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Original article

Exploring the Effects of Amiodarone on Liver Proteins and Lipids and the Enhancing Role of Vitamin E: Spectroscopic Study

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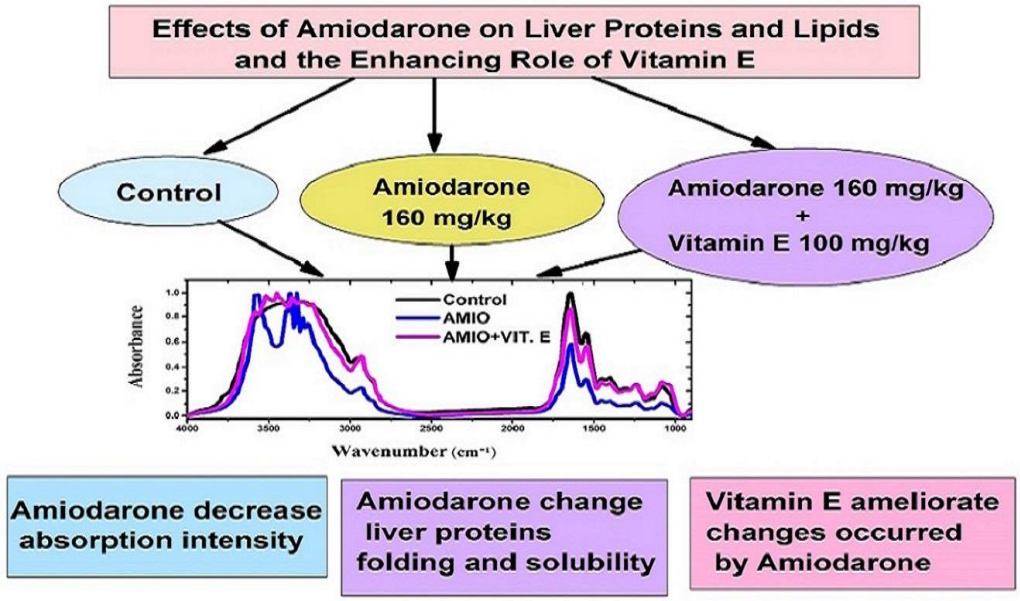
ABSTRACT

Numerous forms of liver damage, from moderate enzyme increase to severe acute liver failure, have been linked to Amiodarone (AMIO), a commonly used antiarrhythmic drug. The present study aimed to evaluate the changes in rabbits' liver resulting from short-term administration of AMIO, in addition to studying the ameliorative effect of vitamin E (VIT. E) on these changes. In this study, three main groups were involved: the control or normal group, AMIO group intraperitoneally (ip) injected with 160 mg AMIO/kg bw/day, and the third group orally dosing 100 mg/kg bw of VIT. E with the 160 mg AMIO/kg bw (ip)/day for two weeks. Fourier transform infrared spectroscopy (FTIR) study was performed on liver samples. The results confirmed that AMIO administration was associated with a change in the absorption intensity after the normalization of all spectra. Additionally, varying effects on the constituents of liver cells in fingerprint and NH-OH regions were noticed. The bandwidth of the CH_2 asym. band showed a significant reduction. Furthermore, the coadministration of VIT.E with AMIO enhanced most of the observed changes. Deconvolution of the amide I region revealed that the percentage of the β-turn was significantly elevated while the α-helix and β-sheet contents were decreased. Coadministration of VIT.E has the reverse effect on α-helix content. So, our study revealed that medication by AMIO could produce changes in liver proteins and lipids. VIT.E coadministration has an enhancement on these changes, which makes it a good supplement for those who are treated with AMIO.

Graphical abstract



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1. Introduction

Amiodarone (AMIO) is considered one of the diiodinated benzofuran derivatives. It was used in the treatment of angina in the mid-20th century. In addition, it has been found to have beneficial effects on heart rhythm, as it can treat arrhythmias that may cause sudden death. Since the approval of AMIO by the United States Food and Drug Administration (USFDA) in 1985, it has been consumed worldwide to medicate ventricular arrhythmia [1-3]. Although potassium channel blockage is AMIO mechanism of action in the heart, it can also block calcium and sodium channels as well as α-adrenergic and β-adrenergic receptors [4].

