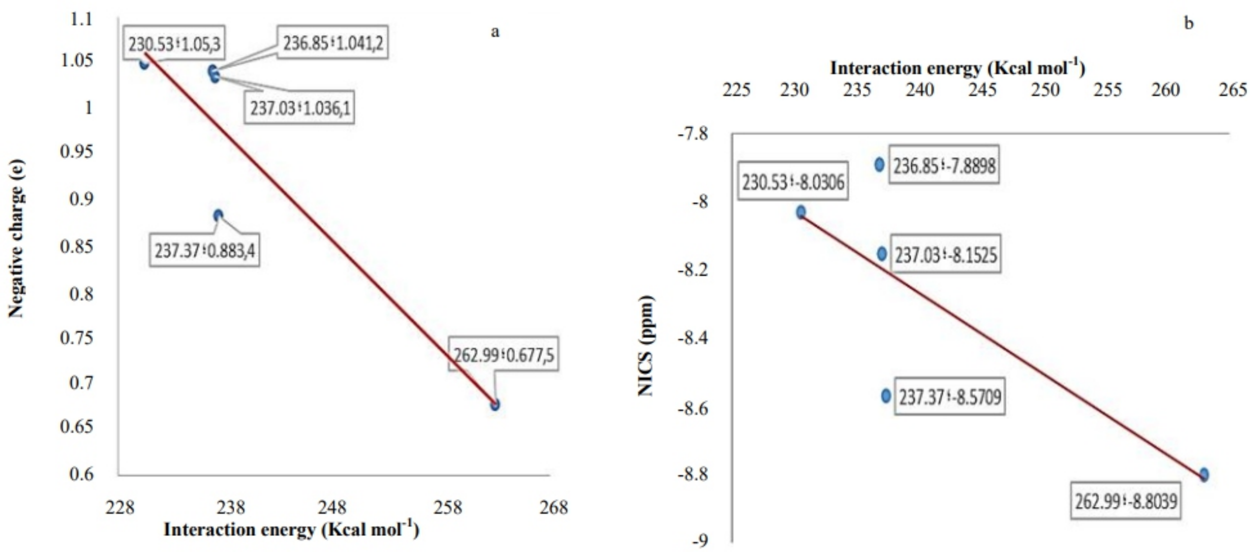


Figure 5. a) Negative charge values of the benzene ring as a function of the interaction energy of benzene ring atoms and b) NICS values of the benzene ring as a function of the interaction energy of benzene ring atoms in compounds 1-5.

CONCLUSIONS

Investigation of the substituent effects is one of the important research aspects in the chemistry of medicinal compounds. In this study, it was attempted to show the role of substituent change at two different MPH positions on structural parameters, chemical reactivity andaromaticity current. The results represented a good and reasonable relation between structural parameters such as intramolecular interactions and atomic charges with chemical reactivity and aromaticity current in Ritalin and its derivatives. The findings indicated that with increasing participation of the studied substitutions in intramolecular interactions, their effect on reactivity indices and NICS has increased. Therefore, substituents NO2 and Cl on the benzene ring, with the highest participation in intramolecular interactions, caused the highest increase in the resonance interactions of the benzene ring. As a result, they increased diatropic currents, enhanced aromaticity in the geometric center of benzene ring NICS (0.0), and increased chemical reactivity. Lone-pair electrons of Chlorine (LP Cls) also had the highest interaction with benzene backbone system compared to other substitutions, which increased diatropic currents and enhanced aromaticity at NICS values (0.5). The NBO results showed that electron-withdrawingsubstituents at positions R7 and R9reduced the accumulation of negative charge on adjacent atoms and benzene ring carbons.

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Conflict of interests

The authors declare that they have no conflict of interest.

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Evaluation of Nitrite Exposure from Meat Products Supplied in Tehran, Iran

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ABSTRACT

Nowadays, due to the increased consumption of meat products, investigation of their additives such as nitrite seems necessary. Nitrate in combination with salt has a synergistic characteristic against pathogenic microorganisms. Nitrite combines with protein, fats, and volatile and non-volatile compounds in meat and plays an important role in flavoring meat products. Excessive use of this substance leads to a more dangerous compound called nitrosamine. The present study aimed to investigate the nitrate content of meat products with different levels of meat. Health risk assessment toward nitrite was also calculated. A total of 108samples of meat products in various commercial brands were collected and analyzed from retail markets of Tehran to detect the amount of nitrite in them. Meat products in terms of their meat percentage were divided into three categories under 50%, 50% to 70%, and more than 70%. Estimated daily intake (EDI), based on per capita consumption rate and hazard quotient (HQ) for adults and children were also estimated. The mean concentration of nitrite in meat products with >70% meat, 50-70%, and <50% were 28.04, 30.07, and 27.02 mg kg-1, respectively that did not resemble any significant difference (p>0.05). The results indicated that the levels of nitrite were lower than permissible levels. The calculated HQ was less than 1 for meat products. However, this value was more for children. So is necessary to take precautions any over-consumption of this type of product in children.

KEYWORDS : Meat products; Nitrite; Red meat; Chicken; Exposure assessment

INTRODUCTION

Food additives are added to food products for a variety of reasons, including color, flavor, consistency, and increased shelf life [1]. In the meat products industry, nitrite is used to stabilize the product's color and prevent the growth of spoilage microorganisms. Nitrite in combination with salt has a synergistic characteristic against pathogenic microorganisms [2]. They inhibit the growth of Clostridium botulinum [3]. Nitrite combines with protein, fats, and volatile and non-volatile compounds in meat and plays an important role in flavoring meat products [4]. Excessive use of this substance leads to a more dangerous compound called nitrosamine. Under acidic conditions in the stomach, nitrite is converted to nitrosamines. Nitrosamine is a cause of bladder, stomach, and esophagus cancers [5]. Assessing and determining the amount of nitrite in food due to the formation of nitrosamine in the stomach is essential. Nitrite also reacts with the heme of myoglobin to form nitrosomyoglobin [6].

Most human exposure to nitrite is through consuming water and food including meat products which in some cases, too much nitrate is added. Nitrite poisoning due to consumption of meat products containing excessive nitrite has been reported in many countries [7, 8]. In the previous study, the amount of nitrite in meat products collected from the Tehran markets was reported in the remarkable amounts and in the range of 2.93–13.9 mg/100 g [9]. In recent years, quantification of nitrite and associated health risks studies has become popular [6-8, 10]. Therefore, the amount of nitrite in meat products and dietary health risks should be evaluated regularly. The main purpose of this study was determination of nitrite in different types of meat products and assesses human exposure to nitrite due to consumption of these products.

MATERIALAND METHODS

Sample collection

A total of 108 samples of meat products were collected from the Tehran retail market with different meat percentages. Meat products were divided into three categories in terms of meat percentage: under 50%, 50% to 70%, and more than 70%. All samples had specific production and expiration date. Samples were sent to the laboratory at cooled transport, immediately. Preparation of the samples10 g of each sample was poured to a 250 ml volumetric flask and then 100 ml water at 70 °C and 5 ml of saturated borax solution (Merck KGaA, Darmstadt, Germany) (50 g of sodium hydrate tetra borate dissolved in some water and then reached to a volume of one liter) was added respectively. This was left for 15 minutes inside the boiling water, then, cooled to laboratory temperature and 2 ml of protein precipitate solution1 (containing 106 g of hydrated potassium Ferro cyanide dissolved in water to a volume of 1 liter) (Merck KGaA, Darmstadt, Germany) was added. After stirring, 2 ml of protein precipitate solution 2 (containing 220 g of zinc acetate and 30 ml of concentrated acetic acid dissolved in water to a volume of one liter) (Merck KGaA, Darmstadt, Germany) was added. After maintaining for 30 minutes at room temperature, the flask was filled with distilled water to the marked line (250 ml). The certain volumes (20, 15, 10, 5 ml) of the filtered solution were added to 100 ml balloons and about 50 ml of water was added. Then, 10 ml of sulfanilamide solution (Merck KGaA, Darmstadt, Germany) and 6 ml of 5 N hydrochloric acid solutions (Merck KGaA, Darmstadt, Germany) were added to each of them and after mixing, the solutions were left in dark for 5 minutes and then 2 ml of N-(1-Naphthyl) ethylene diamine dihydrochloride solution (Merck KGaA, Darmstadt, Germany) was added and placed in the dark for 5 minutes and then the absorbance was recorded at wavelengths of 538 nm (UV-Vis Spectrophotometer, Hach, England).

