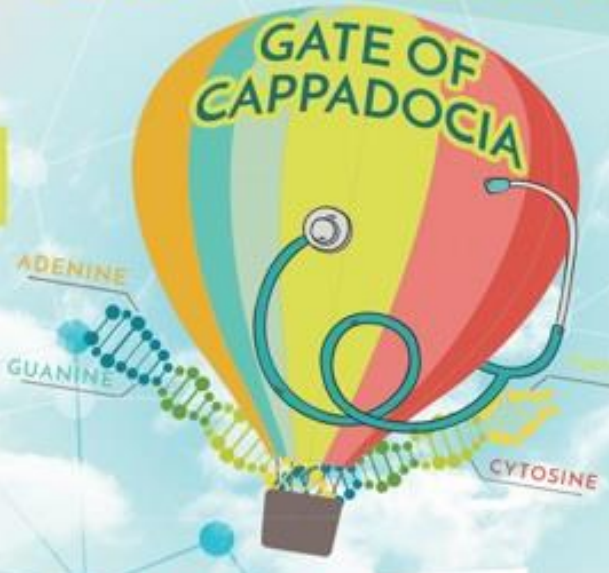
INTERNATIONAL CONGRESS ON

BIOLOGICAL AND MEDICAL SCIENCES

NİĞDE/TURKEY/2018



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ORAL PRESENTATION

Raman Spectroscopy: A Novel Experimental Approach to Evaluating Cisplatin Induced Tissue Damage

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Abstract

The most commonly used alternative treatment method for reducing tissue damage during chemotherapeutic use is the use of antioxidants. The aim of this work is to clarify the effect of curcumin and beta-carotene on cisplatin-induced tissue damage and to demonstrate the potential of Raman spectroscopy to detect tissue changes consistent with liver and kidney histopathology as a potential diagnostic adjunct. In the study,56 Wistar albino female rats were used and randomly divided into 7 groups (n: 8). Sham group received only sesame oil; Cisplatin group, received a single dose injection of cisplatin; Beta-carotene group, treated with beta-carotene; Cisplatin+Beta-carotene group, pretreated with beta-carotene 30 min prior to the cisplatin injection, then received cisplatin; Curcumin group, treated with curcumin; Cisplatin+Curcumin group, pretreated with curcumin 30min prior to the cisplatin injection, then received cisplatin. The second application was performed 1 week after the first application. One of the liver and kidney tissues was taken to 10% form for histopathological examinations and the others were taken to -80ºC for raman spectroscopy. Received sections were hematoxylin-eosin stained. The avidin-biotin peroxidase method was used for to investigate anti-TNF-α and IL1-β activities. TUNEL method was applied to determine apoptotic cells. According to our histopathological findings, beta-carotene and especially curcumin have been found to possess hepatorenal protective activities. These datas were supported by the microscopic damage scores. Although some of these findings were observed in both the cisplatin+curcumin and cisplatin+beta-carotene groups, the incidence and severity of histopathological lesions were less than the cisplatin group. Both TUNEL, immunohistochemical studies and Raman spectroscopy results consistent with histopathological examination of hematoxylen-eosin stained sections. Raman spectroscopy represents a suitable tool to provide insights into structural factors involved in the mechanisms underlying antitumor effects of platinum drug.

Keywords: Cisplatin, Curcumin, Beta-Carotene, Raman spectroscopy

1. Introduction

Cisplatin is a chemotherapeutic agent used in treatment of different type cancers including bladder, ovarian, cervical, testicular, head and neck cancers [1,2]. Curcumin, a polyphenolic phytochemical, is an active compound isolated from Curcuma longa. Curcumin is increasingly being studied for its several therapeutic properties, including antiinflammatory, anti-oxidant and anticancer activities in experimental conditions and in clinical settings [3,4]. Beta-carotene, a well-known antioxidant and precursor of vitamin A [5].

Raman spectroscopy is an optical technique, that provides the biomolecular information of extra and intracellular constituents (for example, minerals, lipids, proteins, etc.) with submicrometer resolution. It has been used to show the distribution of different proteins, lipids, and mineral species in a number of tissues [6,7]. The aim of this work is to clarify the effect of curcumin on cisplatin-induced tissue damage and to demonstrate the potential for Raman spectroscopy to detect tissue changes consistent with liver and kidney histopathology as a potential diagnostic adjunct. Therefore, we assess whether Raman spectroscopy also provides a reliable means of discriminating between tissue that has, from a histological point of view, healed and tissue for which histological inflammation is still observable. We evaluate the ability of Raman spectroscopy to distinguish between quiescent and cisplatin induced inflamed tissue. In this way, we aim to provide a more complete assessment of the utility of Raman spectroscopy as a potential, complementary tool for the assessment of cisplatin induced inflamed tissue.

2. Materials and Methods

Animals and Experimental Protocol: The study was performed at the Experimental and Clinical Research Centre of Erciyes University.

Fifty six female Wistar Albino rats (there was no specific reason for using female rats) aged 8 week old (weighing 190-250 g) were used in the study. Experimental groups were formed by assigning rats randomly divided into 7 groups(n: 8)

Control rats did not receive any treatment until the end of the experiment; Sham group, rats received only sesame oil (1 mg/ kg); Cisplatin group, rats received a single dose injection of cisplatin two times as once a week (5 mg/kg week, ip) [8]; Curcumin group, rats orally treated with curcumin (200 mg/kg) [9] ; Cisplatin+ Curcumin group, rats pretreated with curcumin (200

mg/ kg) for 30 min before cisplatin injection, then received cisplatin (5 mg/ kg/week, ip) Beta-carotene group, rats treated with beta carotene (100 mg/kg) orally [10] ; Cisplatin+Beta-carotene group, rats pretreated with beta-carotene for 30 min before cisplatin injection, then received cisplatin (5 mg/kg/week, ip).The second administration was done 1 week after the first administration and the same injection and ip and/or gavage procedures as in the first administration were applied to the experimental groups for the second time.

Histological examination: After 5 days from the second administration, the rats were sacrificed under an overdose of a combination of xylazin and ketamine; their tissues (liver and kidney) were dissected and fixed in 10% neutral formaldehyde for 2 weeks.The fixed tissues were processed routinely using a standard histological procedure. The resulting paraffin blocks were sectioned at 5 μm thickness using a rotatory microtome. The sections were initially treated with either hematoxylin and eosin (H&E) stain, for assessment of histo-architectural changes.

TUNEL Assay: The terminal deoxynucleotidyl transferase dUTP nick endlabelling (TUNEL) method was used to assess DNA fragmentation in the cells.

Immunohistochemical Procedure: The avidin-biotin peroxidase method was used for the immunohistochemical studies to investigate anti-TNF-α and IL1-β activities.

Raman spectroscopy : Raman spectroscopy analysis of the tissues was performed using the supernatant of the homogenates prepared by centrifugation. The solution of the homogenate was spotted on glass substrates and the Raman spectra were taken following evaporation of water. Raman spectra were taken using the WITec alpha M+ Raman Microscopy system equipped with a 532 nm laser source: spot size=2 μm, integration time=2 s, objective: 50×, NA=0.85. Raman measurements were performed following observation of the focus point through the optical microscope integrated with the Raman system. Baseline correction was not performed for any spectrum. The reported spectra represent the average intensity derived from 10 different points on the samples.

Statistical analysis: Normal distribution of data was evaluated by histogram, q-q graphs and Shapiro-Wilk test. The homogeneity of the variances was evaluated by the Levene test. Kruskal-Wallis test and one-way variance analysis were used for quantitative variables in more than two groups. Dunn-Bonferroni test and Tamhane T2 were applied for multiple comparisons. The datas

were analyzed by Turcosa Cloud (Turcosa Ltd Co) statistical software. $p < 0.05$ was considered to be statistically significant.

3. Results and Discussion

Histopathological examination

Effect of cisplatin on histology of the kidneys: In the control and sham groups, the normal architecture of tissue in sections stained with H&E. Degenerative changes such as hemorrhage, tubular necrosis, apical surface loss in tubular epithelial cells (especially proximal tubules) and mononuclear cell infiltration were observed in kidney tissue in cisplatin group. Although some of these findings were also observed in the cisplatin+curcumine and cisplatin+beta-carotene groups, the incidence and severity of histopathological lesions were less than those of the cisplatin group, tubular dilatations and degenerations were decreased (Figure 1A). A significant protective effect was observed after treatment with curcumin. Kidney tissue showed a normal structure and orderly arrangement and resembled those of control rats. Glomerular and tubulointerstitial lesions of the groups were scored in Table 1. The degree of pathological findings showed a significant difference between groups treated with cisplatin and cisplatin+curcumine, cisplatin+beta-carotene groups (Table 1) ($p < 0.05$).

Effect of cisplatin on histology of the liver: Animals in control and sesame oil group did not display any histological changes when compared with tissue from normal, control animals. In contrast, cisplatin-administered animals in the cisplatin group developed various histopathological changes, including degeneration/necrosis of hepatocytes, cytoplasmic vacuolation, obvious dissolution of hepatic cords and Kupffer cell proliferations. The histopathological alterations in the hepatic tissue were associated with large hepatocellular necrotic areas and focal inflammatory cells. Liver of curcumin and beta-carotene administered rats showed normal hepatic lobules, consisting of a central vein surrounded by radiating hepatocyte plates with normal portal tracts surround the classical lobules. (Figure 1B) However, liver tissue in the cisplatin group administration of curcumin or beta-carotene with cisplatin in the protective group showed an improvement of hepatic toxicity with presence of small degenerated area together with normalization. In the groups of administration of curcumin or beta-carotene

with cisplatin animals also had significantly increased liver pathology scores as compared with vehicle controls (Table 1) ($p < 0.05$).

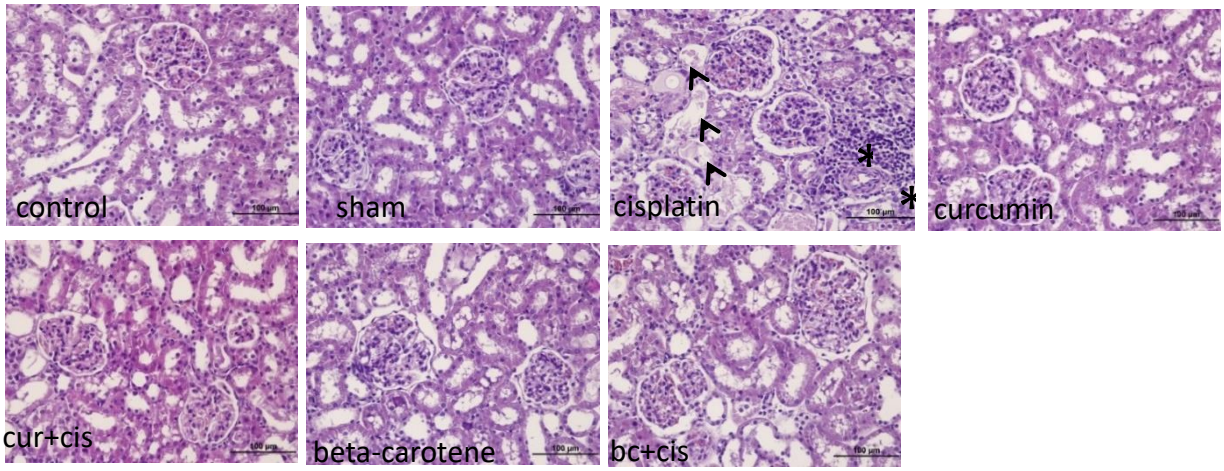
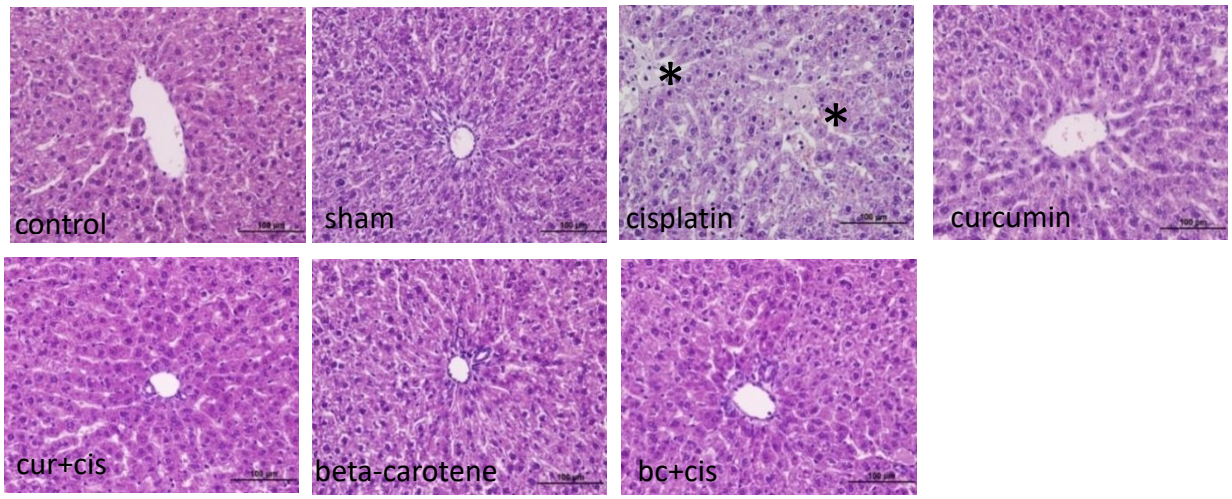


Figure 1. Representative photomicrographs of histopathological changes in the kidney and liver of control and experimental rats. **A:** Renal histopathological microphotographs (×40). In groups control and sham, normal renal architecture are observed. Cisplatin alone treated animals showing degenerative changes within the glomerulus and in tubular cells. administration of curcumin or beta-carotene with cisplatin treated animals showing showing the glomerulus and the tubular structures with mild degenerative changes arrow; tubular damage *; mononuclear cells infiltration



B: Liver histopathological microphotographs (×40). Control and sham groups show normal liver architecture. Cisplatin group, a wide range of changes was noticed vacuolated hepatocyte *. Treatment with curcumin normalised liver histology, represented as nearly normal architecture

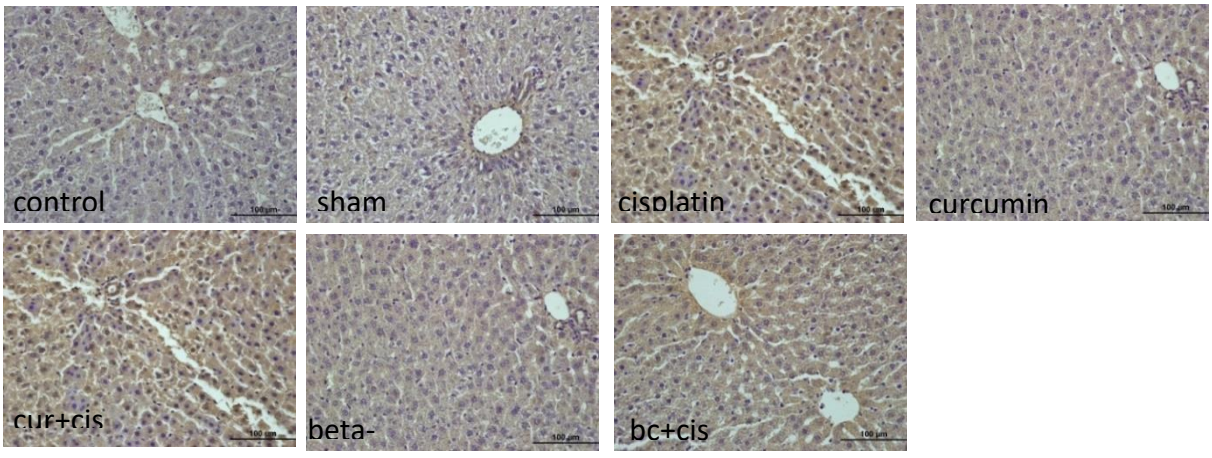
Table 1. Scoring Statistics Analysis Results

	CİS	CUR	CUR+CİS	BK	BK+CİS	*p*
KİDNEY	2.0 (1.0-2.3)[b]	.0 (0.0-0.3)[a]	.0 (0.0-1.0)[ab]	.0 (0.0-1.0)[a]	0.5 (0.0-1.3)[ab]	0.002
LİVER	2.0 (1.0-2.3)[b]	.0 (0.0-0.3)[a]	.0 (0.0-1.0)[a]	.0 (0.0-1.0)[b]	.0 (0.0-1.3)[ab]	0.002

Data are expressed as median (quarter1-quarter3). The same letters in the same row show similarity between groups, and different letters indicate differences between groups, p-value of <0.05 was used for significance.

Effect of cisplatin immunohistochemical analysis: To assess the proliferative and anti-inflammatory effects of cisplatin, we analyzed the secreted levels of several proinflammatory cytokines using immunohistochemical method. As demonstrated in Figure 2.

TNFα and IL-6: Sections stained with TNF-α and IL-1β primary antibody are represented in Table 2 and Figure 2. Diffuse and cytoplasmic stainings were assessed in the kidney and liver tissue slides. As a result of exposure to cisplatin injection caused significant ($p < 0.001$) rise in the immunoreactivity intensity levels of the proinflammatory cytokine TNF-α and IL-1β in both kidney and liver tissues, whereas this induction was significantly attenuated by curcumin treatment ($p < 0.001$). Sesame oil, Curcumin or beta-carotene alone-treated rats exhibited no alterations in the levels of inflammatory markers. Tissue TNF-α and IL-1β immunoreactivity intensity levels of the rats in the cisplatin group markedly upregulated to those of the control group. However, curcumin treatment significantly ameliorated cytokine levels. TNF-α and IL-1β are the proinflammatory mediators and decreased TNF-α and IL-1β levels determined in the curcumin-treated group was associated with the anti-inflammatory functions of curcumin (Figure2). We suggest that, those anti-inflammatory properties of curcumin were one of the most important factors in prevention of histopathological damage induced by cisplatin.