Many users of AMIO drug have noticed that it is associated with some side effects that have arisen, such as its impact on the liver, kidneys, optic nerve, and lungs [5]. Therefore, about 23% began to refrain from using it at its initial appearance. In the long term, after the development of these side effects, nearly half of its users have refrained from using it [6, 7]. Approximately 14% to 82% of patients treated with AMIO experience liver problems, which are characterized by a slight increase in blood transaminase [8]. About 25% of people on the medication had increased liver enzymes, and 3% had "symptomatic" hepatitis [4]. Amiodarone-induced toxicity has been attributed to various mechanisms, such as phospholipidosis, lipid peroxidation, and stimulation of the production of reactive oxygen species; this may lead to increased biomolecule oxidation [8]. Experimental studies have proven that AMIO has oxidative stress due to its effect on mitochondrial H_2O_2, as it works to increase its production [9]. Vitamin E (VIT. E) is a powerful antioxidant since it can scavenge lipid free radicals by participating hydrogen ions from its phenolic group on the chromanol ring [10].

In 2011, Zidane demonstrated that it is possible to overcome lysosomal phospholipidosis caused by AMIO by taking VIT. E [11]. In addition, other studies have shown the beneficial effects of VIT. E on the lungs, which have been negatively affected by the AMIO medication [12]. In 2009, Zaki and others demonstrated that the intake of VIT. E alongside AMIO can reduce the deposits of phospholipids in liver cells and lessen the harmful effects on the nucleus [13].

So, the recent study has focused on providing AMIO to rabbits and evaluating the changes in their livers resulting from AMIO short-term administration, in addition to studying the ameliorative effect of VIT. E on it by using FTIR spectroscopic analysis.

2. Materials and methods

2.1. Materials

Cordarone®, 200 mg (AMIO commercial form) and VIT. E 400 mg was acquired from Sanofi and Pharco Pharmaceuticals Company respectively. The remaining chemicals were obtained from Sigma Aldrich. Animals used in this study were New Zealand white rabbits (male and female) which were haphazardly chosen from the animal house facility located at the Research Institute of Ophthalmology in Giza, Egypt. The methodology was accepted by the local ethics committee in accordance with The Association for Research in Vision and Ophthalmology guidelines (approval number 103/2018). The animals were kept under optimum conditions (proper ventilation, consistent airflow and lighting, a normal feed, and temperature: 25±2 °C).

2.2. Methods

Rabbits were categorized into three groups, with ten rabbits in each group (totaling 20 eyes). One group served as the normal one, and the second group received an intraperitoneal (ip) injection of 160 mg/kg body weight (bw) of AMIO (2 ml for each rabbit) every day for two weeks [14]. Finally, the last group was dosing 100 mg/kg bw of VIT. E orally, along with an ip injection of 160 mg/kg of AMIO per day for the same duration [11].

After ending the dosing interval, rabbits were decapitated and dissected, and their livers were removed to carry out FTIR spectroscopic analysis. To acquire the FTIR spectra, transparent KBr disks were prepared by pressing a combination of 5 mg of liver tissue powder with 95 mg of KBr powder. The device used for this measurement was Nicolet is5 an infrared spectrophotometer with an effective resolution of 2 cm^{-1}. Continuous Nitrogen gas flow was applied while recording the FTIR spectra to eliminate the noising effects of ambient Carbone dioxide and water vapor on the results. Ordinarily, each sample has 100 interferograms. For more noise reduction, the spectra were smoothed using the Savitsky-Golay smooth function (11 points). The spectral data for each sample within the same categorical group were subjected to averaging. Consequently, the comprehensive group spectrum can be derived utilizing the OriginPro 2016 (64-bit) software suite developed by Origin Lab Corporation, Northampton, MA 01060, USA. The resultant group spectrum was meticulously adjusted and assessed by employing the identical software program. [15].

2.3. Statistical analysis

The results were presented as the mean±SD. One-way ANOVA was conducted to compare groups using SPSS-11 for Windows (SPSS Inc., Chicago, IL, USA), with a significance threshold of $p < 0.05$.

3. Results

Figure 1 exhibited the overlaid FTIR graph of the normal liver, AMIO, and Vit. E groups with the range 4000-900 cm^{-1}. By studying this graph, it was observed that the injection of the AMIO drug produced a decrease in absorption intensity compared to the normal group, while co-administration of Vit. E makes it look like the control one.