Dietary survey and risk assessment for nitrite in meat products

It is estimated that Iranians use about 6 kg of meat products per year. Therefore, the daily meat product consumption is about 0.016 kg. The average weight of an adult is 70 kg. Estimated daily intake (EDI) was obtained using equation [1].

$$EDI\text{oral} = Ci \times Cc / \text{BW} \qquad (1)$$

Ci: the mean concentration of nitrite *(mg kg-1)*

Cc: the daily average consumption of meat product *(kg person-1 day-1)*

BW: (body weight)

The average weight of adults and children was considered 70 kg and 15 kg, respectively. JECFA calculated 0 0–0.07 mg kg-1.

BW per day as ADI for nitrite. The dietary risk assessment is performed by using the following equations [2].

$$\text{Hazard Quotient (HQoral)} = \text{EDI/ADI} \quad (2)$$

The HQ displayed possible health hazards from the ingestion of contaminated food.

Statistical analysis

Statistical analysis was performed by SPSS version 21. The Mean±SD was calculated for each group of samples. Kolomogorov-Smirnov test was used to determine the distribution of the data. All data had a normal distribution. Therefore, an independent T-test and one-way analysis of variance (ANOVA) were used to compare means.

RESULTS

Nitrite in meat products based on the percentage of meat

Residual sodium nitrite was measured as 27.02±12.3 mgkg1in products containing less than 50% meat; 30.07±16.2 mg kg-1 in products containing 50 to 70% meat, and 28.04±11.5 mg kg-1 in products containing more than 70% meat (Table 1). There was no significant difference in the amount of nitrite between these groups (p>0.05). Figure 1 shows the average nitrite in red meat and chicken. There was no significant difference between groups (p=0.25).

Table 1. Different products based on the percentage of red meat or chicken

Product type Percent of meat/chicken	Mean ± SD (mg kg^{-1})	Min –Max	Adult		Children	
			EDI	HQ	EDI	HQ
Under 50% N=25	27.02±12.3	5-53	0.0061	0.087	0.028	0.4
50 to 70% N=50	30.07±16.2	0.32-102	0.068	0.97	0.032	0.45
Over 70% N=33	28.04±11.5	11-61	0.0064	0.091	0.029	0.41

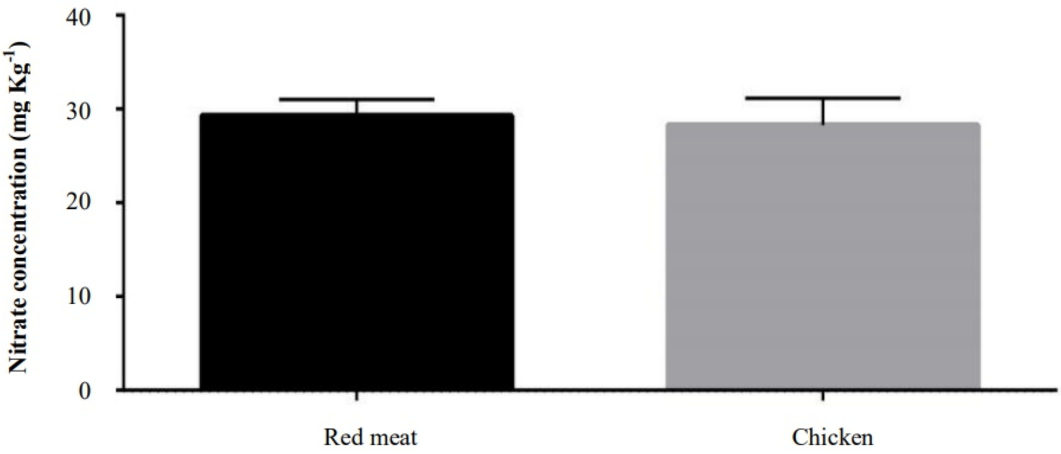

Figure 1. The concentration of nitrite in meat products based on the type of meat (red meat or

Dietary Exposure and risk assessment for nitrite in adult and children

Risk assessment was also performed in the present study for adults and children. The risk assessment of hazards is an effective way to manage the additive residues in foods. Daily dietary intake data of meat products were obtained from meat products traders' union survey. Children are very interested in consuming these types of products; therefore, the EDI of different products for adults and children was also calculated. The EDI was measured in the range of 0.068-0.0061 and 0.028-0.032 mg kg-1 bw day-1for adults and children, respectively (Table 1). Subsequently, the calculated HQ was less than one (Table 1) for adults and children.

DISCUSSION

In present study, the amount of residual nitrite in all samples was less than international and national standards. Similar results were observed in the samples collected in Belgium. Most of the samples were within the permissible limit and below 20 mg/kg [4]. Chetty et al. reported nitrite content in meat product samples in the range of 10.07 to 164 mg kg-1 in Fiji, which was more than the present study [6]. A total of 85% of meat products in China contained nitrite with the range of 0 to 2808.2 mg kg-1 [11]. Nitrite was detected in all 90 analyzed meat samples with an average amount of 46.2±10.1 mg kg-1 [12] that was not exceeded the maximum residue level of the European Union.

Excessive use of nitrite in meat will produce a significant amount of "nitrosamine" which is a carcinogen. Nitrosamine is produced due to the reaction of nitric oxide from nitrite with secondary amines [13]. Nitrites induce methaemoglobinaemia in children [7, 14].

The EDI in the current study was less than the amount approved by the JECFA (the Joint FAO/WHO Expert Committee on Food Additives) for nitrite. The JECFA committee considers a 0.07 mg kg-1 bw day-1 for nitrite. In a study conducted in New Zealand, the amount of nitrite in meat products was much higher than ADI [15], and HQ below one indicates no risk. The amount of HQ for children was less than one that is acceptable, but more than adults. It is necessary to suggest prudent measures in this regard. For example, these products should not be offered in school food stalls.

Elias calculated EDI for nitrite in meat products samples for children aged 3-10 years in Estonia as 0.016 mg kg-1 bw day-1 [16]. Furthermore, in a study conducted in Denmark, EDI for nitrite for the age of 15 to 75 years was calculated 0.003 mg kg-1 bw day-1 [15], which is lower than the current study. But in a study conducted in Sudan, EDI was calculated as 0.026-0.128 and 0.107-0.511 mg kg-1bw day 1 for adults and children, respectively, which were more than the present study [12].