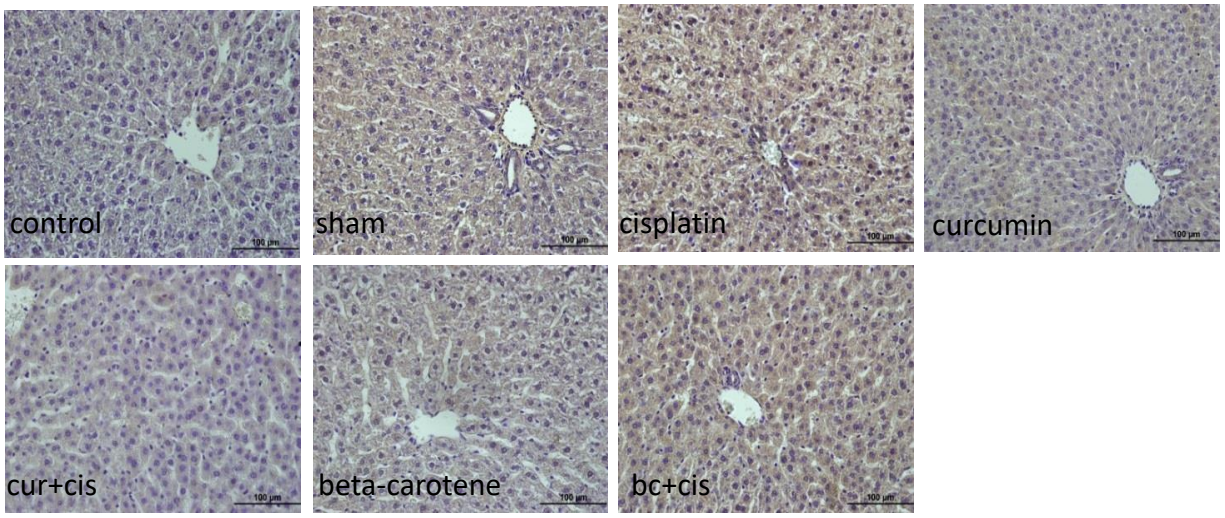


Figure 2 A. First images TNF-α and other IL-1β immunoreactivity intensity (LIVER)

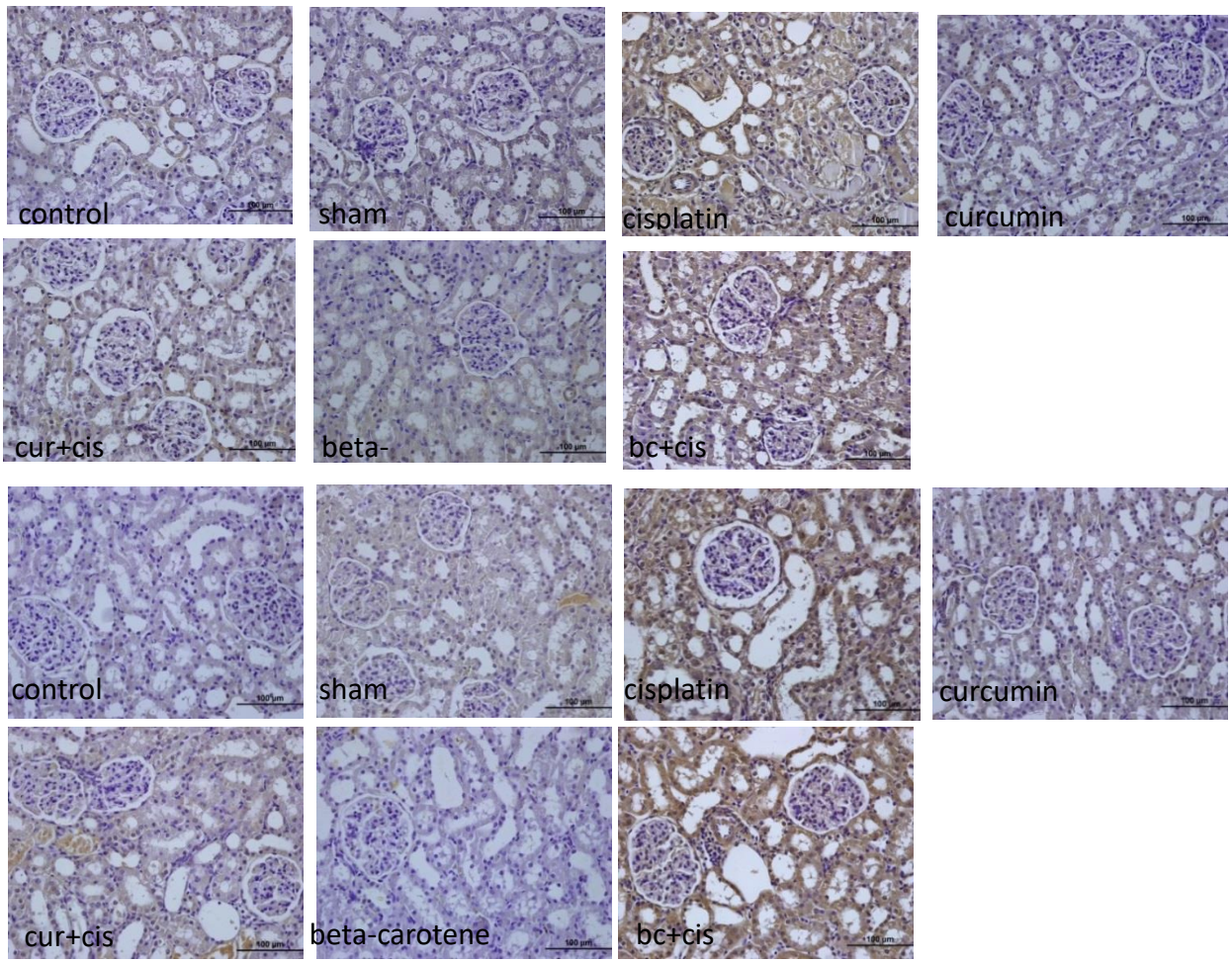


Figure 2 B. First images TNF-α and other IL-1β immunoreactivity intensity (KIDNEY)

Table 2. Liver Immunreactivity And Positive Cellulation Statistics Analysis Results

LIVER	CONTROL	SHAM	CİS	CUR	CUR+CİS	BK	BK+CİS	*p*
TNF-α	102.12±3.86^{a}	104.00±4.70^{a}	109.74±4.92^{b}	103.20±3.37^{a}	102.95±2.75^{a}	103.07±4.96^{a}	106.34±6.97^{ab}	<0.001
IL1- β	101.02±5.03^{a}	102.73±5.14^{ac}	105.41±5.30^{bc}	102.32±4.43^{ac}	103.24±5.02^{ac}	102.98±5.97^{ac}	101.56±3.49^{a}	0.029

KIDNEY	CONTROL	SHAM	CİS	CUR	CUR+CİS	BK	BK+CİS	*p*
TNF-α	101.05±4.00^{a}	101.58±4.41^{ab}	104.29±46^{ab}	101.39±3.45^{ab}	101.37±5.09^{ab}	101.28±5.05^{ab}	104.77±4.69^{b}	0.002
IL1-β	97.13±6.07^{a}	98.87±3.59^{a}	103.67±3.30^{b}	99.77±5.41^{a}	98.93±4.22^{a}	97.97±5.41^{a}	101.10±4.15^{ab}	<0.001

Data are expressed as mean ± standard deviation for seven rats in each group. The same letters in the same row show similarity between groups, and different letters indicate differences between groups, p-value of <0.05 was used for significance.

TUNEL: Quantification of TUNEL-stained tissues indicated that cisplatin treatment alone leds to a significant increases in apoptotic cell number ($p < 0.001$). These datas supported that cisplatin treatment exacerbated cisplatin-induced apoptosis. Quantification of apoptosis is shown in Figure 3. Cisplatin induced increased apoptosis in tubular epithelium as compared to control animal tissues (Figure 3) which was largely suppressed with curcumin. Sham, curcumin or beta-carotene alone did not have an effect on kidney epithelium or liver hepatocytes survival (Figure 3), (Table 3).

Table 3. TUNEL Statistics Analysis Results

TUNEL	CONTROL	SHAM	CİS	CUR	CUR+CİS	BK	BK+CİS	*p*
KİDNEY	.0 (0.0-0.0)^{a}	.0 (0.0-0.0)^{ab}	1.0 (0.0-2.8)^{c}	.0 (0.0-0.0)^{ab}	.0 (0.0-0.0)^{ab}	.0 (0.0-0.0)^{a}	.0 (0.0-1.0)^{b}	<0.001
LİVER	.0 (0.0-0.0)^{ad}	.0 (0.0-0.0)^{a}	.0 (0.0-1.0)^{bc}	.0 (0.0-0.0)^{ad}	.0 (0.0-0.0)^{ad}	.0 (0.0-0.0)^{ad}	.0 (0.0-0.0)^{dc}	<0.001

Data are expressed as median (quarter1-quarter3). The same letters in the same row show similarity between groups, and different letters indicate differences between groups, p-value of <0.05 was used for significance.

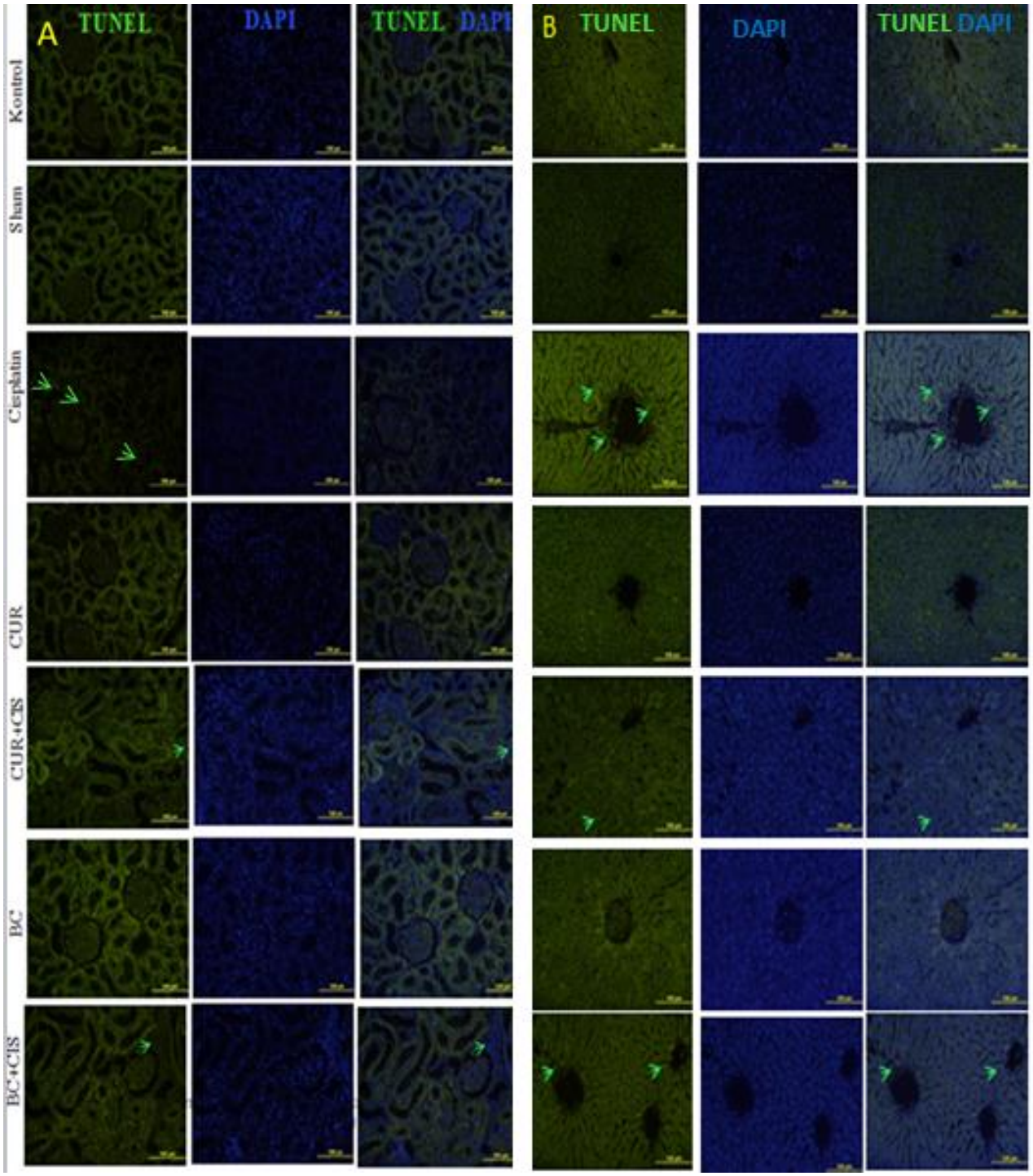


Figure 3A. Apoptotic cells (green arrow), (X40)(KIDNEY), **B;** Apoptotic cells (green arrow), (X40)(LIVER)

Raman Results Kidney

Raman spectra of the homogenates derived from the kidneys of rats that were subjected to different treatments are given in Figure 4A. The characteristic vibrations (at 751, 1131, 1310 and 1585 cm^{-1}) that belong to heam were distinct in the spectra [11]. The breathing vibrations of the porphyrin rings, for example, could be found at 751 cm^{-1} [11]. The intense Raman bands at 1005, 1342, 1448, 1660 and 2930 cm^{-1} were attributed to proteins [11]. The Raman band at 1335 cm^{-1} was assigned to the CH_3CH_2 wagging, which implies the presence of collagens and nucleic acids [12]. The impact of the anti-oxidant molecules and cisplatin on the kidney tissues was investigated using the variation in the intensity of the band 1335 cm^{-1} (Figure 4B). The homogenates derived from the kidneys of the rats exposed to cisplatin exhibited 8-fold lower intensities at 1335 cm^{-1} in comparison with the sample obtained from the healthy kidney. This result likely relates to the DNA damages caused by the cisplatin treatment. The intensity of the band at 1335 cm^{-1} was significantly higher for the homogenates derived from the kidney of rats that were exposed to anti-oxidant molecules and cisplatin. These results strongly suggest that DNA damage is prevented with the use of anti-oxidant molecules.

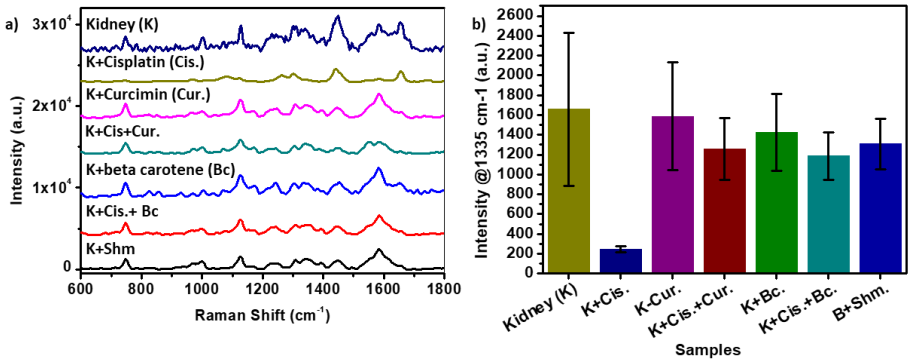


Figure 4A. Raman spectra of Kidney homogenates,**B;**Intensity values at 1335 cm^{-1}spectral position.

Raman Results Liver

Raman spectra of the homogenates derived from the livers of rats that were subjected to different treatments are given in Figure 5A. The characteristic vibrations (at 751, 1131, 1310 and 1585 cm^{-1}) that belong to heam were distinct in the spectra [11]. Similar to the kidney tissues, the impact of the anti-oxidant molecules and cisplatin on the liver tissues was investigated using the variation in the intensity of the band 1335 cm^{-1} (Figure 5B). The intensity

at 1335 cm^{-1} for the homogenates derived from the liver of the rats exposed to cisplatin was roughly half of the samples obtained from the healthy liver. The cisplatin induced reduction in the intensity of the characteristic band was more significant in the case of kidney than liver. The intensity of the band at 1335 cm^{-1} was significantly higher for the homogenates derived from the livers of rats that were exposed to anti-oxidant molecules and cisplatin. These results further confirm that anti-oxidant molecules reduce the DNA damage caused by cisplatin. A similar trend could be observed from the band at 1583 cm^{-1}, which can be attributed to adenine and guanin [13]. The closely positioned haem related bands challenge the use of the band at 1585 cm^{-1}. In our study, results from Raman spectroscopy were consistent with histological findings.