By analyzing the Fourier transform, we can obtain four main regions that are the subject of study. They are as follows; (stretching NH-OH region) 4000 – 3000 cm^{-1}, (CH stretching region) 3000 – 2800 cm^{-1}, (fingerprint region) 1800 – 900 cm^{-1} and (Amide I region) 1700 – 1600 cm^{-1}

NH-OH region.

NH-OH stretching region for control and all studied groups in the range 4000–3000 cm^{-1} were displayed in Figure 2. The overlaid spectra shown in panel (a) illustrated that treatment with AMIO changed the pattern of the bands concerning normal samples. After deconvolution as shown in panel (b), the outline of the control group was divided into six structural bands, which were positioned at 3622±4, 3490±3 cm-1, 3364±5 cm^{-1}, 3250±4 cm^{-1}, 3140±2 cm^{-1}, and 3064±3 cm^{-1}, corresponding to stretching OH ($_{str}$O-H), OH asymmetric (O-H_{asym}), asymmetric N-H (N-H_{asym}), O-H symmetric (O-H_{sym}), N-H symmetric (N-H_{sym}), and finally, C-H $_{ring}$. Assignments stated in Table 1 were founded on Dovbeshko et al. [16]. After the AMIO injunction, the first band corresponding to the ($_{str}$O-H) shifted to a lower frequency, and its width stimulated a significant growth ($p<0.05$). The OH asymmetric band also showed an obvious rise in wavenumber and a clear decrease in bandwidth. In addition to these changes, the N-H_{asym} and O-H_{sym} modes were split into two bands. Likewise, the O-H_{sym} band is centered at 3250±4 cm^{-1}. Moreover, the residual vibrational bands N-H_{sym} and aromatic CH bond were missed in the AMIO group. Coadministration of VIT. E with AMIO showed some enhancements, like the re-emergence of the C-H ring band and the disappearance of splitting occurring in the N-H_{asym} and O-H_{sym} modes. In addition to some recovery in the frequency of the remaining bands.

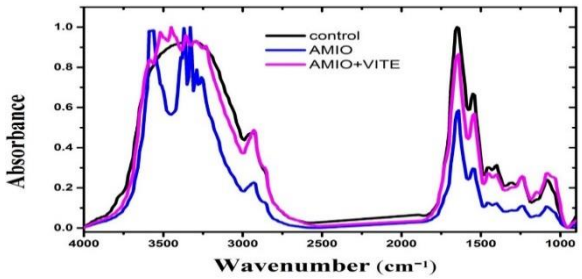

Figure 1: FTIR spectra for liver tissue of control rabbits, AMIO and AMIO with VIT. E groups.

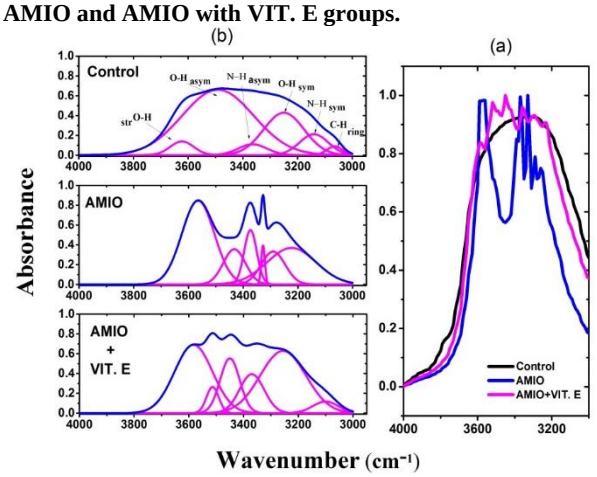

Figure 2: NH-OH region (4000-3000 cm^{-1}) related to control Liver, AMIO and VIT. E treated groups.

Table 1: NH-OH region (4000-3000 cm^{-1}) of Liver tissue for control and all studied groups.

	$_{str}$O–H		O-H_{asym}	N-H_{asym}		O-H_{sym}		N-H_{sym}	C-H_{ring}
Control	3622±4 101±5		3490±3 306±2	3364±5 124±2		3250±4 174±3		3140±2 123±3	3064±3 68±4
AMIO	3564±3* 139±2*		3432±4* 95±3*	3372±4 54±3*	3327±4 15±1	3290±5* 103±3*	3223±3 212±4		
AMIO + VIT. E	3582±5* 163±3*	3513±4 52±1	3450±2* 84±5*	3370±2 105±4*		3255±3 196±6*			3100±3* 110±5*

*** Statistically significance**

Mean±SD expressed for all bands. The first line showed the wavenumber (cm^{-1}), the second line for the bandwidth.