Nowadays, consumption of meat products is increasing due to the increase in the rate of women's employment and the speed and ease of preparation and consumption of meat products. The first effective item in the selection of a meat product by a customer is the color of the product [17]. The main role of nitrite in meat products is to give appropriate color and flavor to the product, preserve fat and protein from the oxidation process, and protect it against microbial hazards [18]. However, its addition leads to the conversion of carcinogenic compounds [19]. Nitrite causes the development of some cancers. The amount of 10 to 20 mg kg-1 nitrite concentration is sufficient for the color stability of the product [4]. But, nitrite concentration for antimicrobial properties should be around 100 to 200 mg L-1 [6].

The maximum permissible limit for adding nitrite to the product is different from the maximum permissible for its residue in the product. According to EU regulations, the limit for potassium nitrite is 150 mg kg-1, while the limit for its residue in the product is 50 mg kg-1 (Table 2). According to Iran's National Standard Organization, the permissible adding amount of nitrite is 120 mg kg-1 in meat products while the residue amount is 60 mg kg-1 (Table 2). According to the codex standard of food additives, the permissible limit of addition has not been announced and the residue amount of nitrite in meat products is 150 mg/kg according to the European Union. Some countries are trying to reduce this amount, including Denmark, which has changed the limit to 60 mg kg-1 [20].

Table 2. The maximum residue level (MRL) of nitrite (mg kg-1) in different countries

Standard	National standard ISIRI, Number; 2303	European union	Codex *	The australian food standard[5]
Maximum adding level (mg kg^{-1})	120	150	-	125
Maximum residual level (mg kg^{-1})	60	50	80	-

*Codex standard on food additives

CONCLUSIONS

The nitrite concentration in 100% of the investigated products was within the permissible limit. Meat products with a lower percentage of meat are cheaper than other products. In this study, there was no significant difference between products with different percentages, so the price of products does not play an essential role in nitrite exposure. The amount of nitrite in these tested products is relatively low, so the possibility of nitrosamine formation is also low. Health risk assessment showed that adults and children are not at health risk of nitrite excess in meat products. Nitrite intake through meat products is less than the limits set by international agencies. Moreover, HQ showed no danger for adults and children. However, consumption of meat products in children must be accompanied with precautions.

Abbreviations

Bw: body weight; EDI: estimated daily intake; HQ: hazard quotient

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Conflict of interests

The authors didn't have any conflict of interest regarding the results of the study.

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Removal of Amoxicillin from Aqueous Solutions by using Synthesized Highly Hydrogel Surface as a Good Adsorbent

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A B S T R A C T

Because of the potential for reversible effects on living organisms and bacterial elaboration resistance, removing drugs from aqueous solutions is critical. Deals with the amoxicillin AMX removal trial using hydrogel. (FTIR), (F.E-SEM), and UV-visible spectroscopy were used to describe the hydrogel of sodium alginate-g-poly (Acrylic acid-fumaric acid). The purpose of the adsorption investigation was to determine the impact of (10-100 mg L-1) conc.of AMX Optimization appear to have the best percentage percent removal at 97.40 percent at concentration 100 mg L-1, and contact duration 2hr. Take a look at two isotherm models. The second order model (R^2= 0.9041) outperforms the Freundlich, Langmuir (R^2= 0.9772), and three types of kinetic models (first order, second order, and Elchovich).

KEYWORDS : Adsorption; Removal; Amoxicillin AMX drug; Isotherm; Kinetic model

INTRODUCTION

Nowadays, pharmaceuticals are considered one of the most important water pollutants because of their widespread use. Pharmaceuticals are classified as a class of health care products and are used all over the world to enhance human health [1-5] They are also applied in animal care and in agriculture, where antibiotics are released into wastewater and consider very dangerous materials [6-8]. Amoxicillin is an antibiotic with widespread use in veterinary and human medicine due to poor metabolism in the organism, where very large amounts of amoxicillin are discharged into effluents [9-11]. Therefore, there are several effective ways to remove drugs from wastewater, including ozone, photo oxidation and adsorption. These methods are characterized as simplest, easiest and cheapest used to remove pollutants, especially medicines, from water and sludge for use on very high efficiency, cheap and easy to prepare surfaces [12, 13]. In this research, a very highly effective hydrogel surface was used to remove amoxicillin, where several techniques were used, including FTIR, FESEM Where the effect of concentration of AMX drug, adsorption isotherms and Kinetic model were studied.

MATERIALS AND METHODS

The calibration curve, solutions of different AMX drug concentrations was prepared via sequential dilutions. The values of absorbance of these solutions were measured at the carefully chosen λmax value as appear in Figure 1.

The calibration in the concentration range is linear according to Beer-Lambert law. The chemical structure of AMX ($C_{16}H_{19}N_3O_5S$). The maximum absorbance of AMX happens at wavelengths of 230 nm. By weighing and dissolving 1.0 g of AMX, the 1000 mg L-1 drug solutions and their diluted working solutions were prepared fresh in 1000 mL elementary flasks. (Figure 2).

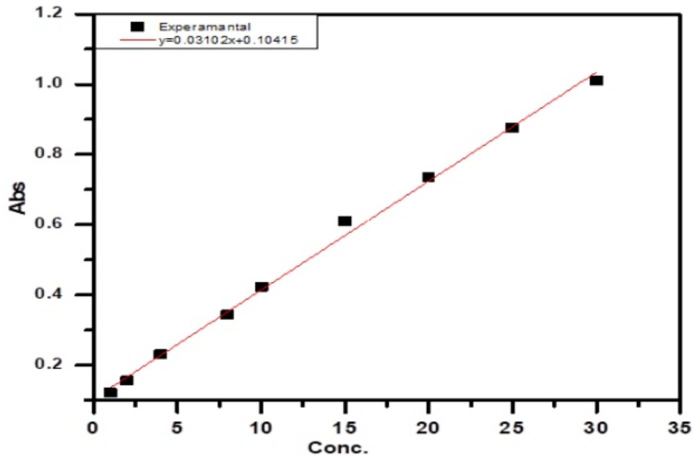

Figure1. Calibration curve for the AMX drug.