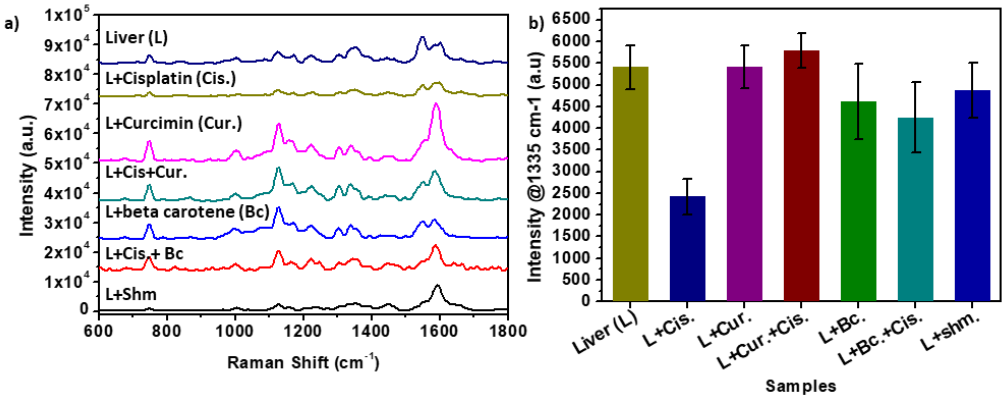


Figure 5 A. Raman spectra of Liver homogenates,**B**; Intensity values at 1335 cm^{-1} spectral position.

Discussion

The effects of curcumin or beta-carotene were tested for the first time against cisplatin hepatorenal toxicity in rats and the importance of Raman spectroscopy has been revealed in this study. The liver is known to accumulate significant amounts of cisplatin, second only to the kidney [14]; thus causes direct and indirect cellular injury [15].

The histopathological evaluation demonstrated that cisplatin treatment produced histopathological alterations in the liver tissue of cisplatin-injected animals, which is inconsistent with the previous results [14,16]. Moreover, the treatment with curcumin or beta charotene ameliorated cisplatin-induced liver damages associated with degeneration/necrosis of hepatocytes, cytoplasmic vacuolation. In this study, cisplatin induced kidney and liver damage in rats as confirmed by the histopathological changes detected in cisplatin group. This is consistent with many former researches [17]. According to our histopathological findings, beta-carotene

and especially curcumin have been found to possess hepatorenal protective activities. These datas were supported by the microscopic damage scores.

In this study, althought cisplatin showed a marked pro-infammatory response as revealed by a signifcant increase in the levels of TNF-α and IL-1β, pre-treatment of curcumin or beta-carotene reduced cisplatin-induced hepatorenal toxicity which was clearly evident from the reduced TNF-α and IL-1β levels. Additionally, these findings were also supported by histopathology of the kidney and liver. Our results showed that the use of curcumin or beta-carotene before cisplatin may be effective in reducing the levels of TNF-α and IL-1β in cisplatin-treated animals.

Apoptosis is a common part of cisplatin-induced organotoxicity, because of the DNA is the main target of cisplatin as it has high affinity to sulph-hydryl groups [18]. The normal balance among pro- and anti-apoptotic pathways in the kidney and liver is shifted in favor of the proapoptotic pathways by cisplatin. Regaining the normal balance through suppression of proapoptotic factors and/or increasing the antiapoptotic ones seems a way of protection against cisplatin toxicity in these organs [19,20]. However, curcumin or beta-carotene before cisplatin treatment restored the apoptotic balance by decreasing TUNEL + cell activities in these tissues. Our study displayed that cisplatin induces liver injury, evidenced by significant increase in TNF-α, IL-1β and TUNEL+ cell number levels Co-treatment with curcumin or beta-carotene displayed significant decreases in TNF-α, IL-1β and TUNEL+ cell number levels. Several studies advocated that infammation, oxidative stress injury and apoptosis undoubtedly participate in renal impairment. Among these pathological changes, instigation of infammatory cascade is the most important issue [21]. Activation of pro-infammatory cytokines and enzymes, including TNF-α, IL-1β, which may eventually cause renal damage

5. Conclusion

In conclusion, we have determined that curcumin co-administration was highly effective in prevention of cisplatin-induced liver and renal damage due to the anti-oxidant and anti-inflammatory effects of curcumin. Moreover, the results of the present work demonstrate that Raman spectroscopy represents a suitable tool to provide insights into structural factors involved in the mechanisms underlying antitumor effects of platinum drugs.

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None.

Conflicts of Interest

There is no conflict of interest

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ORAL PRESENTATION

Childhood Trauma Experiences in Mersin University Faculty of Medicine Students

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Abstract

It was aimed to investigate the sexual abuse and abuse experienced by students in their childhood and factors affecting them. The population of this cross-sectional study is medical faculty students. 650 students were included in the study. Questionnaires and the Childhood Trauma Questionnaire Short Form (CTQ-SF) were administered and taken in sealed envelope. The scale consists of five sub-dimensions: physical–emotional–sexual abuse, physical- emotional neglect. The scale can be scored between 25-125. The increase in score indicates the concentration of childhood abuse experiences. Mean age of participants was 21.7±2.2, 51.8 % were male. The mean total score of CTQ-SF was 37.12±9.07 and the mean score of sexual abuse was 7.12±2.85. Total scores are higher in men, extended family members, who lives with stepmother, whom mother isn't working ($p<0.01$,$p<0.01$,$p=0.02$,$p=0.03$). As the number of siblings and birth orders increased, the total scores were higher ($p<0.01$,$p<0.01$). Low education levels of parents were increased total scores. The total score was high among those who spent most of their childhood in the village/town, who comment perception as 'generally having economic difficulties'. 26.2% of participants had suicidal thoughts, 3.4% had suicide attempts. Suicidal thoughts were high in students who had high total and sexual abuse scores ($p<0.01$,$p=0.01$). Suicide attempts were high in those with high total score ($p<0.01$). In this study, we found being a male, living in an extended family, uneducated parents, unemployed mother, the sense of economic hardship and living in the village/ town increase the risk of childhood traumatisation. Preventive studies should be making about this subject.

Keywords: child abuse, childhood trauma questionnaire, medicine students

1. Introduction

Child maltreatment is the abuse and neglect that occurs to children under 18 years of age. Child maltreatment is negative behaviors that prevent the physical, emotional, mental or sexual development of child. These behaviors can be administered by a parent or other caregivers. Physical, emotional, sexual abuse and neglect are the types of child maltreatment. Physical abuse is any non-accidental/ purposely behaviors causing injury, trauma. Emotional abuse includes humiliating, threatening, blaming a child, persistently ignoring them, etc. Sexual abuse includes touching children for sexual pleasure, exhibitionism, sexual intercourse with the child, using children in pornographic performances etc.[1] According to the World Health Organization (WHO) one out of 4 all adults reported physical abuse, one out of every 5 woman and one out of every 13 men reported having been sexually abused during their childhood. [2] The frequency of sexual abuse varies between 2% and %62 in the studies. [3] According to Research study on Child Abuse and Domestic Violence Research in Turkey; 45% of the children were physically, 51% were emotionally, 3% were sexually abused and 25% were neglected. [4] In a study by Alikasifoglu et al. frequency of sexual abuse was found 13.4%. [5]

In this study, we aimed to investigate the sexual abuse and abuse experienced by Mersin University Faculty of Medicine students in their childhood and factors affecting them, and the sexual abuse knowledge level of medicine students.

2. Materials and Methods

The cross-sectional study was conducted for the undergraduate students of Mersin University Faculty of Medicine during the 2017-2018 academic years. The population size was 1458 medicine students. The minimum sample size was calculated as 616 people with 50% prevalence, 95% confidence interval and 3% standard error. We decided to include 650 individuals in the study. We used stratified sampling; number of participants from each period was determined by weighting to the class size. We calculated that 133 students from first grade, 120 students from second grade, 119 students from third grade, 108 students from fourth grade, 84 students from fifth grade and 86 students from sixth grade must participate in the study.

Childhood Trauma Questionnaire Short Form (CTQ-SF) and another questionnaire including about socio-demographic characteristics and sexual abuse questions were applied to the participants. A study on the reliability and validity of Turkish version of CTQ-SF was conducted

by Kaya S. CTQ-SF is a tool for retrospective assessments of histories of abuse and neglect at the childhood. The scale consists 28 items. 3 questions are not included in the scoring. Responses are provided on a five-point Likert-type scale. The CTQ-SF has five subscales: emotional abuse, physical abuse, sexual abuse, emotional neglect, and physical neglect. Each subscale has five questions and a score ranging from 5 to 25 points. The sum of values of the five yields the CTQ-SF total score. Total score ranges from 25 to 125 points. The increase in score indicates the concentration of childhood abuse experiences. [6]

The pilot study performed with 10 medicine students before collecting data. These questionnaires were not included in the study. Students were reached in their classroom. After obtaining permissions from the participants, questionnaires were distributed. Participants filled out the forms themselves anonymously. The questionnaires received by closed envelops.

Dependent variables were 'CTQ-SF total score, sexual abuse score, suicidal thoughts and suicidal attempts'. The question of parents' profession was asked as open-ended, and the answers were according to the International Standard Classification of Occupations.

In order to measure the level of sexual abuse knowledge, 8 questions were prepared by searching the literature. These questions asked as 'Which of the following questions are sexual abuse'. The questions marked by the participants were evaluated as 'yes'. Before the study was conducted, approvals obtained from Mersin University Clinical Research Ethics Committee and Mersin University Rectorate. Statistical analysis: The normality of the continuous variables were tested with Shapiro Wilk test. Levene test was used for homogeneity of variances. OneWay ANOVA test performed for the groups where the variances were homogeneous, Welch test were performed for non-homogeneous groups. For binary comparisons; Bonferroni test was used for homogenous ones and Games Howell test was used for non-homogenous ones. Mean and standard deviation values are given as descriptive statistics. Statistical significance was taken as $p<0.05$.

3. Results

The mean age of the students was 21.7 ± 2.2 (min=18-max=29). 337 (51.8%) students were boy and 313 (48.2%) were girl. 430 (66.2%) of the students had 2 or less siblings and 220 (33.8%) students had three and more siblings. 254 (39.1%) students were first child, 212 (32.6%) of them

were second child, 184 (28.3%) of them third and more child. 578 (88.9%) of students have nuclear family, 72 (11.1%) have extended family. 21 (3.2%) of students have stepmother. 4 (0.6%) of students have stepfather. 233 (35.8%) of students mother's education level were primary school and under, 227 (34.9%) were middle/high school, 190 (29.3%) were university. 253 (35.8%) of the mothers of the students were not working, 209 were working at a job. 123 (19.0%) of students father's education level were primary school and under, 237 (36.5%) were middle/high school, 289 (44.5%) were university. 12 (1.9%) of fathers were unemployment/not working, 118 (38.1%) were retired, 242 were working at professional jobs, 171 (26.9%) were worked in works that did not require qualification, 93 (14.6%) were working other jobs. 86 (13.3%) of students had generally economic difficulties, 328 (50.5%) had sometimes 235 (36.2%) had never. 77 (11.9%) of students have mostly lived in village/town, 183 in district centre, 389 (59.9%) in provincial centre.

566 (87.1%) participants answered all of questions correctly about sexual abuse knowledge level of students. The question with the least correct answer was: 'Is sexual speaking with a child, a sexual abuse type', 595 (91.5%) of students answered correctly these one. The question 'is sexual exhibitionism a type of sexual abuse', correctly answered by 604 (92.9%) participants. the question 'is viewing a sexual image to a child, a type of sexual abuse', and correctly answered by 611 (94.0%) participants. The question 'Is a sexual relationship without intercourse with a child, a type of sexual abuse', correctly answered by 622 (95.7%) participants. The question 'is penetration with an object to child's genitals a type of sexual abuse', and correctly answered by 623 (95.8%) participants. The question 'is sexual intercourse with the child, a type of sexual abuse', correctly answered by 626 (96.3%) participants. The question 'Is forcing the child to touch to another person for sexual pleasure a type of sexual abuse', correctly answered by 629 (96.8%) participants. The most correctly answered question was: 'Touching the child for sexual pleasure is a sexual abuse type'. 635 (97.7 %) of students correctly answered these one. The level of sexual abuse knowledge was higher in female students ($p<0.01$). This was higher in who live with nuclear family than in the extended family ($p<0.01$). The score of CTQ-SF and sexual abuse were higher in students with low knowledge of sexual abuse ($p<0.01$, $p<0.01$).

The mean; CTQ-SF total score was 37.12±9.07, physical abuse score was 5.81±2.47, emotional abuse score was 5.84±2.00, sexual abuse score was 7.12±2.85, physical neglect score was 7.56±2.60, emotional neglect score was 10.80±2.40.

The mean CTQ-SF scores of males were 38.27±9.66, and it's higher than females (35.89±8.24) ($p<0.01$). The mean CTQ-SF scores of students, who live with extended family (40.78±10.12), were higher than lives with nuclear family (36.67±8.84) ($p<0.01$) (Table 1).

Table 1. CTQ-SF and sexual abuse score averages by socidemographic characteristics

	CTQ-SF total score	**Sexual abuse score**
Gender		
Male (n=337)	38.27±9.66	5.91±2.72
Female (n=313)	35.89±8.24	5.70±2.15
	p<0.01	p= 0.29
Family type		
Extended family (n=72)	40.78±10.12	6.04±2.86
Nuclear family (n=578)	36.67±8.84	5.78±2.41
	p<0.01	p=0.39
Stepmother		
Yes	44.76±13.97	7.05±4.97
No	36.87±8.77	5.77±2.33
	p=0.02	p=0.25
Stepfather		
Yes	45.00±13.95	8.50±5.74
No	37.05±9.03	5.79±2.43
	p=0.81	p=0.41
Mother's education level		
Primary school and under	38.52±8.31	5.76±2.06
Middle school- high school	36.45±9.01	5.77±2.50
University	36.21±9.79	5.91±2.85
	p=0.01	p=0.79
Father's education level (n=649)		
Primary school and under	39.20±7.75	5.63±1.80
Middle school- high school	37.52±9.57	6.00±2.71
University	35.92±9.04	5.73±2.50
	p<0.01	p=0.31

Mother's job		
Not working	37.58±8.91	5.79±2.36
Working	35.97±8.98	5.80±2.52
	p=0.03	p=0.95
Father's job		
Unemployed/Not working	41.75±10.64	7.08±4.54
Retired	35.66±8.23	5.40±1.75
Professional occupation	36.11±8.81	5.78±2.41
Work without qualification	38.87±9.71	6.06±2.70
Others	36.99±8.22	5.63±2.20
	p<0.01	p=0.06
Place where he/she lived most of childhood	40.42±7.94	6.05±2.76
Village/town	37.38±9.02	5.81±2.33
District center	36.36±9.19	5.76±2.47
Provincial center	**p<0.01**	p=0.64
Economic perception		
Had generally economic difficulties	44.84±11.97	6.53±3.55
Had sometimes economic difficulties	36.56±7.95	5.70±2.10
Had never economic difficulties	35.08±7.86	5.69±2.43
	p<0.01	p=0.10

As the number of siblings and birth orders increased, the CTQ scores were increased (p<0.01, p<0.01).

170 (26.2%) of the students stated that they thought suicide at least once in their lifetime. 22 (3.4%) of the students reported suicide attempt at least once in their lifetime. Suicidal thoughts and suicidal attempts increased as the CTQ-SF scores increased (p<0.01, p<0.01). Suicidal thoughts and suicidal attempts increased as sexual abuse scores increased (p<0.01, p<0.01) (Table 2).

Table 2. The relationship between CTQSF score and suicidal thoughts and attempts

	Suicidal thoughts: No (n=479)	**Suicidal thoughts: Yes (n=170)**		**Suicidal attempts: No (n=627)**	**Suicidal attempts: Yes (n=22)**	
CTQSF total score	35.43±9.61	41.85±12.19	**p<0.01**	36.75±8.63	47.45±14.45	**p<0.01**
Sexual abuse score	5.48±1.74	6.74±3.69	**p<0.01**	5.73±2.28	8.05±5.27	p=0.05

4. Discussion

The mean CTQ-SF score was 38.32 ± 9.41 in a study conducted at China and this result reported by Li et al. [7] The mean CTQ-SF score was 31.4±7.4 in a study conducted at Sivas from Turkey and results reported by Guliz et al. [8] Our students' mean score was 37.12±9.07. Differences in scores may stem from cultural or lifestyle differences.

The mean sexual abuse score was 5.96±2.57 reported by Li et al. [7] The mean sexual abuse score was 5.5±1.7 reported by Guliz et al. [8] The mean sexual abuse score 6.38±3.60 in a study conducted at Istanbul from Turkey and this reported by Yoyen. [9] Our students' mean sexual abuse score was 7.12±2.85. Differences in scores may stem from differences in the level of knowing and understanding what is sexual abuse.

In a study conducted at seven universities in Turkey, has stated that 41.3% of the participants had suicidal thoughts at least once during the lifetime, 6.8% of them had attempted suicide, these results reported by Eskin at al. [10] In our study 26.2% of students had suicidal thoughts and 3.4% had suicide attempts.

5. Conclusion

Parents' low level of education, mother's not working, father's unqualified work, economic distress and living in village/town were found to be risk factors for childhood traumas. For prevent of childhood traumas; parents' education level should be increased, level of unemployment should be reduced, and the conditions of village/town such as education level, job opportunities, and child abuse awareness should be improved.

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Conflicts of Interest

The authors declare no conflict of interest.

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ORAL PRESENTATION

Violence, Suicide Behavior and Related Factors in Adolescents in Mersin University

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Abstract

In this study, we aimed to evaluate violence, suicide behaviour and related factors in adolescents in Mersin University. The data of the cross-sectional study was taken from Risky Behaviours Project in Adolescents in Mersin University between September 2015 and May 2016. The population was 21230 students; the minimum sample size was calculated as 1017 people. 1059 people have been reached. Permission has been obtained from Mersin University Clinical Research Ethics Committee. A questionnaire including sociodemographic characteristics and risky behaviours was applied. Chi-square and binary logistic regression analysis tests were used. The mean age was 18.9 ± 0.1 years. The results revealed that violence behaviour in boys was 2.1 times higher than in girls; in students living in extended family was 1.6 times higher than in nuclear family, in students with bad family relations was 2.0 times higher than those who were good; in students who have tried tobacco product, alcohol and addictive substance, was 1.9, 2.2 and 2.4 times higher than those who not tried, respectively. 25 students (2.5%) reported suicide. Suicide attempt in students with bad family relations was 3.2 times higher than those who were good and in students who have tried alcohol was 5.3 times higher than who not tried. Increase the level of the education of the parents and adolescents and prevention of trying addictive products by adolescents is important in terms of protecting adolescents from violent behaviour and suicide attempts.