CH region

As shown in Figure 3, the CH stretching region has been displayed, which ranges from 3000 to 2800 cm^{-1}. From panel (a), it is noticed that AMIO administration causes a reduction in the absorption intensity, and administration of VIT.E with AMIO increases the intensity to mimic the control one. The curve enhancement procedure in the control liver indicated the existence of three bands located at 2967±2, 2928±2, and 2861±1, cm^{-1}, corresponding to asymmetric CH_3 ($_{asym}CH_3$), asymmetric CH_2 ($_{asym}CH_2$), and symmetric CH_2 ($_{sym}CH_2$), respectively. These components have been approved according to what Bozkurt et al. have proven [17]. Band position and width variations according to AMIO and VIT. E coadministration of these stretching vibrational modes was displayed in Table 2. Treatment with AMIO did not show clear changes except for the significant decrease in the bandwidth of $_{asym}CH_2$ mode. This decrease disappeared after the coadministration of VIT. E with AMIO, and its width mimics the control.

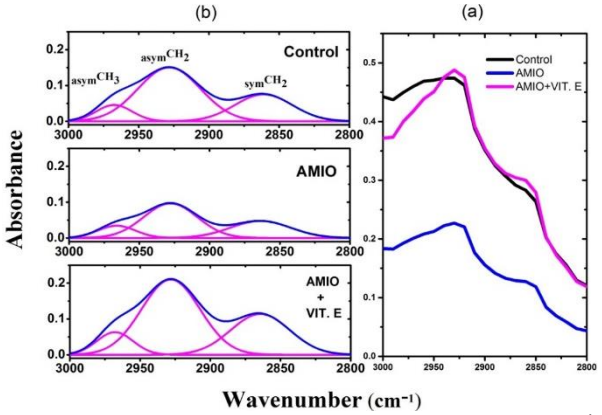

Figure 3: spectra of CH stretching region (3000-2800 cm^{-1}) of control liver and all studied groups

Table 2: CH region (3000-2800 cm^{-1}) of liver tissue for control and all studied groups.

.	$_{asym}CH_3$	$_{asym}CH_2$	$_{sym}CH_2$
Control	2967±2 29±3	2928±2 52±1	2861±1 47±3
AMIO	2966±1 31±2	2927±1 44±2*	2863±1 50±3
AMIO + VIT. E	2967±2 30±5	2928±2 49±2	2864±3 49±2

*** Statistically significance**

Mean±SD expressed for All bands. The first line shows the wavenumber (cm^{-1}), and the second line for the bandwidth.

Fingerprint region

The curve deconvolution methodology facilitated the extraction of eleven vibrational bands from the spectral pattern of the control group: the control group pattern: 1405±1 and 1384±2 cm^{-1} corresponding to COO_{sym}, 1089±2 and 1062±2 cm^{-1} related to PO_{2sym}, 1740±2, 1647±2, 1542±1, 1447±1, 1310±2, 1234±1 cm^{-1} and 1157±2 cm^{-1} corresponding to $_{Ester}C=O$, Amide I, Amide II, $_{bend}CH_2$, $CH_{3\ def}$, PO_{2asym} and $COOC_{asym}$ respectively. These assignments agreed with Cakmak et al. [18].

The fluctuations in vibrational frequency and bandwidth result from the injection of AMIO either alone or with VIT. E relative to the control was illustrated in Table 3. From Figure 4 and Table 3, we can notice the impacts of AMIO on the $_{Ester}C=O$ vibrational mode. There was a shift towards the lower position accompanied by an increase in the bandwidth. Co-administration of VIT.E. improved these outcomes. Lessen in bandwidths of the amide I, $_{bend}CH_2$, CH_{3Def}, and $COOC_{assym}$ modes after AMIO injection. Conversely, amide II, COO_{sym}, and both PO_{2sym} and PO_{2asym} have significant increases in bandwidths. Besides the disappearance of the first band of PO_{2sym} and the second band of COO_{sym} mode.