Preparation of hydrogel

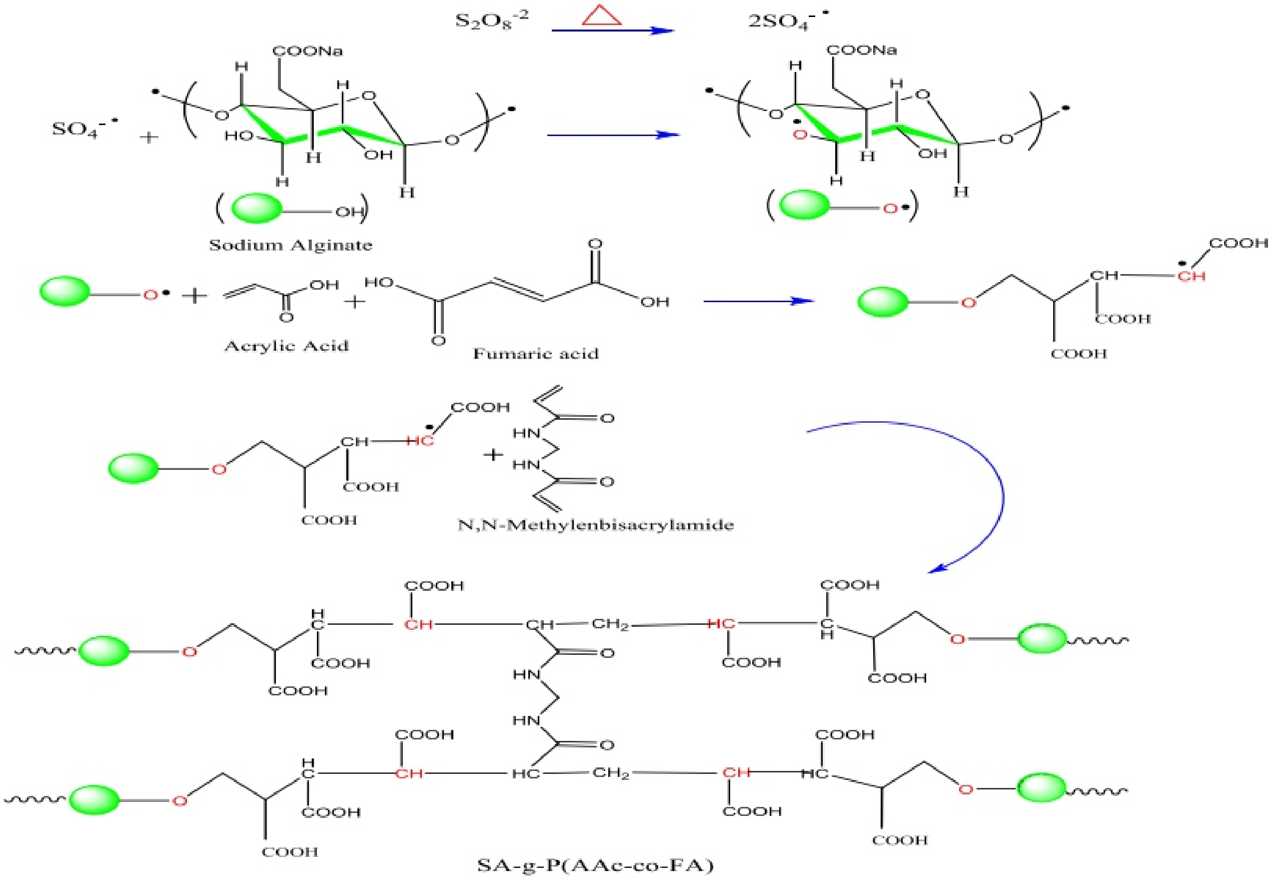

Figure 2. (Hydrogel of sodium alginate-g-poly(Acrylic acid-fumaric acid) preparation.

Effect of initial drug concentration

A series of several concentrations of AMX drug of 100 mL was utilizing in this study (10- 100)ppm, was addingto elementary flask in the presence of 0.05 g of hydrogelthese sequence were putting in a water bath shaker for 2hr, pH= 7.2 ; temp. 25 C; weight of hydrogel 0.05 gm for 100 ml After that, The remaining concentration was determined using a spectrophotometer after the supernatant was centrifuged at the *λmax* 230 nm for drug. The adsorption efficiency was calculated from equation (1): [14]

$$qe = \frac{(C_0 - C_e) * V_L}{m_{gm}} \qquad (1)$$

qe = The amount of AMX adsorbed per gram of hydrogel (mg/g). Co= Primary drug conc. (mg L-1), Ce= Equilibrium conc. drug (mg.L-1).m = weight of hydrogel (g). (E %) of the drug was determined using the decrease in absorbance at λmax [15]

$$E\ \% = \frac{C_{0-}C_e}{C_0} * 100 \qquad (2)$$

RESULTS AND DISCUSSION

FTIR

The hydrogel was studied using FTIR spectroscopy from 4000 to 400 cm-1, with a resolution of 1 cm-1. Figure 3shows the FTIR spectra of hydrogel before and after AMX adsorption. The fact that no new pick appears after the adsorption process, simply a slight change in the degree of adsorption, proves the adsorption process' presence, this is proof of the adsorption process'occurrence, which is of a physic sorption. [16, 17].

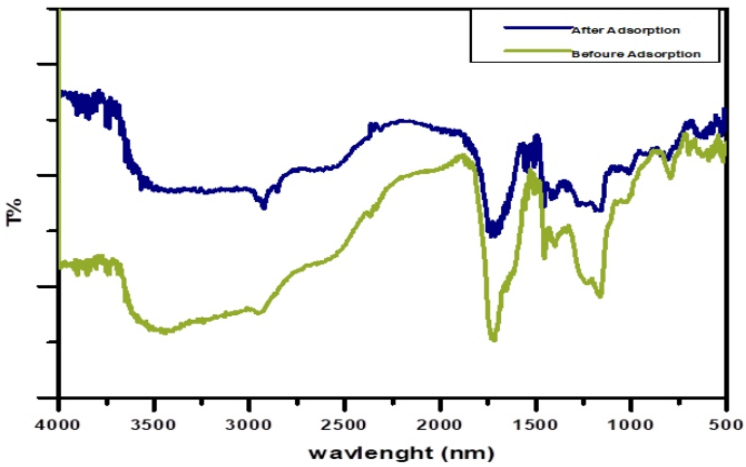

Figure 3. FT-IR hydrogel spectrum before and after AMX drug adsorption.

FESEM

Before the adsorption process, the hydrogel's surface has numerous voids and uneven assemblies, while after the adsorption process, the surface has few voids and uneven assemblies, the surface became smooth and smooth, indicating that the drug was loaded on the surface and the adsorption process occurred. [18, 19] as appear in Figure 4.

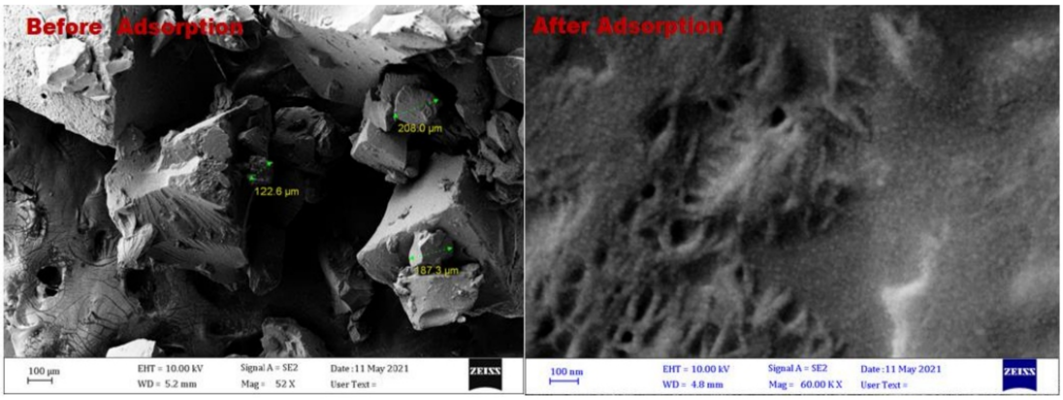

Figure 4. Before and after adsorption FESEM of hydrogel.

Effect of initial concentration of AMX drug

Figure 5 look the plot the amounts of AMX drug adsorbed (qe) and removal ®%) of AMX several initial concentration of AMX drug Co at various experimantal conditions. From the Figure, it can be look that the removal E% of AMX drug de-creased through in creasing in the concentrations of AMX and found the removal percentage E% decrease from (97.87% to 82.11%) but also the adsorption capacity of AMX risewith increase initial drug concentration and found the adsorption efficiency increase from (18.21 to 168.22 mg/g). because when the number of collisions increases with the increase in the initial concentration among drug and the hydrogel increasing, that get better the adsorption method. For the AMX utilized, there was a substantial effect of AMX drug concentration on hydrogel efficiency. [20-23].