Keywords: Adolescent, violence behaviour, suicide attempt

1. Introduction

Violence is defined by the World Health Organization as "the intentional use of physical force or power threatened or actual, against oneself, another person or against a group or community which either results in or has a high likelihood or resulting in injury, death, psychological harm, maldevelopment or deprivation" [1].

Adolescence is expressed as the transition period from childhood to adult life, where the last rapid physical growth, sexual development and psychosocial maturation takes place [2]**.** The World Health Organization (WHO) defines adolescents as those people between 10 and 19 years of age [3].

Adolescents are often thought of as a healthy group. Nevertheless, many adolescents do die prematurely due to accidents, suicide, violence, pregnancy related complications and other illnesses that are either preventable or treatable [3].

We aimed to evaluate violence, suicide behaviour and related factors in adolescents in Mersin University.

2. Materials and Methods

The data of the study was taken from Risky Behaviors Project in Adolescents in Mersin University. The cross-sectional study was carried out between September 2015 and May 2016. Permissions has been obtained from Mersin University Clinical Research Ethics Committee and Rectorate of Mersin University.

The population of the research was 21230 students studying at 32 faculties, colleges and vocational schools in Mersin University. With 50% prevalence, ±3 standard error and 95% confidence interval, the minimum sample size was calculated as 1017 people by using epi-info program. We decided to include 1100 participate in the study and we reached 1059 people.

Schools were stratified according to the number of students, including faculties, colleges and vocational schools.

Inclusion criterias were being registered to Mersin University, being a citizen of the Republic of Turkey, not being a language problem and being in the 16-19 age range.

A questionnaire including sociodemographic characteristics and risky behaviours was applied to the students. The pilot study was performed in a group of 20 people who were not included in the study.

The dependent variables of the study were violent behaviors in adolescents. The independent variables of the study were gender, department, the grade, place of residence, family type, education of parents, social security, perception of income, relationship with family.

Students or groups unpleasant behavior or words to other students in the study (mockery, intimidation, deliberate exclusion, profanity etc.) grouped as "verbal discussion". In the last 12 months, at least once a physical fight and bringing a knife or stick-like tool to the school were grouped as "violent behavior". In the last 12 months, attempted suicide at least once was evaluated as "suicide attempt". Smoking and hookah trial behavior was grouped as "tobacco trial ". Colleges and vocational schools grouped as "vocational school".

Mean and standard error for descriptive statistics, chi-square and binary logistic regression analysis tests were used. A value of $p < 0.05$ was considered significant.

3. Results

Students were evaluated according to their sociodemographic characteristics. A total of 1059 students with a mean age of 18.9 years were included in our study. 563 (53.2%) were girls and 496 (46.8) boys. 288 (27.2%) of the students were studying at the faculties and 771(72.8%) of them were studying at the vocational school. 74 (7%) of the students were in preparatory grade, 500 (47.2%) of them were in first grade and 485(45.8%) of them were in second grade. 515 (48.8%) of the students were living with their family, 190 (18%) in home -alone or with a friend- and 351 (33.2%) in dormitory. 816 (79.4) of the students' family type were nuclear and 212 were (20.6%) extended family. Education level of 622 (59%) of the mothers' were primary school and under, 382 (36.2%) were middle and high school and 51 (4.8) were university. Education level of 442 (41%) of the fathers' were primary school and under, 496 (47.2%) were middle and high school and 113 (10.8) were university. 974 (93%) of students had social security while 73 (7%) didn't. 551 (53.1%) of them stated that they found their income insufficient. 594 (56.6%) of students have good, 363 (34.6%) moderate and 92 (8.8%) bad family relationship.

We evaluated the violent behavior of the students. 226 (21.4%) of students have a verbal discussion at least once in the last year. 94 (8.9%) of the students stated that they brought a damaging tool to the school in the last year. Last year, 147 students (13.9%) had at least one physical fight and 44 students (4.2%) reported physical violence by their girlfriend or boyfriend. 25 students (2.5%) reported suicide attempt.

We found that violent behavior in boys was 2.1 times higher than in girls and in those living in extended family was 1.6 times higher than in nuclear family (Table 1).

Table 1. Factors related to violence behaviour

Variables	B	OR	%95 CI	p
Gender				
Girl		1.0		
Boy	**0.75**	**2.1**	**1.38-3.29**	**0.01**
Family type				
Nuclear		1.0		
Extended	**0.48**	**1.6**	**1.06-2.46**	**0.02**
Family relationship				
Good		1.0		
Moderate	0.31	1.4	0.92-2.04	0.12
Bad	**0.69**	**2.0**	**1.11-3.59**	**0.02**
Tobacco trial				
Not trying		1.0		
Trying	**0.62**	**1.9**	**1.11-3.11**	**0.02**
Alcohol trial				
Not trying		1.0		
Trying	**0.79**	**2.2**	**1.43-3.41**	**< 0.01**
Addictive substance trial				
Not trying		1.0		
Trying	**0.89**	**2.4**	**1.13-5.31**	**0.02**
Constant: -0.827				

Considering the factors associated with suicidal behavior, suicide attempt in students with bad family relations was 3.2 times higher than those who were good. In students who tried alcohol, the suicide attempt was 5.3 times higher than those not tried. The fact that the father was

educated in primary school and higher was found to be protective from suicide attempts (Table 2).

Table2. Factors related to suicidal behavior

Variables	**B**	**OR**	**%95 CI**	**p**
Family relationship				
Good		1.0		
Moderate	0.23	1.3	0.48-3.29	0.64
Bad	**1.17**	**3.2**	**1.07-9.69**	**0.03**
Alcohol trial				
Not trying		1.0		
Trying	**1.68**	**5.3**	**1.41-2.25**	**0.01**
Education of father				
Illiterate		1.0		
Literate	-0.52	0.6	0.95-3.71	0.58
Primary school	**-2.78**	**0.1**	**0.01-0.33**	**<0.01**
Secondary school	**-1.86**	**0.2**	**0.03-0.74**	**0.02**
High school	**-2.82**	**0.1**	**0.01-0.36**	**<0.01**
University	**-1.72**	**0.2**	**0.03-0.99**	**0.05**

Constant: -3.643

4.Discussion

According to a study conducted with data from 27 countries, the prevalence of getting involved physical fights in adolescents has been reported between 15.9% and 57.7% as discussed by M.H.Swahn et al [4]. In the studies in Turkey, physical fight rates were reported as between10.1% and 50% as discussed elsewhere [5,6]. We found that the 13.9% of students had a physical fight in our study.

In a study conducted in the USA, the rate of suicide attempt in the last 12 months was reported as 1.9%, as discussed by Meehan et al [7]. This rate was reported as 7.4% in the Youth Risk Behavior Survey 2017 [8]. In Turkey, suicide attempt was reported as 1.3% and 4.4% as

discussed elsewhere [9,10]. In our study, we found that the rate of attempted suicide is 2.5%. Our findings were consistent with other studies.

5. Conclusion

We found that the gender, family type, family relations, trying addictive substance and education level of parents are related to violence behaviour and suicide attempt. Adolescents living in extended families and boys should be evaluated as risk groups. Preventive studies against violent behavior should be planned for this group. Establishment of positive relationships between parents and adolescents, increase the level of education of parents and prevention of trying addictive products by adolescents is important in terms of protecting adolescents from violence behavior and suicide attempts.

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Conflicts of Interest

There is no conflict of interest

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ORAL PRESENTATION

Glucosinolates in Cruciferous Vegetables and Their Health Benefits

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Abstract

Cruciferous vegetables belong to *Brassica oleracea* family that includes different genus. The vegetables in this family contain chemically stable glucosinolates that has a protective role of both in plant and human body. Variation in the glucosinolates compound of Brassica vegetables can be influenced by variety, maturity at harvest, growth conditions, environmental stress, storage, processing and cooking methods. Glucosinolates are group of nitrogen and sulphur containing compounds that are biologically inactive when tissue is intact. However, when tissue is ruptured by pests, harvesting, food processing or chewing enzyme myrosinase is activated which leads to hydrolysis of glucosidic bond of these compounds. The isothiocyanates (sulphoraphane, benzyl isothiocyanates-BITC and phenethyl isothiocyanates-PEITC) and indoles (indol-3-carbinol) are the important and most investigated hydrolysis compound. Epidemiological studies indicated that isothiocyanates are modulating the balance of Phase I and II xenobitic metabolizing enzymes that are excrete in liver and epithelial cells. Recent studies have provided evidence that glucosinolates brake down products can play a crucial role in the prevention of cancer, chronic and degenerative diseases. It is stated that bladder cancer was decreased 51% by high intake of cruciferous vegetables per week. Similarly, prostate cancer was decreased 41% by three or more serving of cruciferous vegetables per week. Although Brassica vegetables are good sources of nutrition excess amount of consumption may cause some toxic effects, such as decreasing reproductive performance and growth, goiter and limiting effect of trace mineral absorption. As a result, further research is needed to understand both production system to increase amount of glucosinolates content of plant and health benefits of them.

Keywords: Brassica vegetables, antioxidant activity, isothiocyanates, indoles

1. Introduction

Cruciferous vegetables are in the *Brassica oleracea* family in the order of Capparales. This family is also known as Mustard family. Most of the family members, such as *Brassica oleracea* (cabbage, broccoli, cauliflower, kale, brussel sprout and kohlrabi), *Brassica rapa* (turnip, Chinese cabbage), *Brassica napus* (rutabaga, rapeseed), *Brassica nigra* (black mustard), *Sinapsis alba* (white mustard), *Raphanus sativus* (radish), are globally economical vegetables within the family. These plants are rich in β-carotene, vitamin C, fibers (including pectin and cellulose), calcium, lutein and zeaxanthin and phenolics. In addition, Brassica vegetables contain sulfur and nitrogen containing secondary metabolites called glucosinolates (Figure 1).

Glucosinolates are composed of a β-D-thioglucose moiety, a sulfonated aldoxime moiety and variable side chain (Figure 1). There are three classes of glucosinolates depending on amino acid structure of precursor: 1. Aliphatic synthesized from methionine, valine, leucine or isoleucine, 2. Aromatic synthesized from phenylalanine or tyrosine, 3. Indole synthesized from tryptophan [1]. Glucosinolates are stored in vacuole where they are biologically inactive and chemically stable in intact cells. However, tissue damage activates enzyme called myrosinase (β-glucosidases) resulted hydrolyses of glucosinolates. Breakdown products of glucosinolates where they protect plant against herbivores, pest and pathogens include isothiocyanates (mustard oils), thiocyanates, epithionitrils, oxazolidines and nitriles are shown in Figure 2 [2]. The end product of hydrolyses depends on side chain of the glucosinolates, pH, availability of ferrous ions and plant species [3,4].

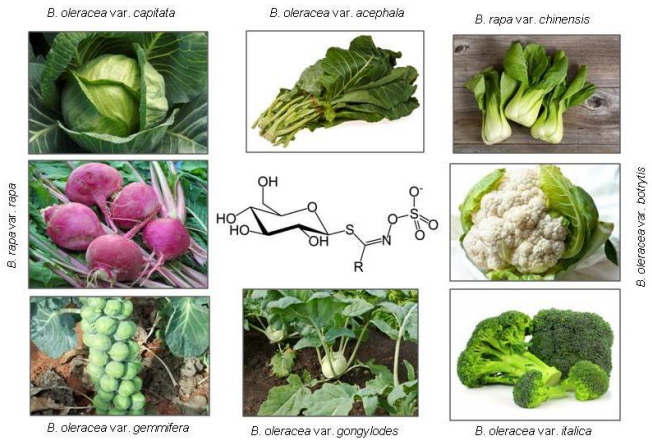

Figure 1. Chemical structure and sources of glucosinolates

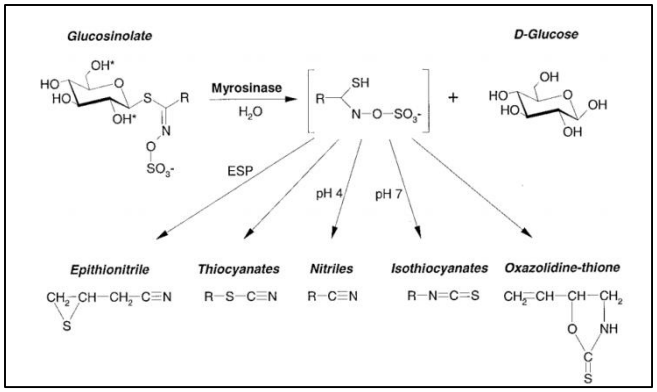

Figure 2. Enzymatic degradation of glucosinolates, unstable intermediates and reaction products [2].

The concentration and chemical form of glucosinolates in Brassicaceae vegetables varies with genotypes [5], environmental stress [6], cultural practices [7] and both storage and processing as well as cooking methods [4]. Bioavailability level of glucosinolates in these foods depends on the amount of myrosinase activity. However, physical disruption of tissue by pest, harvesting, food processing or chewing will lead release of enzyme myrosinase stored in apoplast to hydrolyze glucosinolates at the damage surface. Thus, rest of the intact tissue will be affected minimal loss of glucosinolates until cooking process. As a result, when these vegetables are eaten raw, hydrolysis of glucosinolates will occur in digestive tube with active myrosinase.

Epidemiological studies indicated that breakdown products, especially isothiocyanates (sulphoraphane, benzyl isothiocyanates-BITC and phenethyl isothiocyanates-PEITC) and indoles (indol-3-carbinol), have effective in inhibiting carcinogenesis. Recent studies have provided evidence that the risk of bladder cancer was decreased by consumption of intake cruciferous vegetables [8,9,10]. The Health Professional's Follow-Up study, included over 47000 men, showed that risk of bladder cancer reduced by consumption of cruciferous vegetables, especially broccoli. In this study, they compare the effect of different serving of broccoli. The result indicated that more than 1 serving of broccoli per week was correlated with a 29% reduction compare to less than 1 serving per week. Furthermore, the result indicated that 2 or more serving per week reduced the bladder cancer risk up to 39%. The study that has been conducted on men greater than 65 year old showed that high fruit consumption did not affected prostate cancer incidence [11]. In same study, high overall vegetable intake was associated with reduced risk of

prostate cancer. However, cruciferous vegetables were clearly protective where 3 or more serving per week statistically (41%) reduced prostate cancer compare to less than 1 serving of cruciferous vegetables. Epidemiologic evidences indicated that high consumption of cruciferous vegetables (more than 3 serving per week) were correlated statistical reduction in lung cancer [12, 13, 14]. However, several prospective studies concluded inverse relation between consumption cruciferous vegetables and lung cancer depends on study group in those studies [13,15]. The author point out that genetic variation possibly have power on glucosinolates hydrolysis of final products that may have impact on effect of cruciferous vegetable intake on lung cancer [16,17].

There is much evidence in regards to brassica vegetables and their role in the protective effects of vegetables against the risk of cancer. The biological effects of glucosinolates breakdown product action involve modification of Phase I and Phase II enzymes. Phase I enzymes, such as cytochrome p450, metabolizes procarcinogens to highly carcinogenic compounds. On the other hand, Phase II enzymes, such as glutathione transferase family (GST), causes conjugation of products that are released by Phase I enzymes by making them more water soluble discharge form urinary system. There is a growing evidence that isothiocyanates, breakdown product of glucosinolates, induces transcription level of Phase II enzymes [18, 19, 20]

2. Conclusion

It is well known that naturally occurring compounds are possessing composite chemical variety with prominent drug-like action that prevent or inhibit certain health problems. Further research is needed to define the biological activities of the glucosinolates breakdown products. Also, glucosinolates concentration of cruciferous vegetables can be manipulated by plant breeders in order to increase the nutritional properties of these crops for better health benefits.

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ORAL PRESENTATION

Development of Molecular Imprinting Technology and The Effective Use of Molecular Imprinted Polymers

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Abstract

Highly selective molecules used for antibodies or enzymes have great importance in chemistry, diagnosis and biology. However, the production of these natural receptors is difficult and expensive. Their longevity and applicability are also limited. Molecular imprinting technique (MIT) has been developed to overcome these limitations. The functional groups of the polymerizable monomers are combined with the template molecule to enable the desired selectivity. After polymerization in the presence of cross-linkers, template molecules in the polymer are removed to obtain molecularly imprinted polymers (MIPs) recognizing the size, shape and surface chemistry of the template molecule. Polymers that are selective to template molecule are cheaper, simpler and more durable than their counterparts. Polymers with different properties can be produced using a wide variety of monomers. MIT development has been ongoing for over 30 years and it's an effective method for preparing synthetic molecular recognition systems with similar binding properties like natural antibodies. MIPs used as initial separation methods are polymers, synthetic enzymes, biological receptors and biosensors with catalytic activity under the influence of progressive studies and technological developments. MIT can be adapted to the Enzyme Linked Immunosorbent Assay (ELISA), an immunological assay based on antibody-antigen interaction. MIPs are used in drug development studies, drug delivery and medicine as biomimetic antibodies. In our study, we showed that MIP imprinted against template molecule, can bind its target molecule in *in vitro* cell culture assays and can also be used in an ELISA.