Coadministration of VIT. E with AMIO caused an improvement in the frequency of $_{Ester}C=O$, Amide I, Amide II, and bend CH_2 and $CH_{3\ def}$ modes, but they still need little enhancement in their widths but are better than the AMIO group. Additionally, two missed bands that correlate to COO_{sym} and PO_{2sym} have reappeared; nevertheless, the position and width of the PO_{2sym} second band still require improvement. After VIT. E co-administration, most of the fingerprint region components became near normal values.

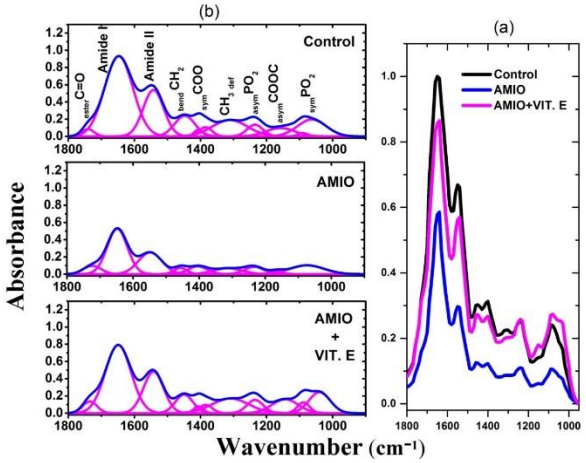

Figure 4: FTIR spectral region (1800-1000 cm^{-1}) corresponding to fingerprint region of control liver and all studied groups

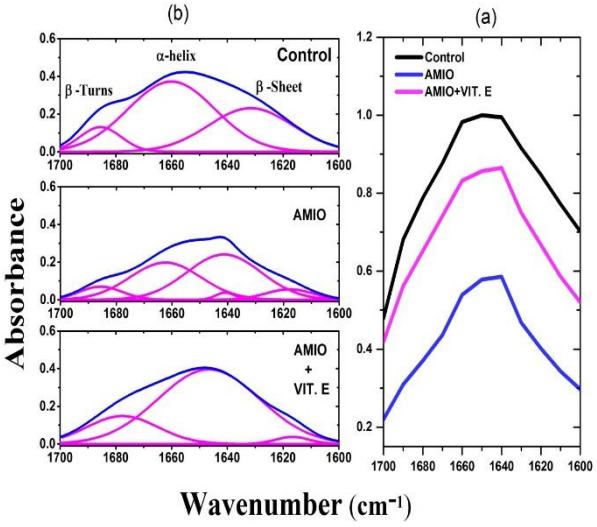

Figure 5: Amide I region FTIR spectra (1700-1600 cm^{-1}) assigned to control liver and all studied groups

Table 3: Fingerprint region (1800-900 cm^{-1}) of the liver tissue for control, AMIO, and VIT. E. treated groups.

	Ester C=O	Amide I	Amide II	bend CH_2	COO_{sym}		CH_3 def	PO_2 asym	COO_{asym}	$PO_{2\ sym}$	
Control	1740±2 42±3	1647±2 101±4	1542±3 73±1	1447±1 71±3	1405±1 30±1	1384±2 45±3	1310±2 124±4	1234±1 56±2	1157±2 101±5	1089±3 35±3	1062±2 101±2
AMIO	1723±1* 63±2*	1647±1 71±3*	1548±2 83±4*	1456±2* 44±1*	1404±2 67±1*		1313±2 99±2*	1233±2 67±1*	1165±1* 43±2*		1073±3* 121±1*
AMIO + VIT. E	1736±2 49±4	1648±2 93±3	1542±2 71±1	1449±2 64±3	1406±1 33±2	1384±2 46±2	1309±2 130±3	1234±1 58±3	1143±1* 94±3	1088±1 43±5	1139±1* 75±3*

*** Statistically significance**

Mean±SD expressed for All bands. The first line shows the wavenumber (cm^{-1}), and the second line for the bandwidth.

Amide I

Amide I spectra were represented as shown in Figure 5, which ranged from 1700 to 1600 cm^{-1}. This region is critical as a result of its sensitivity in providing insights regarding the secondary structure of proteins. It originates from the stretching vibration of protein functional groups. The resulting curve was enhanced and separated the amide I area into three structural components: β-turn structure, α-helix, and β-sheet modes.