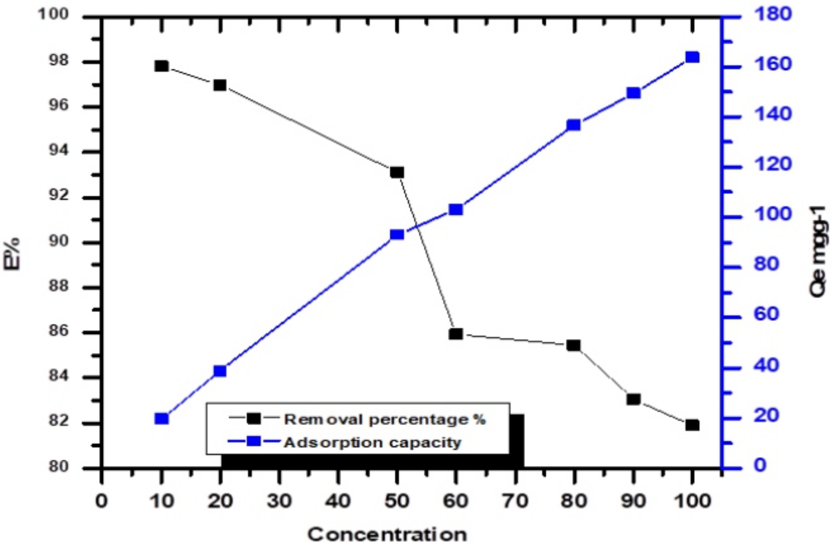

Figure 5. Effect of AMX drug adsorption concentration in a hydrogel (25°C, pH 7.2, weight of hydrogel 0.05 g).

Adsorption model

Freundlich isotherm

The Freundlich equation is one of the utmost significant utilized models in the case of adsorption of solution. [24]:This Isotherm accepts that the surface of the hydrogel is hetero-geneous because of the variance energy levels for adsorption sites Freundlich isotherm model has been defined in equation [25, 26]

$$q_e = K_f C_e^{1/n} \qquad (3)$$

Langmauir Isotherm: isotherm Langmauir has a widespread use to absorbe contaminants from the solution liquid [27, 28]. The adsorption isotherm of Langmuir single-layer models can be applied to solidliquid adsorption methods [29]. Here, adsorption Langmauir model has been defined in equation (6).

$$q_e = \frac{q_0 K_L C_e}{1 + K_L C_e} \qquad (4)$$

qe is for the amount absorbed (mg g-1), Ce stands for the adsorbent content in the solution after absorption (mg/L), and qo stands for the Empirical constant Langmauir, which represents the highest absorption efficiency (mg g-1). Furthermore, the Freundlech model and the KL empirical Langmauir constant (L mg-1) indicated a strong fit to absorb AMX onto hydrogel, R2 (0.9773) values and KF increase as adsorption temperature rises (Table 1), the model parameter values are shown in the figure of (Qemg g-1) vs. (Cemg L-1) (Figure 6) and (Table 1).

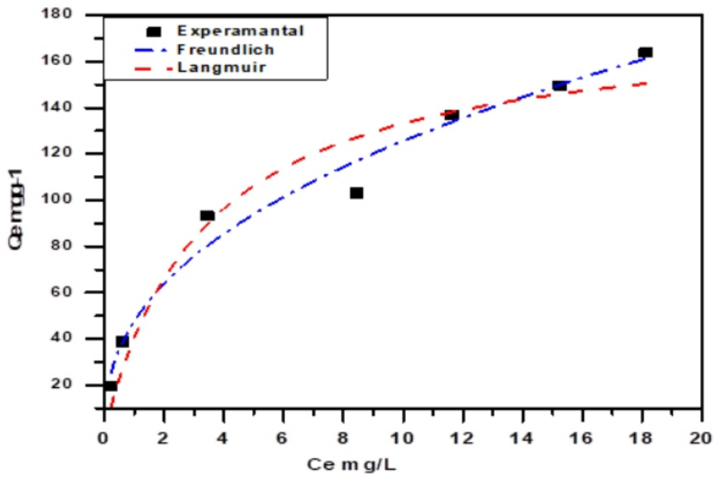

Figure 6. Different non-linear isotherm absorption model patterns for absorbing the AMX medication on hydrogel, main concentration = 100 mg L-1, temperature =25°C, hydrogel mass = 0.05 g

Table 1. At 25°C, the model variables for AMX medication absorbed on to hydrogel (Freundlech and Langmuair)

Isotherm models	Parameters	AMX
Langmauir	qm (mg g^{-1})	178.578± 20.092
	K_L(L mg^{-1})	0.2931±0.1241
	R^2	0.9333
Freundlech	K_F	47.772±5.377
	1/n	0.4197±0.0445
	R^2	0.9773

Kinetic models

The kinetics of adsorption provides details and information on the adsorption mechanics. Three kinetics adsorption models were used in this study: first models, second models, and the Elcovich model.

$$qt = qe\ [1\text{-}exp\ (kf\ t)] \qquad (5)$$

The kinetics adsorption process model is also known as a second order equation[30] . The nonlinear form of the t equation is as follows:

$$qt = \frac{K2qe2t}{1 + K2qet} \qquad (6)$$

Nonlinear of the Elcovich model (Chemi-sorption model kinetic) [49] as appear in equation 7:

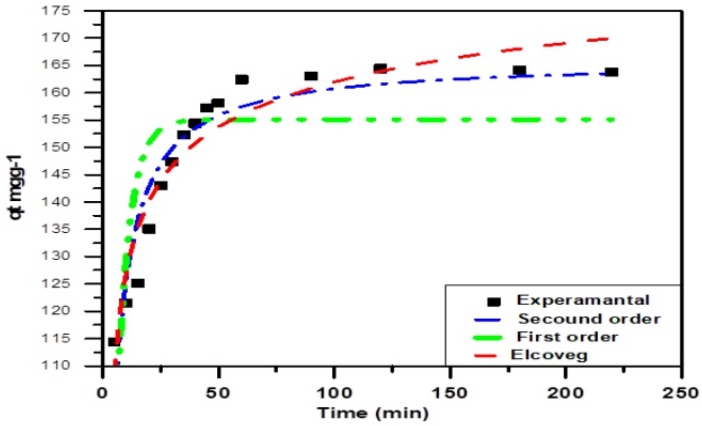

Figure 7. AMX drug adsorption on hydrogel (first and second order reaction kinetics, and Elcovich model).

Table 2. AMX drug adsorption on hydrogel, Elcovich model and correlation coefficients utilizing first and second order reaction kinetics

Model	Equation	Parameters	Value
First	qt = qe [1-exp (kf t)]	Kt(min^{-1})	0.1797 ± 0.0285
		qe(calc)(mg g^{-1})	155.114 ± 3.4333
		R2	0.5351
Second	$qt=\frac{K2qe2t}{1+K2qet}$	K2(g mg^{-1} min^{-1})	0.3103 ± 0.0410
		qe(calc)(mg g^{-}1)	165.903 ± 2.563
		R2	0.9045
Elcovich	$qt=\frac{1}{\beta}1\beta ln(\alpha\beta)+\frac{1}{\beta}lnt$	α (mg g^{-1} min^{-1})	5.340± 1.321
		β (g min^{-1})	50.576± 4.374
		R2	0.8751

CONCLUSIONS

1- In this study, to eliminate amoxicillin, a hydrogel surface with a high effectiveness was utilized.
Two types of adsorption isotherms have been studied, and the best obey is the Frendlig isotherm.2-3- Three kinetic first and second order reaction kinetics, as well as the Elcovich model, were investigated, with the second model proving to be the most effective.

4-The adsorption efficiency rises with increasing conc. Of AMX drug, with increasing concentration, the percentage E percent removal drops.