Keywords: Molecular Imprinted Polymer, Biomimetic Antibody.

1. Introduction

MIPs have gained importance due to their wide applications in chemical sensing, separation, drug delivery, and extraction [1]. In the presence of template molecule, MIPs can be synthesized by co-polymerization of functional monomers and cross-linkers. The cross-linkers have function of stabilizing the binding sites after removal of the template molecule and forming recognition cavities for MIPs to detect similar molecules [2]. MIPs have important properties, such as specific recognition and high stability at high temperatures, compared to other analysis techniques [3]. In antibody-antigen interactions, , the antibody recognizes an epitope of the antigen [4–6]. The use of commercially available antibodies for isolation and purification is quite expensive and difficult to store for long periods [7]. MIPs obtained by the polymerization process around the surface of template molecule with the cross-linker can perform an antibody-like function after template molecule has been removed and recognize the same molecule using specific binding sites [8]. MIPs can now be used in a wide range of applications, such as separation (e.g., chromatography, capillary electrophoresis, solid-phase extraction, and membrane separation, etc.), immunoassays, antibody mimics, artificial enzymes, (bio)sensors, catalysis, organic synthesis, drug delivery and drug development [1].

2. Materials and Methods

Materials

Template Molecules

The purpose of molecular imprinting is to produce MIPs with affinity and specificity that are comparable to those of biological receptors, and ultimately alter these biological entities in real applications. An ideal template molecule should contain functional groups that do not inhibit polymerization, exhibit excellent chemical stability during the polymerization reaction and contain functional groups capable of complexing with functional monomers [2]. MIPs have recently been successfully applied for the identification and detection of various small organic molecules. In addition, large structured species such as viruses and cells have also been reported for MIPs [9–12]. However, great challenges remain for imprinting of proteins and other bio-macromolecules [13, 14].

Functional Monomers

Functional monomers providing functional groups have a role in forming a pre-polymerization complex with the template molecule. Therefore, it is important to select suitable functional monomers which can interact strongly with template molecule and may form specific donor-receptor or antibody-antigen complexes prior to polymerization [1]. Among the functional monomers, methacrylic acid (MAA) has been used as a functional monomer because of its hydrogen bonding, receptor properties and its dimerization modestly increases its imprinting effect [15]. It was also shown that high molar fractions of MAA will result in large pore size of the polymeric materials and further enhance the binding capacity of the polymers [16].

Cross-linkers

In polymerization process, cross-linkers are used to fix the functional monomers around template molecules, so that even after removal of template molecules, a highly crosslinked solid polymer is formed. The amount and type of cross-linker has a significant effect on selectivity and binding capacity of MIPs [17].

Solvents

The fluorogenic solvents generally act as dispersing media and pore forming agents in the polymerization process therefore play an important role in polymerization. Generally, the solvents used for the MIP synthesis are 2-methoxyethanol, methanol, tetrahydrofuran (THF), acetonitrile, dichloroethane, chloroform, N, N-dimethylformamide (DMF) and toluene [18].

Initiators

Most MIPs are widely prepared by free radical polymerization (FRP), photo-polymerization and electro-polymerization. FRP can be thermally or photo-chemically initiated for various functional groups and templates. As well as peroxy compounds, azo compounds are also widely used as initiators. One of is the azo compound is azobisisobutyronitrile (AIBN), which is optimally used at decomposition temperatures around 50-70 °C. In order to achieve the polymerization reaction, it is very important to remove dissolved oxygen from polymerization solutions prior to proliferation. Cleaning the oxygen can be achieved by bubbling an inert gas such as nitrogen or argon [1].

Preparation of MIPs

Molecular imprinting is carried out by polymerization of functional monomer around the template molecule in the presence of cross-linker [17]. First, template molecule-monomer complex is obtained by using different molecular imprinting technologies between selected molecule and complementary functional monomers [19]. After the polymerization reaction around the complex, template molecule is extracted and as a result, the three-dimensional polymer with complementary binding sites is obtained with the geometry and position of template molecule functional groups. In the production of MIPs, two main methods are usually used based on covalent and non-covalent interactions between template molecule and functional monomers. Covalent imprinting provides the formation of functional monomer residues in the imprinted cavities. However, covalent imprinting is considered to be a less flexible method because of its limited reversible reactions. In addition, the strong covalent interactions result in slow binding and dissociation, making it difficult to reach the thermodynamic equilibrium [20]. Non-covalent imprinting may occur by ionic interactions, hydrogen bonding, van der Waals forces and π-π interactions. The most common non-covalent interaction is the hydrogen bonding between MAA groups and primary amines in non-polar solvents [21]. Recently, non-covalent imprinting has become the most popular synthesis strategy due to its ease and quickness of binding and extraction.

Characterization Methods

Morphologic properties of MIPs are widely studied by scanning electron microscopy (SEM) and transmission electron microscopy (TEM). In addition, atomic force microscopy (AFM) and various fluorescence techniques are also used for the characterization of thin film MIPs. However, there has been a recent trend in spectroscopic studies of ligand-MIP interactions [1].

3. Results and Discussion

In this study, fundamentals of MIPs are summarized briefly and production processes are emphasized. Solid phase extraction (SPE) is widely used for MIPs called molecular printed SPEs. Molecularly imprinted SPE absorbers are available in a variety of forms such as cartridges, discs, SPE pipette tip, 96-well SPE microtiter plates [22]. Solid phase micro extraction (SPME) is widely used for sample preparation in analytical laboratories due to its simple, solvent-free and short-term results. Stir bar sorption extraction (SBSE) derived from SPME has a similar extraction mechanism

like SPME. SBSE has some advantages as high enrichment factor, reproducibility, high adsorption capacity and solvent-free and has been applied in environment, food and biological samples [23, 24]. In addition to their wide application areas in pre-treatment techniques, MIPs are also used as stationary phases in chromatography techniques such as HPLC [25], capillary electrochromatography (CEC) [26] and capillary LC (CLC) [27]. On the other hand, similar tests have been developed with enzyme-linked immunosorbent assay (ELISA) by coating microplate wells with MIPs [28]. MIP-based sensors are first proposed by Mosbach for specific binding of vitamin K_1 to the silicon surface by surface-imprinting method and using an optical surface ellipsometry [29]. In addition, MIP-based sensors can be developed by designing and preparing MIP particles or films. MIPs are widely applicable but their high volume production and large-scale applications are rarely reported. For these reasons, computational and combinatorial tools are required for synthetic MIPs.

5. Conclusion

This study shows that molecularly imprinted polymers can be used in chemical detection, separation, drug delivery and extraction applications. In addition, MIPs can be used in pseudo ELISA assays however further investigation is needed to produce high volume and large scale of MIPs.

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Conflicts of Interest

There is no conflict of interest

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ORAL PRESENTATION

In Vitro Anti-Tumorigenic Effects of Silver Nanoparticles Synthesis with Allium Sativum Extract

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Abstract

In recent years, Allium vegetables have been widely used to treat cancer types and microbial infections. Silver nanoparticles, on the other hand, are also known as a therapeutic agent for cancer therapy. This work aims to evaluate the antiproliferative capacity of silver nanoparticles synthesis with Allium sativum (Garlic) extract (G-AgNPs). To characterize the production of silver nanoparticles, UV–vis spectroscopy were used. The size and morphology of the synthesized nanoparticles were examined using transmission electron microscopy (TEM). Cell viability was monitored using MTT reduction assay. The mode of cell death was investigated through acridine orange and propidium iodide (AO/PI) staining. Cells were treated with AO/PI were imaged on inverted microscope with fluorescence attachment. G-AgNPs exhibited absorption maxima at 428 nm. TEM images revealed bimodal size distribution of G-AgNPs. MTT assay and apoptotic analysis were done after 24, 48, and 72h incubation of cells with different dilutions of G-AgNPs. As compared to control, MTT results showed that there were no significant differences between dilutions groups across all time intervals. Within the 72h, G-AgNPs-D6 ($p<0.05$) and G-AgNPs-D7 ($p<0.01$) groups were significantly difference from control group. Also, the results of the AO/PI staining were supported MTT assay. Late apoptotic cells appeared orange or red in colour owing to their condensed nuclei. These results revealed the potential drug like efficiency of G-AgNPs for cancer therapy.

Keywords: Allium sativum; silver nanoparticles; cancer treatment; cell proliferation.

1. Introduction

With the widespread use of herbal supplements, the usage of herb-based threapy in medicine, especially in cancaer threapy is a growing trend in medical application. The health effects of dietary garlic have been utilized throughout the centuries to offer protection against infections, heart disease and cancer [1]. In cancer prevention, numerous epidemiological studies have demonstrated a link between garlic consumption and decreased risk of cancer especially cancer of the colon and stomach [2].

The unique physico-chemical properties of silver nanoparticles (AgNPs) have attracted increasing interest from the scientific community [3] due to their high thermal conductivity, plasmonic properties, chemical stability and antibacterial ability. AgNPs are a promising tool as anticancer agents in diagnostics and probing [4], with strong effects against different cancer cell lines offering many advantages. For these reasons, the goal of nanomedicine is to identify cost-effective molecules that have high specificity and sensitivity in cells.

Starting from these assumptions, in this study we focused to evaluate the antiproliferative capacity of silver nanoparticles synthesis with Allium sativum (Garlic) extract (G-AgNPs) on T98G cell line.

2. Materials and Methods

To characterize the production of silver nanoparticles, UV–vis spectroscopy was used. The size and morphology of the synthesized nanoparticles were examined using TEM.

Cell viability was monitored using MTT reduction assay. The mode of cell death was investigated through AO/PI staining. Cells were treated with AO/PI were imaged on inverted microscope with fluorescence attachment.

3. Results and Discussion

The surface plasmon resonance peaks in absorption spectra for G-AgNPs synthesized with garlic extract showed that the absorption maximum range was at 428 nm (Figure 1). TEM images revealed bimodal size distribution of G-AgNPs. The mean particles sizes are found to be approximately 47.2 nm and 7.4 nm respectively. The G-AgNPs were monodispersed in the colloidal solution and displayed a distribution of sizes in the range of 7-47 nm. G-AgNPs were

surrounded by a thin layer of materials which indicates the possibility of organic-based capping agents inherent in the aqueous extract (Figure 2).

MTT assay and apoptotic analysis were done after 24, 48, and 72h incubation of cells with different dilutions of G-AgNPs. The control group with no particle treatment was referred to as having 100% cell viability. As compared to control, MTT results showed that there were no significant differences between dilutions groups across all time intervals. Within the 72h, G-AgNPs-D6 ($p<0.05$) and G-AgNPs-D7 ($p<0.01$) groups were significantly difference from control group (Figure 3). Also, the results of the AO/PI staining were supported MTT assay. Control cells were mostly viable and showed uniform green fluorescence. The nuclei and membranes of early apoptotic cells were intact and showed bright green patches as an indication of perinuclear chromatin condensation. Late apoptotic cells appeared orange or red in colour owing to their condensed nuclei (Figure 4).

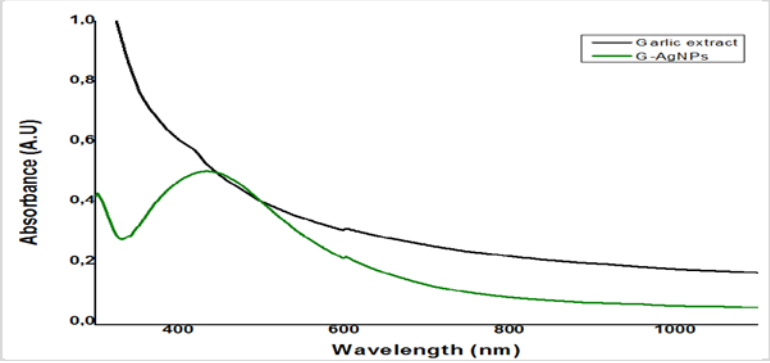


Figure 1. Figure shows the UV-vis spectra of the G-AgNPs synthesized with the help of garlic extract as a reducing agent.

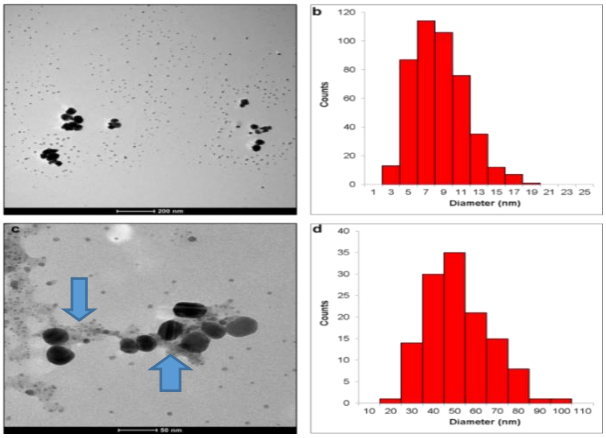


Figure 2. TEM images of G-AgNPs. The mean particles sizes are found to be approximately 47.2 nm and 7.4 nm respectively.

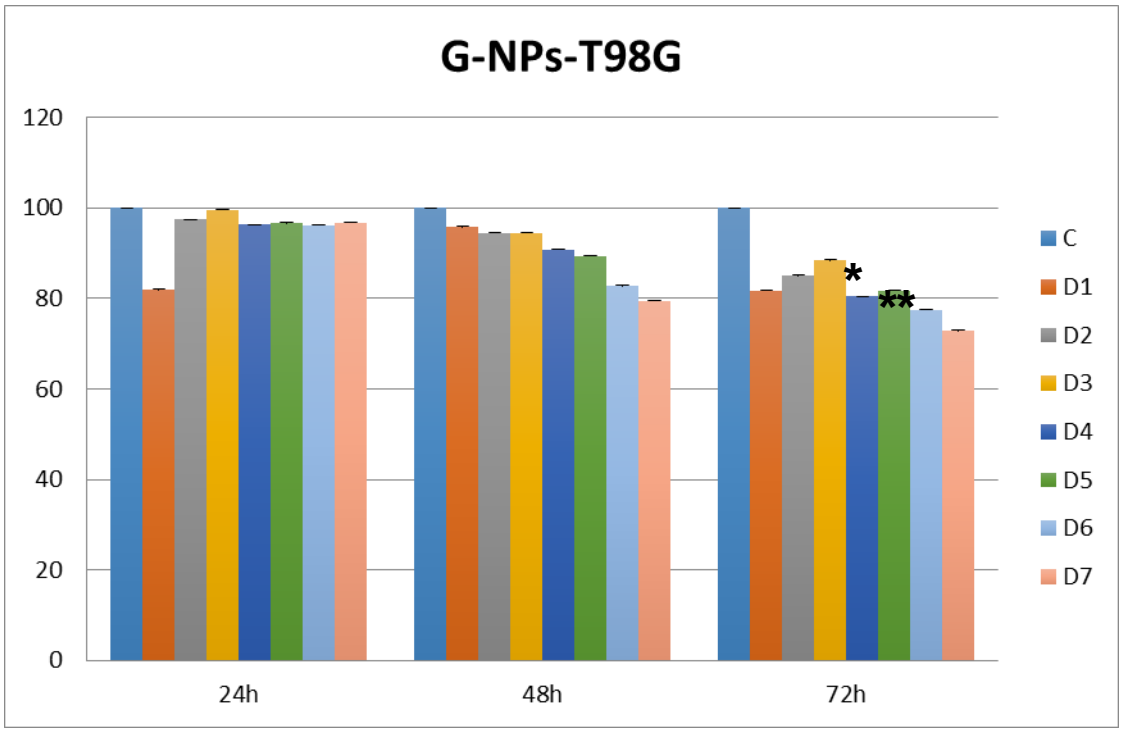
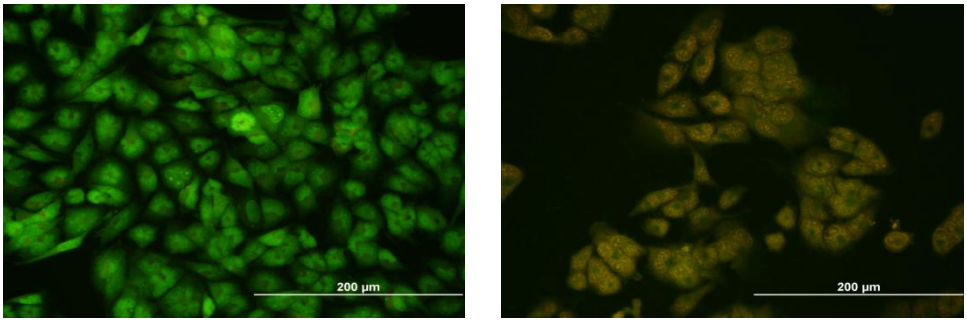


Figure 3. MTT results of cell proliferation (after treatment with various concentrations of nanoparticles) in different time duration.



Control group Experimental group

Figure 4. AO/PI stainings of control and experimental groups.

4. Conclusion

This study aims to evaluate antiproliferative capacity of silver nanoparticles synthesis with G-AgNPs. The outcomes of our studies suggest that, depending on doze, G-AgNPs effects negatively cell proliferation. These results revealed the potential drug like efficiency of G-AgNPs for cancer therapy.

Acknowledgements

None.

Conflicts of Interest

There is no conflict of interest.