The area percentage of Amide I components, which suffer from many fluctuations due to AMIO injection,

was exhibited in Figure 6 and Table 4. The β-turn percentage area has obviously risen, whereas the β-sheet and α-helix content were significantly decreased. Coadministering VIT.E. with AMIO reduced protein content changes and returned to normal levels, but enhancing was still required. Additionally, the α-helix quantity increased. Subsequently a reduction in the total intensities of absorbed spectra of all bands of the Amide I region after AMIO injection was noticed, but this deficiency was overcome after VIT. E coadministration and absorption intensities were restored to normal levels.

4. Discussion

Recently, the importance of using FTIR in studying the structure and properties of biomacromolecules in biological tissues has increased. This is due to its ability to highlight fine details in the structures of proteins and lipids, their composition, the arrangement of their functional groups, and their quantities. Additionally, it accurately exposes protein chains and detects saturation and unsaturation within fats, revealing any imbalances or disorders in the biological system [19].

In the current study, many changes appeared in the NH-OH region, which is a clear indication of the disruption in the secondary structures of proteins, fats, and polysaccharides. Liver samples were dried thoroughly to prevent interference and the effect of water molecules from appearing in the Fourier spectroscopic analysis. After spectra analysis in the group injected with AMIO, many changes appeared, especially in the $_{str}$O-H vibrational mode. This often indicates numerous changes in the hydrogen bonds and their sites in liver proteins. The fluctuations in positions and bandwidths of O-H_{asym} and O-H_{sym} bands give us information about the development of new hydrogen bonds or the destruction of the original ones, which can be among the most important links between liver tissue and drug components. Moreover, AMIO treatments also affected liver membrane constituents by affecting the symmetric and asymmetric vibrational modes of NH groups, indicating a molecular alteration in liver proteins [20].

Furthermore, the CH region is one of the regions where, through its study, we can obtain a precise and detailed description of the phospholipid's components. Since any variations in the wavenumber and width of $_{asym}CH_2$ and $_{sym}CH_2$ bands are directly and sensitively related to the quantity and concentrations of lipid components in liver tissue; As a result, any decrease in the bandwidths or intensity of the $_{asym}CH_2$ band following AMIO administration means a lessening in the lipid concentration and alignment of liver tissue acyl chains, which may cause disturbances in lipid metabolism and produce lipid content reduction[21, 22]. After VIT. E coadministration, CH region has significant improvement in $_{asym}CH_2$ width and contour absorption intensity. In the fingerprint region, the absorption band 1740 ±4 cm^{-1} corresponding to C=O vibrational band of the carboxylic acid found in amino acid was decreased in wavenumber and increased in bandwidth. This may be associated with the destruction of original hydrogen bonds and the construction of new ones [23].

Table 4: Area percentage for the secondary structure of protein components for the liver of Amide I (1700-1600 cm^{-1}) region for control and all studied.

	β-turn (%)	α-helix (%)	β-sheet (%)
Control	**9.4±3**	**56.7±3**	**33.9±2**
AMIO	**44.4±2***	**48.7±1***	**6.9±3***
AMIO + VIT. E	**20.3±2***	**77.2±2***	**2.5±2***

*** Statistically significance**

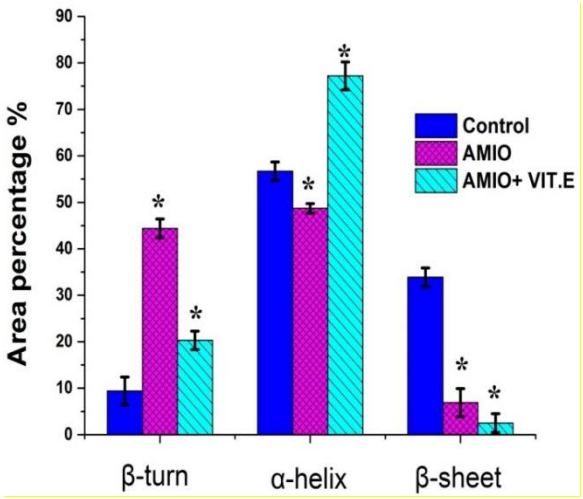

Figure 6: Area percentage for secondary structure of protein components β-turn structure, α-helix, and β-sheet of the liver for control and all studied groups.