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Epidemiology of Childhood Cancer Based on the Databases of Population-Based Cancer Registries in City of Erbil, Iraq

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A B S T R A C T

Cancer disease in children is very rare and includes less than one percent of all cancers. However, it is one of the main causes of death among children. The aim of this study is to investigate the epidemiology of various cancers in children in Erbil city, Iraq. In this epidemiological study, in which the cancer registry data of Erbil city was used, the frequency distribution of cancer between 2014 and 2021 (for 8 years) in children under 18 years of age was investigated and the incidence rate per one million people was calculated. Data analysis was done using SPSS version 23 software and MS Excel 2016 software. The registered cancer cases related to the residents of Erbil city during these years were 1766 cases, among which blood cancers had the highest frequency. 59.7% of the cases have been observed in men, and the age group of 15-18 years (34.6%) had the highest frequency in comparison with other age groups. The average age at the time of diagnosis was estimated at 11.2 years. The minimum and maximum age-standardized incidence in this 8-year period is estimated to be 73 cases (year 2014) and 241 cases (year 2019) per million people, respectively. Based on the results of this study and contrary to our expectations, it was found that the incidence of cancer among children under 18 years old in Erbil city is not much different from developed countries.

KEYWORDS : Neoplasm;Children;Cancer registry;Epidemiology;Incidence rate

INTRODUCTION

One of the most crucial difficulties in urban environmental management is the accumulation of dust on impermeable surfaces, and air pollution, a major issue in contemporary society, is a form of pollution that may be fatal [1, 2]. In the city of Erbil, street dust pollution by heavy metals (HMs) and polycyclic aromatic hydrocarbons (PAHs), as well as their mineralogical and morphological characteristics, were studied [3]. According to the findings, there are significant ecological and health problems in the research region due to the high levels of Cu, Pb, Zn, Hg, and PAH contamination in street dusts, particularly in the city's high-traffic and industrial zones. It was also showed that exposure to PAHs in street dust puts people of the Erbil city at risk of developing cancer. The prevalence of chronic diseases has increased despite recent advances in the management and prevention of infectious diseases [4, 5]. In the meantime, cancer is regarded as one of the most significant public health issues in several nations, coming in second to cardiovascular illnesses as the second leading cause of death [6, 7]. In developed nations, cancer ranks as the second most common cause of death for children. The likelihood of cure has increased significantly over the past few decades thanks to highly specific diagnostic techniques, the development of multimodal treatment approaches, and their ongoing improvement. For patients, their families, the oncologists who are treating them, as well as from the perspective of public health, childhood cancer and its treatment have nevertheless remained a challenge. In 2020, there were 12,400 cancer diagnoses among American children and adolescents under 20 [8]. Between birth and the age of 20, there is a 1 in 300 chance that a particular child will develop cancer [9–11]. In this age group, cancer-related mortality was thought to account for 2,300 deaths in 2021, or about 8% of all deaths [12]. Cancer is the second most common cause of mortality for children aged 5-14 and the third highest cause of death for children aged 1-4 [13–15]. However, cancer is the third leading cause of mortality in Iraq, following cardiovascular disease and accidents [16]. Although it is known that some postnatal exposures (radiation, viruses) and prenatal (such as radiation, diethylstilbestrol [DES]) can raise the risk of various childhood cancers, the exact cause of most cases of childhood cancer is still unknown[17,18]. The International Classification of Childhood Cancers (ICCC) divides childhood cancers into 12 major histological types (Figure 1) [19].develop cancer [9–11]. In this age group, cancer-related mortality was thought to account for 2,300 deaths in 2021, or about 8% of all deaths [12]. Cancer is the second most common cause of mortality for children aged 5-14 and the third highest cause of death for children aged 1-4 [13–15]. However, cancer is the third leading cause of mortality in Iraq, following cardiovascular disease and accidents [16]. Although it is known that some postnatal exposures (radiation, viruses) and prenatal (such as radiation, diethylstilbestrol [DES]) can raise the risk of various childhood cancers, the exact cause of most cases of childhood cancer is still unknown[17,18]. The International Classification of Childhood Cancers (ICCC) divides childhood cancers into 12 major histological types (Figure 1) [19].

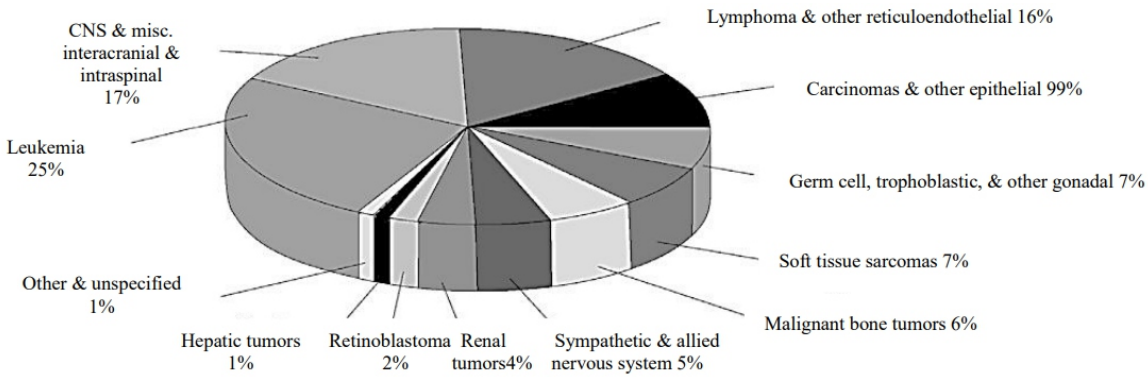

Figure 1. The prevalence of childhood cancer (age 0-18).

The epidemiology of cancer in children differs from that in adults, making blood cancers, lymph node cancers, and cancers of the central nervous system the most prevalent types of cancer in children. According to reports, there are between 62-121 and 71-156 cases of cancer per million people for girls and boys, respectively, in different geographic regions of Iraq [20]. Based on information from the Erbil cancer registry, the goal of this study is to look into the incidence rate of cancer in children under the age of 18 as well as the frequency distribution (age and sex) of different cancer types in children.

MATERIALAND METHODS

The Kurdistan Region of Iraq, often known as Iraqi Kurdistan, is an autonomous region in northern Iraq with Erbil as its capital and three additional governorates: Halabja, Sulaymaniyah, and Duhok. It is formally recognized by the Iraqi constitution under the name Kurdistan Region of Iraq. The city of Erbil, which is the largest in northern Iraq and is situated around 350 kilometers north of Baghdad, is situated between latitudes 36° 07′ and 36° 15′ N and longitudes 43° 55′ and 44° 07′ E (Figure 2).

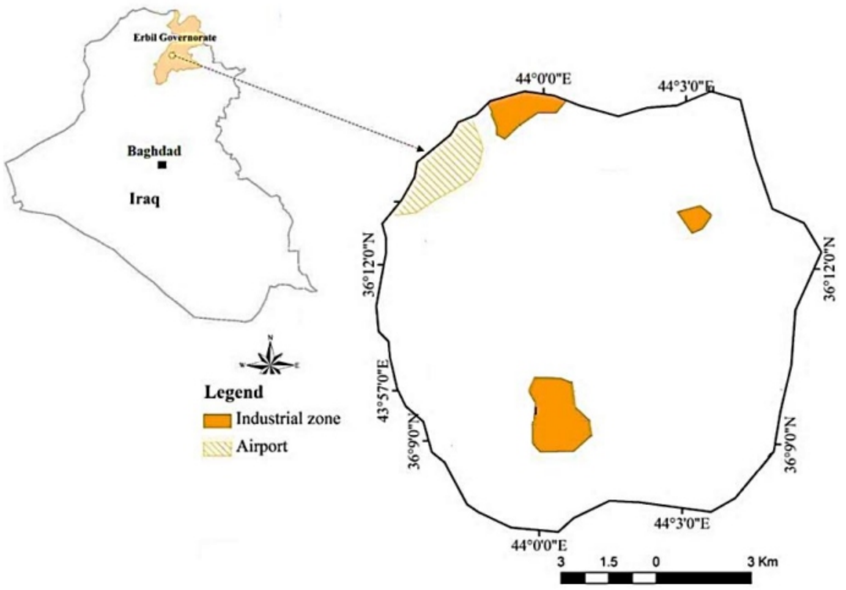

Figure 2. Industrial zones of Erbil metropolis.