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INVITED PRESENTATION

Plant-Originated Molecules as Promising Enzyme Inhibitors: *in vitro* and *in silico* Approaches

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Abstract

Nature has always afforded many drug molecules to treat human diseases. Among them, some reputed drugs such as aspirin, morphine, quinine, artemisinin, taxol, etc have been gifted from plants. On the other hand, enzyme inhibition has been a quite attractive target for scientists in drug discovery as it is one of the common mechanisms of action for many clinically available drugs. Relevantly, during our extensive screening of natural products to explore new enzyme inhibitory molecules using *in vitro* methods using ELISA microplate reader, up to date, we have reported a good number of molecules with promising inhibitory effects against various target enzymes comprising tyrosinase, elastase, collagenase, cholinesterases (acetyl- and butyryl- derivatives), xanthine oxidase, phosphodiesterase, carbonic anhydrase, urease, etc. Then, the inhibiting molecules were subjected to molecular docking (*in silico*) experiments to examine possible interactions at molecular level. During these efforts, we have recently revealed a number of promising molecules such as coumarin derivatives (*e.g.* pteryxin), isoflavone derivatives, tanshinones (diterpene derivatives) as selective butyrylcholinesterase inhibitors, luteolin 5-*O*-*beta*-glucoside as potent carbonic anhydrase type-II inhibitor, quercetin as tyrosinase inhibitor, etc. In the present talk, current data obtained from our enzyme inhibition experiments on natural compounds will be discussed.

Keywords: Enzyme inhibition, natural molecules, *in vitro* study, molecular modelling

1. Introduction

Natural sources with an enormous potential have been always a great interest to scientists from the point of drug development view. In fact, many drugs used in clinic in past and present have been isolated generally from plants as well as other biosources such as marine organisms, microorganisms and animal species, *i.e.* venoms in particular. Consistently, natural products have been proven to be very fruitful sources for new drugs as confirmed by a report presenting that 48% of 877 new drug molecule discovered between 1981-2002 were declared to be either directly

natural molecule (6%) or their derivatives (27%) or synthetic analogs (16%) [1]. For instance; tubocurarine became a clinically used myorelaxant drug from an arrow poison (known as curare) obtained from the bark of *Chondrodendron tomentosum* [2]. Another important example would be acetylsalicylic acid, popularly known as aspirin, which was isolated from the willow bark (*Salix alba*) [3]. Taxol, another striking molecule from the bark of *Taxus brevifolia* (yew tree), was approved as anticancer drug by FDA for ovary cancer in 1992 and for breast cancer in 1994 [4]. On the other hand, more modern natural molecules such as epigallocatechin gallate (EGCG) from green tea (*Camellia sinensis*), curcumin from turmeric (*Curcuma longa*), and resveratrol from the grape skin (*Vitis vinifera*) seem to be pretty auspicious novel drug candidates in near future [5-7].

It is well-known that enzyme inhibition is a popular strategy for drug development and a lot of drugs of natural or synthetic origins available in clinic are enzyme inhibitors. For instance, a current case of clinically available drugs of herbal origin with enzyme inhibitory action is galanthamine used for the treatment of Alzheimer's disease (AD), an anticholinesterase alkaloid found in the bulbs of several Amaryllidaceae species, *e.g. Galanthus* sp., *Narcissus* sp., and *Leucojum aestivum* [8]. Besides, statins as antihyperlipidemic agents were firstly discovered in 1979 from a microorganism, *e.g. Aspergillus terreus* [9]. Captopril, an inhibitor of angiotensin-converting enzyme (ACE) is a popular antihypertensive agent, whose precursor compound was initially found in the venom of the snake species *Bothrops jararaca* [10].

2. Results and Discussion on Enzyme inhibitors from plants during our studies

Since the year of 2000, we have been working on finding novel enzyme inhibitors from natural sources, mainly Turkish medicinal plants. Being very rich in number of plant species, Turkey has a great plant biodiversity with ethnobotanical use in Anatolian folk medicine. In this great endeavor, we have so far screened over 400 plant species and at least 300 natural molecules in order to find new enzyme inhibitors. In our large screening studies, we started with inspecting all *Salvia* species (over 95 species) growing naturally in Turkey for their anticholinesterase effect as *Salvia officinalis* has been recorded to be used for memory-enhancing purpose in European folk medicine [11-17]. Later on, in addition to acetyl- (AChE) and butyrylcholinesterase (BChE); our studies on screening natural products have continued against more enzymes including tyrosinase (TYR), elastase, collagenase, xanthine oxidase (XO), lipoxygenase (LOX), phosphodiesterase-I (PDE-I), carbonic anhydrase-II (CA-II), urease, hydroxymethylglutaryl-coenzyme A (HMG CoA)

reductase, etc. These studies conducted by our group have led to identification of numerous plant species along with many pure molecules as new enzyme inhibitors. According to our data, we have revealed that coumarins from plants in particular seem to be quite promising selective inhibitors of BChE. Among them, we have discovered imperatorin from *Angelica officinalis*, pteryxin and hyuganin C from *Mutellina purpurea*, etc as strong BChE inhibitors (Figure 1) [18-24]. Besides, we performed our screenings not only by *in vitro* assays, but also *in silico* experiments using molecular modelling. By the same approaches, we have newly elucidated a number of xanthohumal derivatives isolated from *Humulus lupulus* growing in Poland as potent BChE inhibitors [25]. On the other hand, flavonoid-type of inhibitors of CA-II from medicinal plants that have been also revealed by our group were luteolin 5-O-β-glucoside, methyl rosmarinate, apigenin, and vicenin 2 [26]. Many diterpenes obtained from *Perovskia atriplicifolia* Benth. and *Salvia glutinosa* have been shown to be potent and selective inhibitors of BChE [17].

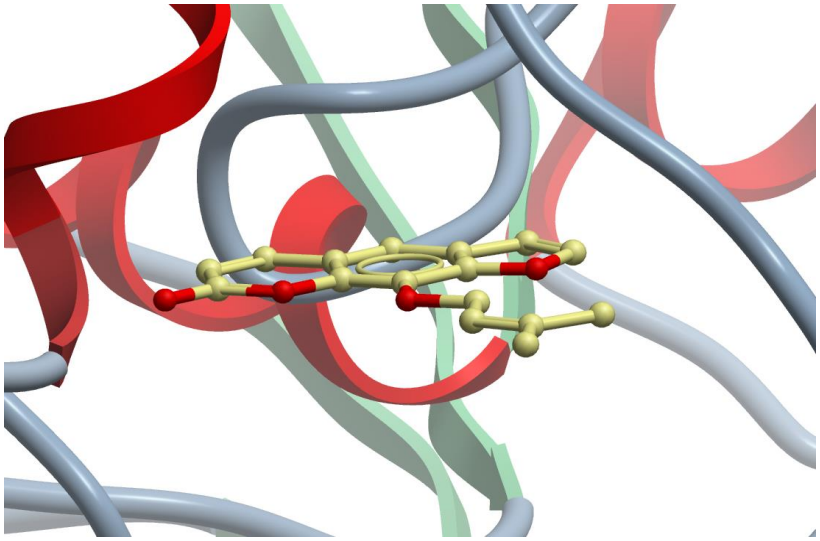

Figure 1. Imperatorin docked into the active site of huBChE.

3. Conclusion

The outcomes obtained from our extensive studies on finding novel enzyme inhibitors from plants have indicated that plant-originated molecules are very promising candidates with a marked inhibitory potential. Our experience underlines that some specific plant metabolites are also selective inhibitors of some enzymes. As aforementioned, coumarins appear to be the selective and

potent inhibitors of BChE, a valid target enzyme for AD. Moreover, polyphenols might be stated to be strong inhibitors of TYR or CA-II. Therefore, plant metabolites will always draw a huge attention from researchers working on exploring new enzyme inhibitors.

Acknowledgements

Contribution of a number of scientists and co-workers (F.S. Senol, A. Rauf, K. Skalicka-Wozniak, E. Banoglu, S. Shekfek, R.A. Salmas, S. Durdagi, W. Oleszek, S. Slusarczyk, A. Matkoswki, and H. Perez-Sanchez) to our studies mentioned above are gratefully acknowledged.

Conflicts of Interest

There is no conflict of interest

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ORAL PRESENTATION

Mitochondrial DNA HVR I and HVR II Variations in a Turkish Populations

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Abstract

Recently, mitochondrial DNA (mtDNA) mutations or alterations have also been identified in bladder cancer, breast cancer, esophageal cancer, head and neck cancer, hepatocellular carcinoma, lung cancer, ovarian cancer, prostate cancer, renal cancer, thyroid cancer and a number of blood cancers. Various types of molecular alterations in mtDNA such as point mutations, polymorphisms, deletion, insertions, microsatellite instability and changes in mtDNA copy number have been characterized throughout the mitochondrial genome in human cancers. In the present study, we investigated two hypervariable mitochondrial hypervariable region I (HVR I) and hypervariable region II (HVR II) in a total of 100 samples. A total of 47 nucleotide polymorphisms at were detected in both regions, and 39 out of 47 sites were in HVR I region, whereas 8 were in HVR II region. Transition, transversion, insertion, and deletion were observed in the examinations. In HVR I region, the most frequently observed nucleotide alterations were at the positions 16221 (84.2%) (nucleotide transversion from C to A). In HVR II, the most frequently observed nucleotide alterations were at the positions 263 (nucleotide transition from A to G) with the frequency of 100%. Overall, these findings support a role for mitochondrial genome variations. Databases from the present study will abet the expanding role of mtDNA typing in different genetic information.

Keywords: Variation, Mitochondrial DNA, HVR I, HVR 2, Humans, Turkey.

1. Introduction

The human mtDNA is a closed circular genome, approximately 16.5 kb in length with about 1.1 kb-long noncoding DNA [1,2]. The complete nucleotide sequence of human mtDNA has been reported [2], and it has been established that the mtDNA control region represents a highly variable sequence [3,4]. mtDNA is maternally inherited, and exists in a high copy number (1000–10000 copies) in each cell with rapid mutation (5–10 times faster compared to nuclear DNA) [5,6]. Studies about sequence polymorphism have mostly been focused on the control region or displacement loop (D-loop). The mtDNA control region includes two hypervariable regions: HVR I and HVR II. The D-loop region is a hot spot for mtDNA alterations, and the sequence analysis of these two regions is used not only in forensic analyses, but also in medical diagnosis [7].

Analyses of the frequency, variation, and distribution of mtDNA have shown that mtDNA polymorphism can play an important role in modulating disease expression [8]. Mutations in the mtDNA have been reported to occur in human cancers [9-11]. For example, Alonso et al. detected mutations in the mtDNA D-loop region in colorectal and gastric malignancies [9]. There are other reports that have analyzed alterations of the D-loop in lung cancer, colon cancer, ovarian cancer, hepatocellular carcinoma and breast cancer [10]. And also Kirches et al. worked on direct sequencing of the complete D-loop from frozen glioblastoma samples and corresponding blood [11].

Keeping in view the importance of polymorphism in mtDNA, we investigated two hypervariable mitochondrial HVR I and HVR II regions in the healthy population.

2. Materials and Methods

Sampling

A total of 100 unrelated individuals living in Denizli province were participated in this study. All the individuals were included in the study after giving informed consent. This study was approved by the Ethics Committee of Pamukkale University Medical Faculty. The blood samples from each individual were collected in and stored at -80ºC until the DNA extraction.

DNA extraction, amplification, and sequencing

Total genomic DNA was extracted from peripheral blood with standard phenol chloroform extraction protocol [12]. PCR (polymerase chain reactions) amplification of mtDNA control region was performed using two primer sets as follows: L15997 5'-CACCATTAGCACCCAAAGCT-3' and H16401 5'- TGATTTCACGGAGGATGGTG-3' for HVR I; L29 5'-GGTCTATCACCCTATTAACCAC-3' and H408 5'- CTGTTAAAAGTGCATACCGCCA-3' for HVR II [13]. PCR products were determined first by agarose gel electrophoresis and then by a capillary electrophoresis system (ABI Prism 310, PE Biosystems) to define polymorphic sites.

Data analysis

Sequences were individually checked by using Chromas version 1.41 (http://chromas.software.informer.com/), and all the detected polymorphisms were checked with the original electropherograms. When possible polymorphisms were unclear, independent PCR reactions and sequencing were performed for confirmation. Sequences were aligned manually with MEGA6 (with ClustalW alignment option) [14], and CLUSTAL X (with default alignment option) softwares [15] following the similarity criterion as suggested by Simmons et al. [16].

3. Results and Discussion

Nucleotide sequence changes in the poly-cytosine tract from 16184 to 16193 in HVR I region were examined (Fig.1).

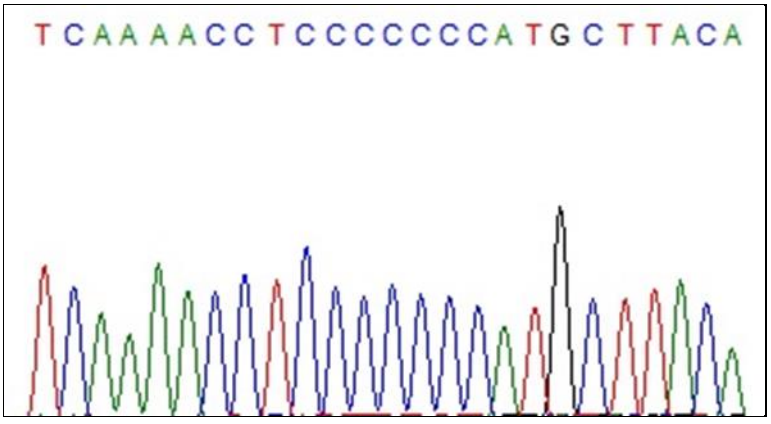

Figure 1. DNA Sequencing of poly-cytosine tract from 16184 to 16193 in HVR I

Nucleotide sequence changes in the poly-cytosine tract from 303 to 315 in HVR II region were examined (Fig 2).

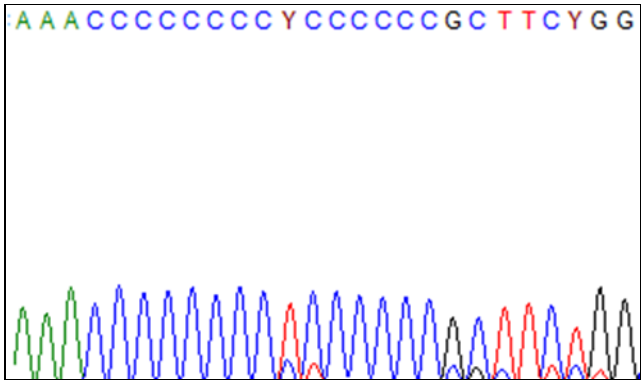

Figure 2. DNA Sequencing of poly-cytosine tract from 303 to 315 in HVR II

DNA alterations in mitochondria are believed to become fast hotspots of cancer research. Numerous mutations in mtDNA has now been observed in multiple cancer types (17]. For example, the first somatic mtDNA mutation was detected by Bert Vogelstein's group in human colorectal cancer cells 15 years ago [18]. After these determinations, mtDNA mutations or alterations have also been identified in bladder cancer, breast cancer, esophageal cancer, head and neck cancer, hepatocellular carcinoma, lung cancer, ovarian cancer, prostate cancer, renal cancer, thyroid cancer and a number of blood cancers [19-22]. Various types of molecular alterations in mtDNA such as point mutations, polymorphisms, deletion, insertions, microsatellite instability and changes in mtDNA copy number have been characterized throughout the mitochondrial genome in human cancers [22].

According to our unpublished data we determined variations both glioblastomas and blood samples in mtDNA. We observed nucleotide alterations were at the positions 16221 with high frequency. The mtDNA sequences were aligned and compared with the complete Cambridge Reference Sequence (rCRS; GenBank accession no: NC_012920.1), this nucleotide alteration was rare among the populations. Interestingly we observed this variation with high frequency both tumor and blood samples. On the other hand, Seo et al. reported this variation in the Japanese population study [23]. Kirches at al. worked on glioblatoma mtDNA alterations, observed nucleotide alteration results were diffrent comparision to our results [20]. Although they determined A185G, T195C, C204T, T295C, C16126T, A16293G, T16356C, and T16519C variations in D-loop region, however we

did not observed T195C, C204T, T295C, T16356C, and T16519C variations in our tumor and blood samples. Also, they found 17 patients (31%), the brain was homoplasmic; and in 12 of these cases, the polycytosine tract was unchanged in the corresponding tumor. We also observed the unchanged polycytosine tract corresponding tumor samples.

5. Conclusion

Taken together, these findings raise the possibility that the genotype distributions within the Turkish population living in different regions and/or with different origins may be different. In conclusion, this preliminary study shows the importance of mtDNA in between the populations. Databases from diverse populations will abet the expanding role of mtDNA typing in different genetic information. Further investigations are necessary to determine the importance of mtDNA alterations in the development and maintenance of cancers.