In addition to Amide I, COO_{sym}, and CH_3 deformation bands have reductions in bandwidths, which may produce protein insolubility and aggregation with less folding; also, the absence of the second band assigned to the COO_{sym} mode may indicate a drop in the liver fatty acid content. Due to this instability in the COO mode (asymmetric and symmetric), most fatty acids will suffer from instability in the confirmation, likewise cholesterol esters, lipids, and nearly all of the biomolecules in liver tissue [17].

Furthermore, the alterations found in nearly all of the fingerprint bands were indicative of changes in the lipid constituents produced after AMIO injection since the length of the fatty acid chain is sensitive to the absorption location of the $_{Ester}$C=O band, lipid ester is assigned to the width of the COO_{sym} and COO_{asym} modes, and the phosphodiester backbone of phospholipids is owned by the asymPO2 and $_{sym}PO_2$ bands [5**].** The changes in bandwidth related to liver lipids (specifically $_{asym}CH_2$, $_{bend}CH_2$, and COO modes) resulting from AMIO administration may be linked to the fact that AMIO is a highly lipophilic compound. This property enables it to accumulate in adipose tissue and organs such as the liver. When present, AMIO can disrupt normal lipid metabolism, leading to alterations in the structural organization of lipid acyl chains within liver cells. [24].

Obviously, the changes observed in previous bands were a sign of an alteration in the assembly and conformation of lipids, proteins, fatty acids, and genetic material in liver tissue due to AMIO administration. In the VIT.E. group, most of the fingerprint region bands have improved in their wavenumbers and bandwidths. The damage caused by the consumption of AMIO in liver

cells may be attributed to hepatic dysfunction that may lead to apoptosis and necrosis of the hepatocytes [25]. This may be related to the fact that adipose tissues and high blood perfusion organs like the skin and liver mostly have concentrated AMIO; this is due to the lipophilicity of the AMIO drug, Long-term use may cause a variety of adverse effects like photosensitivity, hypothyroidism, and hepatic impairment [26]. In the same context, Saleem et al. (2017) [27] stated that AMIO also activates the magnesium-dependent neutral sphingomyelinase pathway by reducing glutathione levels, which then induces oxidative stress-increasing and hepatocyte apoptosis [28].

Protein structure is mainly related to Amide I absorption intensity; by spectra analysis, we can monitor the secondary structures of proteins and polypeptides. This is due to the sensitivity of protein structure to C=O stretching mode. So we can say that the variations in α-helix, β-sheet, and β-turn contents in the AMIO group belong to the significant changes in the liver protein solubility and folding leading to protein structure with different compositional characteristics. This might be a transitional state, progressively resulting in an increase in β-turn structural content which may indicate protein aggregation and liver tissue geometry disorders [29]. Amiodarone can lead to liver damage, which ranges from mild elevations in serum aminotransferases to severe liver failure requiring transplantation. The incidence of liver toxicity is reported to be between 15% and 30%, with some cases progressing to cirrhosis [30]. Amiodarone administration in rats resulted in significant liver damage, including disrupted hepatocytes, increased vacuolations, and damaged organelles. The microvilli of bile canaliculi were disrupted, and there was an increase in lipid content within hepatocytes [13].

Recent studies have proven that vitamin intake has numerous beneficial and healthy effects in overcoming many diseases and improving the functions of organs such as the eyes, heart, and optic nerve [5, 20, 31]. Especially VIT. E, when taken alongside AMIO, shows clear improvements in various organs, such as reducing lysosomal phospholipidosis and AMIO toxicity. Systematic reviews indicate that VIT. E supplementation significantly reduces liver enzymes and improves histological features such as steatosis and inflammation in nonalcoholic fatty liver disease patients, although its effect on fibrosis remains uncertain [32]. Additionally, VIT. E, particularly α-tocopherol, can protect the retinal tissue from oxidation-harmful effects and lower the chance of age-related macular degeneration emerging [28]. These effects also align with what has been studied and proven: that the VIT.E. coadministration reduces oxidative stress hepatotoxicity and cell apoptosis induced by AMIO [25].

5. Conclusion

In conclusion, our current experimental study demonstrated that temporary injection of AMIO led to liver tissue alterations, including hydrogen bond deformability, changes in lipid content, and protein degradation. VIT. E-coadministration with AMIO effectively addressed these observations. This makes it an effective complement for AMIO users.

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