Kurdistan Region's economy is dominated by the tourism, agriculture, and oil industry. It has a population of roughly 900,000 people and a governorate with a permanent population of 2,110,419 as of 2018. Erbil's climate is categorized as hot-summer Mediterranean because It has an average annual temperature of 20.2°C and experiences 540 mm of precipitation [21]. Table 1 provides annual emission of air pollutants from various sources in Erbil city [22].

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Table 1. Annual emissions of various pollutants in Erbil.

Years	CO (ton)	HC (ton)	NO_x (ton)	CO_2 (ton)	Mass Particulate (Pm) (ton)
2014	17.12	2.02	1.30	267.75	0.12
2015	35.30	4.15	2.69	553.35	0.24
2016	58.29	6.87	4.47	934.50	0.51
2017	80.64	9.51	6.24	1316.70	0.84
2018	113.44	13.40	8.85	1890.00	0.84
2019	171.86	20.35	13.59	2953.65	1.38

The data used in this epidemiological study is from the cancer registration program of Erbil city, between 2014-2021. Prior to 2019, pathology centers were used in this province for cancer registration; however, since thenpreparations have been made for population-based cancer registration. A group of relevant experts assists in implementing the cancer registration program. The necessary data for cancer registration is gathered from pathology and non-pathology centers, with pathology centers providing 80% of the information and nonpathology centers providing 20%. It should be mentioned that there are about 77 pathology centers in Iraqi Kurdistan, of which 54 are located in the center of the province, city of Erbil. In this province, non-pathology centers include the deputy health department's death registration office, hospital medical records, private and public hematology-oncology clinics, legal medicine, immunohistochemistry clinics, flow cytometry clinics, imaging clinics, drug and alcohol monitoring departments, and blood transfusion facilities. The incidence rates were calculated per million people, and the incidence rate was standardized by direct standardization method and using the standard population of the United States of America in the year 2000 [23,24]. The collected information was coded using the International Classification of Diseases for Oncology (ICD-O) method [25], and after entering the data into the software, the patients were sorted alphabetically in order to check for repeated registration, and the duplicates were deleted. Also, the patients who came to the treatment centers of Erbil city from other regions were not considered in the calculations, although it should be mentioned that a small percentage of the patients of this region may have gone to the neighboring provinces for treatment. Using the exponential estimation method, the population of the Erbil city was calculated for these years, and the rate of population growth between 2004 and 2019 was 1.31 [26]. SPSS version 23 was used to describe the data.

RESULTS

A total of 2433 cases were registered during the 8 years under review (2014-2021), of which 516 cases (21.2%) were connected to cases outside the province that were excluded from the study and 1766 cases were looked into in Erbil city. As shown in Table 2, the most common cancer was blood cancer (44.34% of cases), followed by eye, brain and other parts of the central nervous system and lymph nodes with 9.40% and 9.23%, respectively.The least common types of cancer, with 1.81% of cases, were those of the lip, mouth, and pharynx. The most cases were diagnosed in 2019 (21.1%). Men made up 59.7% of the cases, and compared to other age groups, the 15-18 age groups had the highest frequency, with 531cases (34.6%). Figure 3, breaks down childhood cancer incidence rates by gender and age group by year. The highest incidence of cancer has been observed in men aged 15-18 years. The mean age at the time of diagnosis (± standard deviation) was estimated to be 11.2±4.6 years.

Table 2. Frequency distribution of tumor location in cancer patients registered in the registration center of Erbil city between 2014-2021.

	Contaminated location	**Number**	**Percent**
Tumor location	Blood cancers and endothelial network	783	44.34
	Eye, brain and central nervous system (CNS)	166	9.40
	Lymph nodes	163	9.23
	Bones and joints	128	7.25
	Urinary tract and kidney	66	3.74
	Digestive system	66	3.74
	Skin	55	3.11
	Soft tissues	53	3.00
	Thyroid and endocrine glands	50	2.83
	Respiratory system	40	2.27
	Lips, mouth and pharynx	32	1.81
	Unknown primary member and other cases	164	9.29
	All patients diagnosed	1766	100

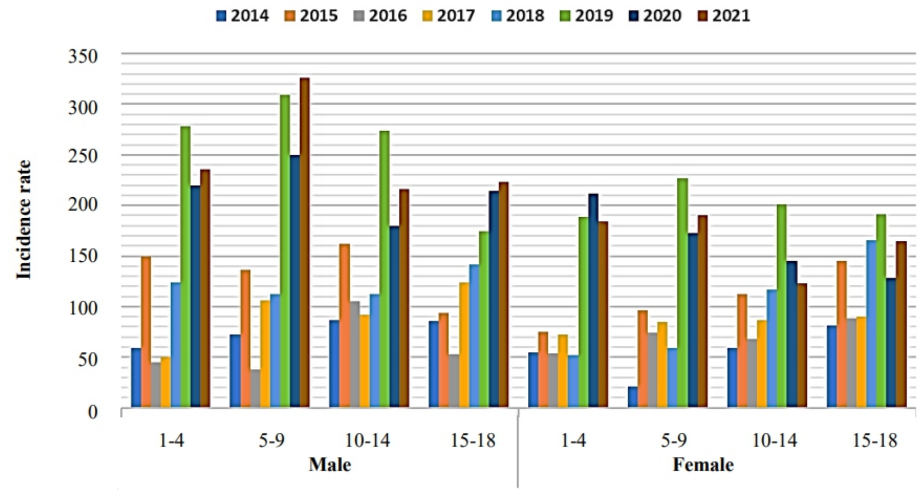
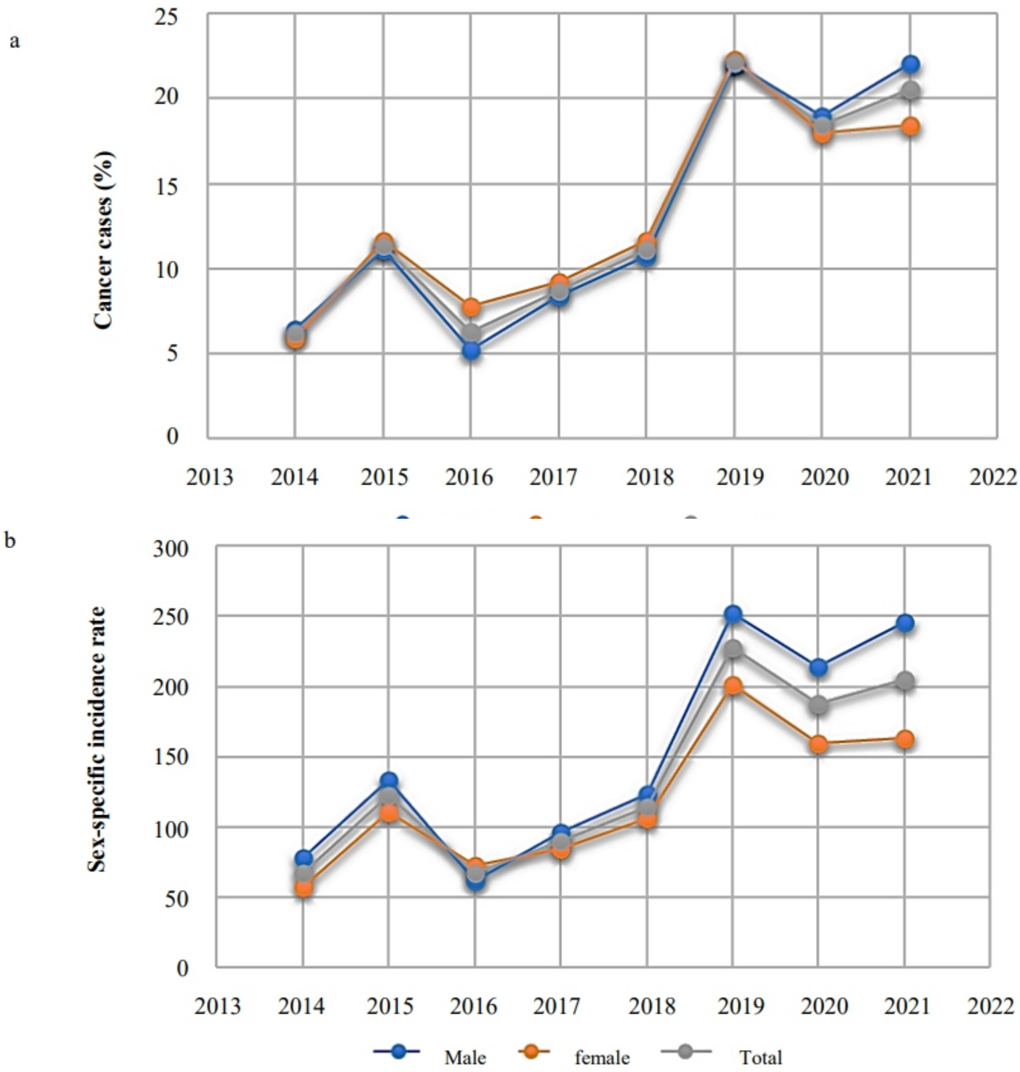