Acknowledgements

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Conflicts of Interest

There is no conflict of interest

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ORAL PRESENTATION

Effects of Paclitaxel on Lipid Peroxidation and Antioxidant Enzymes in Tissues of Mice Bearing Ehrlich Solid Tumor

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Abstract

Several chemotherapeutic drugs have been studied for anticancer activity. Paclitaxel is one of the chemotherapeutic drugs of high medicinal interest.This study was performed to investigate effects of paclitaxel on lipid peroxidation and antioxidant enzymes in tissues of mice bearing Ehrlich solid tumor. In this study, 36 Balb/C male mice aged 8-10 weeks were used.Six mice were kept as cancer stock to produce Ehrlich ascites tumor (EAT) cells.Thirty mice were distributed to three groups as healthy control, tumor control and paclitaxel treatment. The animals in tumor control and Paclitaxel treatment groups received $1x10^6$ EAT cells via s.c. route through nape skin on the first day of the experiment.After EAT cells application,10 mg/kg Paclitaxel injected via intraperitoneal route on days 4, 9 and 14.At the end of the study animals were sacrificed. The liver, kidney, brain and testis tissues were collected and analyzed for malondialdehyde (MDA) by TBARS method, superoxide dismutase (SOD) and catalase (CAT) activities spectrophotometrically. Paclitaxel treatment significantly reduced the increased MDA levels in kidney and liver. Paclitaxel had no effect on testis MDA but brain MDA level reduced. Paclitaxel returned the brain MDA level close to the level of healthy control.EAT cell injection reduced CAT activity in kidney and liver and Paclitaxel had no effect on CAT activities in these tissues.In EAT cell injected mice;testis and brain CAT activities were higher than healthy controls by Paclitaxel treatment. Paclitaxel had no significant effect on decreased kidney and liver SOD activities whereas significantly reduced the increased SOD activities in testis and in brain. Paclitaxel alleviated the lipid peroxidation in kidney and liver but had no effects on antioxidant status in these tissues of Ehrlich solid tumor-bearing mice.

Keywords: Paclitaxel, Ehrlich solid tumor, lipid peroxidation

1. Introduction

Cancer, a genomic disease, is a major public health problem worldwide. Cancer, which is accepted among chronic diseases, is frequent and is the second leading cause of mortality following cardiovascular diseases [1-3]. Cancer is one of the most important health problems in Turkey as it is in the whole world. In Turkey, the standardized cancer rate for age in 2013 is 186.5 per hundred thousand for women and 267.9 for men. Total cancer incidence is 227.2 per hundred thousand. In 2002, deaths from cancer in our country constituted 12% of all deaths whereas it increased up to 21% in 2009. When the 2012 data is evaluated, over 175.000 new cancer cases have emerged in our country within one year. If a similar course continues, it is expected that there will be 22 million new cases annually in 2030 [2].

A number of experimental cancer models have been developed for use in cancer-related studies and among them Ehrlich solid carcinoma is a commonly used tumor model [4-5]. Ehrlich ascites carcinoma is a spontaneous murine mammary adenocarcinoma adapted to ascites form and carried in outbred mice by serial intraperitoneal (i.p.) passages [6].

Ehrlich solid carcinoma is an undifferentiated tumor [5]. It has been reported that Ehrlich ascites tumor (EAT) cells undergo rapid proliferation in almost any mouse host because they lack H-2 histocompatibility antigens [4]. Morphological and metabolic changes occur following implantation of EAT cells. It has been shown that subcutaneous implantation of EAT cells into mice causes changes in oxidant and antioxidant status in the tissues [5].

Several chemotherapeutic drugs have been studied for anticancer activity. Paclitaxel, also known as Taxol, is a chemotherapeutic drug of high medicinal interest. Paclitaxel, one of the most commonly used chemotherapeutics in the clinic. It is a broad spectrum anticancer drug that is effective in various solid tumors such as ovarian and breast cancer, lung cancer, melanoma, head and neck cancer and bladder cancer [7-9]. Paclitaxel has potent anti-proliferative action against tumor cells [10]. And this agent shows its functions by stabilizing microtubules, blocking mitosis and inducing apoptosis [9-11]. Reactive oxygen species (ROS) are produced continuously in the body as a result of aerobic metabolism as well as external factors [12]. and these are balanced by antioxidant defense systems.When ROS are produced in excess, they cause tissue damage. Cellular damage resulting from oxidative stress is involved in the initiation and progression of cancer [13]. Cancer cells increase production of ROS compare to normal cells [14]. and it is

speculated that tumorigenic signaling also increases expression of antioxidant proteins to balance the high ROS production to maintain redox homeostasis [15-16]. Studies indicated that the levels of oxidative stress markers increase in cancer cases [17-20]. Several adverse effects of chemotherapy treatments have been reported and most of these effects are associated with oxidative metabolism [18]. Anti-cancer drugs can also cause oxidative stress as the side effect [13]. The previous studies investigating the effects of taxol on lipid peroxidation and antioxidant status in different cancer types in animal models and human have revealed distinct results [21-23]. Therefore, this study was performed to investigate the effects ofPaclitaxel on tissue MDA levels and antioxidant enzyme activities in mice bearing Ehrlich solid carcinoma.

2. Materials and Methods

Animals, management and experimental design

In this study, 42 Balb/C male mice aged 8-10 weeks and weighing 25-30 g were obtained from Erciyes University Experimental and Clinical Research Center (DEKAM). The study was held at DEKAM with the permission of Erciyes University Experimental Animals Local Ethics Committee, Approval No. 15/03 and dated 14.01.2015.

Animals were maintained in polycarbonate cages sized 42x26x15 cm (five mice in each) at this center that provides appropriate standard conditions (21±2^{o}C room temperature, 50±5% humidity, environmental ventilation systems providing air flow rotation of 12 per hour and 12 hours light/dark light cycle) for highest health status throughout the study. A commercially available pellet diet containing 24% crude protein, 3.85% crude cellulose, 5% fat, 6.98% ash as well as amino acids and vitamin-mineral mix that met the daily nutritional requirement of the mice and routinely used in DEKAM was provided throughout the experiment. Water and feed were supplied ad libitum during the study.

In the beginning of the study, all animals were weighed. Before starting the experiment, 12 mice were kept as cancer stocks to obtain sufficient EAT cells. The remaining 30 animals were assigned into three experimental groups consisting of 10 mice in each. Individually labeled five mice were maintained in one cage.

Group I was kept as healthy control and a 0.1 ml of physiologic saline solution was administered subcutaneously (s.c.). On the first day of the experiment, a single dose of $1x10^{6}$ EAT cells in 0.1 ml of phosphate buffer saline (PBS) was injected via s.c. route through nape skin to each animal

in groups II and III for solid tumor development. Mice in group II were kept as solid tumor control following the EAT injection. Mice in groups III received 10 mg /kg Paclitaxel via i.p. route on days 4, 9 and 14. The animals in control group also received physiologic saline solution via i.p. route on the same days.

Sample collection and preparations

At the end of the experiment (on day 15th the study), all of the animals were sacrificed with ketamine-xylazine under general anesthesia and the liver, kidney, brain and testis tissues from each animal were collected into sterile plastic bags for determination of MDA levels, SOD and CAT activities. The samples were transferred immediately to the laboratory under cold chain and stored at -80ºC until biochemical analyses.

Homogenization of tissues

Tissue samples (500 mg) were thawed and homogenized in a glass-glass homogenizer with physiological saline solution (pH=7.4) (1/10, w/v). The homogenates were centrifuged at 12.000 rpm for 20 minutes under 4 °C. Some parts of the supernatants were separated for MDA and CAT analyses. The remaining supernatants were mixed with ethanol/chloroform mixture [5/3 (v/v)] at a 1/1 ratio and were centrifuged again at 12.000 rpm for 20 minutes in a refrigerated centrifuge. The supernatants were separated for SOD enzyme activity.

Determination of MDA level, CAT and SOD activities

Malondialdehyde, a secondary product of lipid peroxidation, is an important indicator of lipid peroxidation. Malondialdehyde forms a pink-colored complex with thiobarbituric acid (TBA) under aerobic conditions at pH=3.4 following the incubation at 95°C. The absorbance of this complex was measured at 532 nm by a UV-Visible spectrophotometer (Shimadzu, UV 1601, United states) using freshly prepared 10, 20, 40, 60, 80, 100 nMol/ml of 1,1,3,3-tetramethoxypropane (density: 0.99 g/ml) solutions as standards according to the method described by Ohkawa et al. [24]. Briefly, 100 µl of tissue homogenate was mixed with 8.1% of sodium dodecyl sulfate (SDS), 20% of acetic acid (HAc) (pH=3.5) and 0.8% of TBA (pH=3.5) and incubated at 95 °C for 30 minutes. Then cooled and n-Butanol-pyridine (nBu-Pri) solution

and distilled water were added and strongly vortex mixed. The supernatant was separated following the centrifugation at 4.000 rpm for 10 minutes and the absorbance was read. The result was expressed as nMol/mg protein.

The activity of SOD was measured spectrophotometrically according to the method described by Sun et al. [25]. This method is based on the reduction of nitrobluetetrazolium (NBT) by superoxide radicals, which is formed by the enzymatic reaction of xanthine oxidase (XO). The colorless NBT ion is transformed into a blue-coloredformazan giving maximum absorbance at 560 nm when reduced with the superoxide radical.The tissue was homogenized with 1/10 of distilled water. The sample was mixed with the chloroform/ethanol mixture 1/1 (v/v) and centrifuged at 12.000 rpm for 2 hours at +4 ºC. The supernatant was separated to determine SOD activity. A 50 µl of tissue supernatant and a 50 µl XO in 2 M ammonium sulfate solution (1/100, v/v) were added to 2.9 ml of the reagent mixture consisting of xanthine solution + NBT + Na_2CO_3 + BSA. After incubation at 25 °C for 20 minutes, a 1 ml of 0.8 mM $CuCl_2$ was added to the tube and the optical density of the sample was read at 560 nm. The SOD activity was expressed as Unit/mg protein (1 unit=50% inhibition of NBT reduction) and % inhibition was calculated with the following formula: % inhibition = [(blank abs-tissue abs)/blank abs] x 100.

Catalase enzyme catalyzes the conversion of H_2O_2 to H_2O. This conversion can be followed by a decrease in absorbance at 240 nm. The decrease in absorbance at 30 second is related to catalase activity. The CAT activity was determined as described previously by Aebi [26]. The CAT assay was performed briefly as follows: Tissue homogenate was mixed with H_2O_2 solution (30 mM) + freshly prepared PBS (50 mM, pH=7.0) then the absorbance was measured spectrophotometrically at 240 nm after 30 second against blank. The extinction coefficient was 0.004 (0.0039) mM-1mm-1. The CAT activity was expressed as U/mg protein/min for tissue.

Analysis of the data

IBM SPSS Statistics 22.0 (IBM Inc., ILL, USA) program was used for statistical analysis of the data. The normal distribution of the data was evaluated by histogram, q-q graphs and Shapiro-Wilk test. The variance homogeneity was tested by the Levene test. One way ANOVA and Kruskal Wallis test were used in the intergroup comparisons. Tukey and Dunn-Bonferroni tests were applied for multiple comparisons. The data were evaluated using the R 3.2.3 program. Data

were presented as means ± standard deviation of the means and median (25% -75% percentiles) where appropriate. Significance level was accepted as $p < 0.05$.

3. Results and Discussion

Compare to healthy control, tumor development slightly but not significantly increased kidney and liver MDA levels. Paclitaxel treatment significantly reduced the increased MDA levels in kidney and liver ($p<0.001$). Paclitaxel had no effect on testis MDA but brain MDA level reduced by EAT cells injection and Paclitaxel returned the brain MDA level to the level of healthy control ($p<0.001$). EAT cell injection reduced catalase activity in kidney and liver ($p<0.001$) and Paclitaxel had no effect on catalase activities in these tissues. In EAT cells injected mice; testis and brain catalase activities were higher than healthy controls that were returned to control levels by Paclitaxel treatment. Paclitaxel had no significant effect on decreased kidney and liver SOD activities whereas significantly reduced the increased SOD activities in testis ($p<0.05$) and brain ($p<0.01$).

Organs		**Healthy control**		**Cancer Control**		**10 mgPaclitaxel**	**p**
				MDA			
Kidney		0.87^{ac} (0.83-0.89)		0.94^{bc} (0.91-.96)		0.60^{a} (0.42-0.62)	0.000
Liver		12.02^{ac} (11.69-12.47)		15.93^{bc} (14.54-16.77)		10.90^{a} (9.89-11.40)	0.000
Testis		0.79 (0.69-0.93)		0.97 (0.89-24.24)		0.94 (0.69-21.24)	0.146
Brain		8.20^{a} (7.80-8.42)		6.23^{b} (5.85-6.45)		8.20^{a} (7.90-9.10)	0.000
				Catalase			
Kidney		29.26 ± 2.53^{a}		23.97 ± 2.92^{b}		22.07 ± 2.13^{b}	0.000
Liver		47.96 ± 3.10^{a}		41.76 ± 5.96^{b}		33.89 ± 1.93^{c}	0.000
Testis		12.95 ± 0.64^{b}		15.97 ± 0.39^{a}		12.17 ± 0.91^{b}	0.000
Brain		25.53 ± 1.32^{b}		32.64 ± 1.65^{a}		26.64 ± 1.32^{b}	0.000
				SOD			
Kidney		5.45^{b} (5.38-5.68)		3.70^{a} (3.50-3.90)		3.60^{a} (2.90-4.2)	0.002
Liver		6.50^{b} (6.40-6.90)		4.50^{a} (4.30-4.50)		4.70^{a} (3.50-4.90)	0.001
Testis		2.45^{ab} (2.40-2.95)		2.75^{b} (2.70-3.33)	0	2.40^{a} (2.10-2.63)	0.020
Brain		4.10^{ac} (3.71-4.53)		4.40^{bc} (3.98-4.83)	0	3.25^{a} (2.86-3.63)	0.006

Cancer cells demonstrate alterations in oxidative metabolism characterized by the increased production of ROS compare to normal cells [22]. And it is speculated that tumorigenic signaling increases expression of antioxidant proteins to balance the high ROS production to maintain redox homeostasis [15,16]. It has been reported that oxidative stress, chronic inflammation and cancer are closely related [27]. Ehrlich ascites carcinoma, a spontaneous murine mammary adenocarcinoma, is adapted to ascites form by serial intraperitoneal passages [6]. Ehrlich ascites tumor cells rapidly proliferate in almost all mouse species because ofthe lack of H-2 histocompatibility antigens [4]. Ehrlich ascites tumor cells cause morphological and metabolic changes including alterations in oxidant and antioxidant status in the animals [5]. Previous studies have shown that the oxidative stress especially the lipid peroxidation increases in cancer cases [13,14,17,19,20,28]. Therefore, in the present study, Ehrlich solid tumor model was chosen to investigate the effect of paclitaxel on lipid peroxidation and antioxidant status in the tissues of solid tumor bearing mice.

Taxol has been used effectively in the treatment of various cancers including ovarian and breast cancer, lung cancer, melanoma, head and neck cancer, bladder cancer and other cancer types [7-9]. Oxidative stress is defined as an imbalance between ROS and the anti-oxidant capacity of the cell (13). Patmavathi et al. [7], have shown the increases in the level of lipid peroxidation in the breast and liver of breast cancer bearing rats. Didziapetriene et al. [14], detected elevated MDA level in ovarian cancer. In the present study, the MDA levels in the kidney and liver were increased by the tumor development in the EAT cell injected mice. The elevated kidney and liver MDA levels were reversed with Paclitaxeltreatment, which was lower than the healthy controls. However, Paclitaxel had no significant effect on the testis MDA level. On the other hand, brain MDA level was found to be lower than healthy control mice but returned to the level of controls after paclitaxel treatment.

In cancer bearing animals, significant decreases were reported in the SOD and CAT activities inthe breast and liver of breast cancer bearing rats [7]. Didziapetriene et al. [14], reported lower CAT activity in ovarian cancer patients. Similarly, in the present study, in kidney and liver SOD and CAT activities were decreased by the cancer development but in contrast to the findings of Patmavathi et al. [7], paclitaxel had no effect on the activities of these antioxidants.In testis and brain, the activities of SOD and CAT were higher in cancer group than both control and paclitaxel treated cancer bearing animals.Catalase is a tetrameric protein, which consist of four

similar subunits containing heme group. It is excessively expressed in some tissues to protect the cells against excess ROS formation. It has an oxidase activity as well as is involved in ROS generation [10,29]. Thus the higher CAT levels in cancer group may be attributed to the modification of the CAT levels in cancer cells resistant to some chemotherapeutics or hydrogen peroxide [29]. In the study of Cosan et al. [23], Paclitaxel treatment returned the altered MDA level and SOD and CAT activities to control levels and paclitaxel restored the damaged kidney and liver structure. Campos et al. [21], determined decreases in catalase activity and metahemoglobin levels after paclitaxel infusion in rats. Panis et al. [18], detected high oxidative stress status characterized by elevated lipid peroxidation and reduced CAT activity in advanced breast cancer and these authors found that paclitaxel enhanced lipid peroxidation due to systemic oxidative stress and red blood cell oxidative injury with anemia development.

5. Conclusion

The results of this study have shown that Paclitaxel alleviates the lipid peroxidation in kidney and liver but had no effects on antioxidant status in these tissues whereas significantly reduced the increased SOD activities in testis and brainof Ehrlich solid tumor-bearing mice.

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Conflicts of Interest

There is no conflict of interest

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ORAL PRESENTATION

Antioxidant and Physicochemical Properties of Chestnut Honeys from Turkey

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Abstract

Thanks to its rich flora, it is possible to produce various monoflorial honey in Turkey and one of these honey is chestnut honey. The purpose of this research was to determine the physicochemical and antioxidant properties of chestnut honey collected from different geographical regions of Turkey. The color, humidity, HMF, diastase number, proline, acidity and electrical conductivity values of honey samples were determined as 82.1 mm, 17.33 %, 23.83 mg/kg, 15.12 diastase number, 754.12 mg/kg, 28.68 meq/kg and 0.93 MS/cm respectively. The fructose, glucose and sucrose content in honey samples were determined as % 37.09, 30.41 and 0.02 respectively, while other sugars were changed between % 0.02 and 1.99. The total phenolic content of chestnut honey samples was determined as 154.12 mgGAE/100g with Folin Ciocalteu method, while antiradical activities (DPPH method) were found as 37.65 %. There is a need to investigate the biological activities of chestnut honey has important production potential in Turkey.