Figure 3. The age-sex specific incidence rate of cancer (per one million people) in children aged 0-18 between 2014-2021.

As shown in Figure 4, the age-standardized incidence rate of cancer between 2014 and 2021 was equal to 73, 118, 73, 89, 112, 241, 199, 213 cases per million people, respectively.



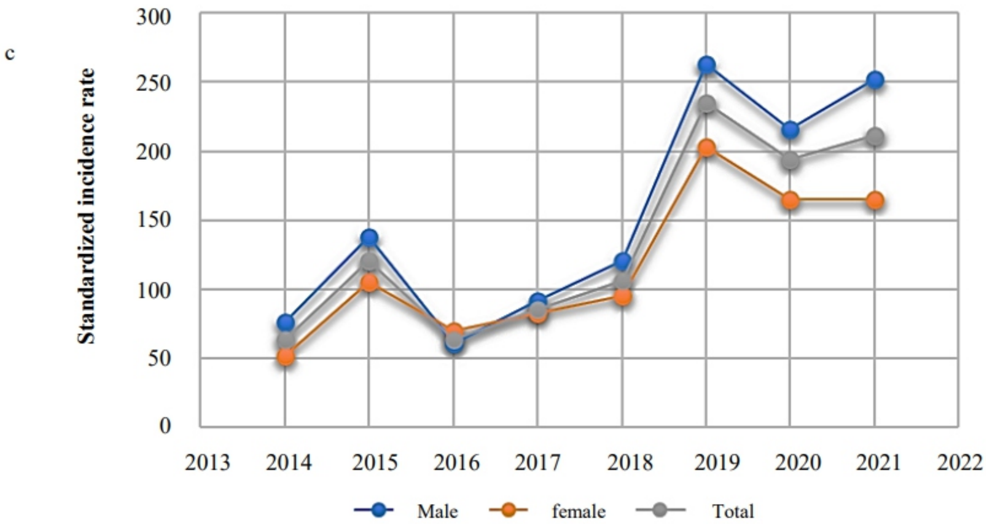


Figure 4. In children of 0-18 age range in Erbil city between 2014-2021: (a) the percentage of cancer cases, (b) sex-specific and © age-standardized incidence rate of cancer (per one million people).

DISCUSSION

Although all forms of cancer in people under the age of 18 are extremely uncommon and make up less than one percent of all cancers, they are still one of the leading causes of death in children, and since Iraq is one of the nations with a young population, with a sizeable portion of that population falling under the age of 18, it is important to pay attention to the issues that this group faces. The results of a census carried out in 2019 by the Iraqi statistical center revealed that more than 31% of the population in the Erbil city is comprised of individuals who are younger than 18 years of age. Blood-related cancers made up the highest proportion of new cancer cases during the 8 years under investigation in this study, similar to other studies carried out in various societies[27–31]. Following that, cancers of the eye, brain, CNS,lymph nodes, bones, and joints, urinary tract, kidney, digestive system, skin, soft tissues, thyroid and endocrine glands, respiratory system, lip, mouth, and pharynx, as well as breast and other cancers, were ranked next. Other studies found that blood cancer was most common, followed by lymph node, eye, brain, and CNS cancers[32–35]. A study in the city of Basrah using cancer registry data came to similar conclusions [36]. In this study and over the course of 8 years (2014–2021), the age-standardized incidence rate of cancer in individuals under the age of 18 was equal to 73, 118, 73, 89, 112, 241, 199, 213 cases per million people, respectively.These figures demonstrate a rising cancer incidence in this age group. Of course, a significant portion of this rise in incidence rates may be attributable to a modification in the process of recording new cases of cancer; as discussed in the methodology section, preparations for population-based cancer registration have been made since 2019, whereas prior to that, cancer registration in Erbil was based on pathology centers.According to a study of the Basrah there are 100 new cases of cancer for every million people under the age of 19 [36]. It was calculated to be between 126.7 and 87.8 cases per million people in a study done in India between 2010 and 2018 [37]. Also, in the study conducted in Switzerland (1985-2014), the incidence rate of cancer was calculated as 142 cases per one million people [38].Another study that looked into the epidemiology of childhood cancer in Europe found that the British Isles had the lowest incidence rate (130 cases per million people) and Scandinavian countries had the highest incidence rate (160 cases per one million people) [39].According to the GLOBOCAN report from 2018, developing nations like Kenya, Iran, Egypt, India, and Zimbabwe have the lowest incidence rates of most cancers (at all ages) compared to developed nations (in both sexes) [40]. The highest incidence rates are found in developed nations like the United States of America, Canada, and Denmark for women and Hungary, Poland, and Belgium for men. The insufficient recording of data on some variables and the patient referral to other provinces are two of this study's limitations that could be mentioned.

CONCLUSIONS

Based on the findings of this study, it was discovered that Erbil city, a region of a developing country, has a different incidence rate for the under 18 age group than developed countries, particularly in the study's most recent years, which are based on registered information based on population. This might be as a result of the alteration in lifestyle and the greater impact it has on children. Although the difference in the incidence of cancer between developing countries and advanced countries is greater in old age, this is likely due to the fact that people who have reached this age belong to older cohorts and are, by nature, less affected by changes in lifestyle.

Conflict of interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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