Keywords: Chestnut honey, chemical analysis, antioxidant activity

1. Introduction

Honey is a natural, sweet and functional food that meets the energy needs of the human body. The chemical content of honey depends on the type of bees as well as the botanical and geographical origin [1]. According to the source is obtained, honey types are classified under two groups; flower honey and honeydew honey. The honey made by honey bees from the nectars of various flowers is the flower honey. Cotton, chestnut, citrus, astragalus, trifolium, acacia, thyme, and sunflower honey types are included in the flower honey group [2]. Carbohydrates, water, organic acids, minerals, enzymes, vitamins, aromatic substances and antioxidants constitute the main components of honey [3]. There are basic monosaccharides such as glucose and fructose which are among the energy-giving carbohydrates as well as 25 different oligosaccharides such as panose, melezitose, and raffinose [4]. Although proteins are not high in honey, the amino acids in honey are important for the origin of honey. Proline, lysine, phenylalanine, γ-amino butyricacid, β-alanine, arginine, glutamine, serine, glutamic acid and aspartic acid are among the amino acids that exist in honey [5]. In addition, honey has many beneficial effects in terms of health and one of them is the activity of antioxidants. Honey's antioxidant activity is attributed to phenolic substances. The botanical origin of honey has the greatest influence on its antioxidant activity, while processing, handling and storage affect honey antioxidant activity [6].

Chestnut honey is widely produced in Turkey, especially in Marmara and Western Black Sea regions. Among the monofloral honey produced in the world is the most delicious and highest quality honey. It is known that it has floral, woody, spicy, floral and fruit-specific taste as its sensory characteristics [7]. Chestnut honey is a honey that has dark (amber) color, sharp taste and distinctive intense aroma that leaves a slight burning effect on the throat after it is eaten. Compounds responsible for the taste and aroma of chestnut honey; 1-phenylethanol, cinnamyl alcohol, p-hydroxyacetophenone and aminoacetophenone compounds responsible for biological activity are phenolic acids, such as caffeic acid, ferulic acid and p-coumaric acid [8]. This honey, which is rich in K, Ca and Mg, was found to be high in diastase enzyme activity than other monofloral honey [9, 10]. Dark-colored chestnut honey has high pH, electrical conductivity and ash value. This honey is characterized by a high fructose low glucose content. Because the F/G ratio is high, G/water ratio is low, it crystallizes late [11]. In addition, there are many researches on the biological effects of chestnut honey. Perna et al. [12] reported that chestnut honey had

higher antioxidant activity because it contained higher phenolic content, flavonoids and vitamin C than other monofloral honey. Chestnut honey has antimicrobial effect against pathogenic bacteria such as *Erwinia carotowora, Yersinia enterocolitica* and *Aeromonas hydrophila* as well as bacteria such as *S. aureus* and *E.coli* [13]. Furthermore, the effect of anti-inflammatory, wound healing and acetylcholinesterase (AChE) inhibitor has been reported [14, 15].

In this study, it was aimed to determine the physicochemical properties, sugar profile and antioxidant activity of chestnut honey with significant production potential and commercial value in Turkey.

2. Materials and Methods

Honey Samples

Chestnut honey samples are obtained from the provinces where Turkey's chestnut honey production is the most (Zonguldak, Yalova, Bursa). Samples were collected in accordance with the method specified by the Turkish Food Codex Regulation and labeled with harvest date, botanical and geographical origin.

Melissopalynological Analysis

The palynological analysis of honey samples was carried out according to Louveaux's method [16]. Palynological analysis of honey samples has been found to contain more than 80% of all samples of chestnut pollen.

Chemicals and Biochemical Analysis of Honey Samples

The 2,2-Diphenyl-1-picryl-hydrazyl (DPPH), 3,4,5-Trihydroxybenzoic acid (gallic acid; GA), Folin–Ciocalteu reagent, ascorbic acid (AA) and ethanol were obtained from Sigma Chemical Co. (St. Louis, MO, USA). The sulfuric acid (H_2SO_4), sodium phosphate (Na_3PO_4), ammonium molybdate ($(NH_4)_2MoO_4$), sodium carbonate (Na_2CO_3) and methanol (MeOH) were obtained from Merck (Darmstadt, Germany). The color value of the honey was determined using a Hunter

spectrometer (CR-400, Minolta, Osaka, Japan). Moisture content was measured using 4 refractometer (Atago, Tokyo, Japan), electrical conductivities with a conductometer (WTW inoLab Cond/720, Germany) and optical activity or rotation with a polarimeter (Beta PPP7, England). Sugar analysis of the samples was performed using a refractive detector (RID) with HPLC (Elite La Chrom, Hitachi, Japan) and a reverse phase–amide column (200/4.6 Nucleosil 100-5 NH2). Quantitative and qualitative sugar analyses were performed using the method described before. The calibration curves of all analyzed sugars were between 0.994 and 1.000. All the analyses were carried out according to the principles of EU legislation [3]

HPLC-RID Analysis for Sugar profile of honey samples

HPLC-RID analyses were performed by Hitachi, LaChrom Elite® (Hitachi High Technologies America, Inc., San Jose, California) equipped with RI detector (Hitachi, L-2455 Diode Array Detector). HPLC-RID analyses were performed on a reverse phase NH_2 column (200 mm × 4.6 mm id, 5 μm particle; Nucleosil). Fructose, glucose, sucrose, turanose, maltose, theralose, isomaltose, erlose, melezitose, and maltotriose were determined and normalization calibration method was used [17]. Mobile phase was applied as an isocratic elution; 79-21% acetonitrile/water mixture. Injection volume was 25 μL, column temperature was 80°C and flow rate was 1.5 mL/min. For sample analysis, about 1 g honey was dissolved with 10 mL ultra-pure distilled water and the solutions were filtered by 0.45μm filter (Sartorius, Goettingen, Germany)

Determination of total phenolic content of honey samples

TPCs were determined using the Folin-Ciocalteau procedure with gallic acid as standard [18]. Briefly, 0.2 mL extract was mixed 1.8 mL distilled water and 1 mL of 0.2 N Folin-Ciocalteu reagent, and the contents were vortexed. After 3-min incubation, 1.5 mL of 2% Na_2CO_3 (w/V) solution was added. After vortexing, the mixture was incubated with intermittent shaking for 2 h at room temperature. Absorbance was measured at 760 nm and TPC concentration was calculated as mg of gallic acid equivalents per gram of 100 g sample, using a standard calibration graph.

Determination of radical scavenging activity (DPPH)

Scavenging activities of the honey samples toward DPPH radical were assessed by using the method described by Molyneux [19] with a minor modification. Briefly, various concentrations of 0.200 mL of extracts of honey were mixed with 3.9 mL of 0.1 mM of DPPH in methanol. The

setup was left in the dark for 30 min, at room temperature for allowing to react with stable free radical. After incubation period, the decrease in absorbance at 517 nm was measured spectrophotometrically against control, using a UV-Visible spectrophotometer. The inhibitory effect of DPPH was calculated according to the following formula:

$$\% \text{ Inhibition} = [1 - (\text{Abs}_{\text{Sample}} / \text{Abs}_{\text{Control}})] \times 100$$

Statistical Analysis

SPSS 22.00 package programme was used in statistical analyses. Data was presented as arithmetic means and standard deviations.

3. Results and Discussion

The physico-chemical analysis summarized in Table 1. Color is one of the important parameters to determine the Botanical origin of honey. In this research, the color of chestnut honey was determined as light amber [20]. It is known that dark-colored honey contains more minerals and has higher phenolic content. The color value obtained was also consistent with a study conducted with 62 chestnut honeys [1].

Table 1. Physochemical properties of chestnut honeys

Parameter	**Mean ±SD**	**Parameter**	**Mean ±SD**
Colour (Pfund)	81.82±6.85	Proline	740.00±94.44
Moisture %	17.31±0.83	Acidity meq/kg	28.87±4.78
HMF (mg/kg)	22.38±17.55	Electrical conductivity MS/cm	0.89±0.36
Diastase number (DIN)	15.65±3.60		

Moisture in honey is one of the most important quality parameters. The moisture content of honey may vary according to botanical origin, climatic conditions, season and honey processing methods. Honeybees do not cap off the comb under normal conditions without falling below 18% of the moisture value of honey in the comb. However, if the bees harvest the honey without being fully cap off during the harvest, the humidity of the honey will be high. High humidity content in

honey causes the crystallization and fermentation of honey. Therefore, the high moisture content in honey is an undesirable property. The moisture content of chestnut honey was found to be 17.31 % on average. Devillers et al. [1] investigated with 469 mono-floral honey samples and their quality data and found the mean of moisture values as 17.60 %.

The indication of the wrong procedures performed by honey can be understood with honey diastase activity and HMF content. If honey is stored for a long time or exposed directly to heat, the HMF value increases and diastase enzyme activity decreases. These two parameters are important in the quality of honey. The results obtained in this study for HMF (22.38 mg/kg) and diastase activity (15.65) are consistent with Turkey and world honey Codex values [21, 22]. Proline is an amino acid and honey is passed through the saliva during honey processing. It is an important indicator of whether or not honey is associated. In this research, the proline value obtained from chestnut honey is a high value (740 mg/kg). This result shows that the honey being tested is natural and mature. Researchers have determined the proline value of 43 honey, 6 of which are chestnut honey, between 590-609 mg proline /kg honey.

Honey is usually responsible for the taste characteristics while the acidification of honey varies according to the Botanical origin. Electrical conductivity is an analysis used to determine the source of honey. The result obtained in this study for acidity (28.87 meq/kg) is consistent with Turkey and world honey Codex values. Electrical conductivity value obtained was also consistent with a study conducted with Saric et al. [23]; 0.538-1.38 mS/cm

In this study, fructose content, which is the basic sugars of chestnut, was found to be 37.09%, glucose content was 30.41% and saccharose content was 0.02 %. However, turanose, maltose, isomaltose, erlose, melesitose were among the other identified sugars (Table 2). The contents of the total disaccharides were determined as 7.29 %. Among these sugars maltose was detected at the highest rate. In addition, Fructose+Glucose content, which is important parameters in crystallization, was found to be 67.50, Fructose/glucose content was found to be 1.23 and glucose/water content was found to be 1.76. Chestnut honey is a late crystallized honey. Crystallization is a significant parameter for the market value of honey. In temperate climates, honey can crystallize even under normal storage temperatures and crystallization negatively influences consumer preferences. The majority of honeys are supersaturated solutions with glucose and this glucose can spontaneously crystallize into glucose-monohydride at room

temperature [24]. The crystallization trend of honey from different botanical origins is closely related to some physical and chemical parameters, some of these parameters are glucose, glucose/water, glucose-water/fructose, fructose/glucose ratios and melezitose content. Specifically, honey crystallizes faster when the glucose content is >28–30 %, glucose/water ratio is ≥2.1, fructose/glucose ratio is <1.14 and melezitose ratio is over 10 % [25]. Beside these parameters, the existence of dust, pollen, comb and propolis particles in honey also influences the crystallization of honey [1, 26]. Furthermore, botanical origin, processing conditions, storage conditions, storage temperature, relative humidity and the container in which the honey is kept also influence the crystallization of honey [27].

Table 2. Sugar profile of chestnut honeys

Sugar %	**Mean ±SD**	**Sugar %**	**Mean ±SD**
Fructose	37.09±2.29	Erlose	0.13±0.13
Glucose	30.41±4.08	Melesitose	0.02±0.06
Sucrose	0.02±0.06	Maltotriose	0.00±0.00
Turanose	1.38±0.38	Fructose+Glucose (DIN)	67.50±6.12
Maltose	1.99±0.28	Fructose/Glucose	1.23±0.12
Trehalose	0.00±0.00	Glucose/Water (DIN)	1.76±0.29
Isomaltose	0.68±0.51	Total disaccharides	7.29±2.66

One of the most important activities of phenolic compounds is antioxidant activity [28]. Honey contains not only phenolic compounds, but also other enzymatic and nonenzymatic compounds that promote antioxidant activity.

Phenolic compounds exert their beneficial health effects mainly through their antioxidant activity and also honey is known to be rich in both enzymatic and non-enzymatic antioxidants. Antioxidant capacity of honey depends on the floral source, seasonal and environmental factors [29]. The total phenolic content of the samples was found between 122.93-174.19 mg GAE/100 in this study. These values are higher than those obtained in previous studies [12, 30, 31]. In this

research, the antioxidant activity of honey samples tested was determined between 27.52-43.13 %. Sagdic et al. [32] and Perna et al. [12] reported the inhibition level of authentic chestnut honeys were 67.92 % ± 8.58 and 75.37 % ± 7.87, respectively.

5. Conclusion

In the present study, the physico-chemical properties sugar profile and antioxidant activity of chestnut honey were investigated. The results obtained from this research will give an overview of the properties of chestnut honey has an important production potential in our country. The results obtained from this research will give an overview of the properties of chestnut honey has an important production potential in our country. In addition, it provides data for use in the field of health because it has a high level of total phenolic content and antioxidant activity

Acknowledgements

None.

Conflicts of Interest

There is no conflict of interest

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ORAL PRESENTATION

Antioxidant Pigments and Their Micro-Encapsulation

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Abstract

Plant pigments with high antioxidant activities have free radical scavenging actions. Various novel products were developed by studying antioxidant activities in vegetables. For a long time, the consumption of fresh and processed vegetables were known for protecting the human body from various critical diseases such as diabetes, brain and heart diseases, cancer and also, neurodegenerative diseases. Currently, it is believed that the protective properties of these foods resultant of low-molecular antioxidants found in these vegetables which protect the human organ cells and their structures from oxidative damage. A new technology known as micro-encapsulation is carried out for protecting these antioxidants against degradation, controlling their release, as well as masking their taste and flavor. Microencapsulation is a process in which active ingredients are enclosed by various small coated materials. The success of this technology depends upon the correct source of wall and core materials. Therefore, in this review specific microencapsulation techniques will be explained for encapsulation of well-known antioxidant pigments.

Keywords: Anthocyanin, industrial vegetable, fruit, microencapsulation techniques.

1. Introduction

From previous decade, the research has been mainly promoted in the field of horticulture and food science by exploring naturally occurring antioxidants in fruits and vegetables to avoid the multifaceted health related complexities arising in human body due to reactive oxygen species (ROS) that are overproduced [1, 2]. The antioxidants especially in vegetables aimed an important role in the upkeep of our health and prevention of diseases [3]. A variety of vitamins A, C, E, as well as anthocyanin, carotene and phenolic etc are excellent antioxidants sources, which also pay to our good health through other mechanisms, i.e involvement in oxidation-reduction reactions and being co-factors for certain enzymes [4,5]. While the production of these ROSs in an excess amount can bring serious issues related to human health as their surplus generation can lead to various pathophysiological situations such as heart-related disorders (i.e., cardiovascular disorders-CVDs), by destroying nucleic acids causes fast aging process and change in the justification of protein molecules, diverse types of cancers, inflammation, neuro-degenerative disorders, oxidation of membranous lipid, weakening of hydro peroxide synthesis, kidney and lungs infection, osteoporosis (bone-related problems) and also health related disease called "oxidative stress" [6]. Also a direct correlation between insulin confrontation (key factor for type-II DM) and oxidative stress has also been explained by the researchers [7].

It has been roughly calculated that rise in vegetable consumption decreases the hazards of cardio-vascular disease up to 30%, mortality rate up to 20% and cancer risk till 15%, [8, 9]. A fresh vegetables diet provides protection from most familiar kind of epithelial cancer that includes the digestive and non-digestive neoplasms. Particular β-carotene, antioxidants and vitamins C and E revealed an important reverse relation with the hazard of pharyngeals, oral, breast and esophageal cancer, while the more defensive reaction were recorded by riboflavin carotene, and vitamin C against colorectal cancer, [10]. In addition, anti-carcinogenic means also available in vegetables that include several trace nutrients, i.e. dietary fiber, flavonoids, glucosinolates and indoles, phenols, selenium, protease inhibitors and plant sterols.

Approximately 5000 known plants and model studies have been carried out that many of them have antioxidant activity [11]. The phenolic antioxidant activity is mostly due to its redox reactions, which let them to perform as phenolics reducing sources, hydrogen donors, metal chelators and singlet oxygen quenchers [12]. Its antioxidant activity is generally based on the

existence of a 2, 3 double bond & 4-oxo-function as well as location and number of hydroxyl groups present [13]. The flavonoids, a large family with low molecular weight of polyphenolic complexes, include the flavones, flavonones, flavonols, flavan-3-ols isoflavones, and anthocyanins [14]. Attention in these substances has increased because of their possible effects on human health, although flavonoids are normally reflected as non-nutritive agents, [15]. Additionally to flavonoids anti-oxidant activities, it inhibits enzymes i.e. cyclooxygenase, prostaglandin synthase and lipoxygenase that is highly associated to tumorigenesis, and might encourage detoxifying enzymes like glutathione S-transferase [16]. Numerous classes of flavonoid were stated in fruits and vegetables and their kinds where its subjects differ with cultivar and maturation [15].The carotenoids play vital role against cancer because of their ability to quench singlet oxygen [17, 18]. Dark-green leafy, orange and yellow vegetables contain flavonoids and carotenoids. Muller during 1997 [19] investigated 22 species of various vegetables where leaf of parsley, kale, lamb's lettuce, red paprika, tomato, spinach and carrot were found very rich in carotenoids (over 10 mg/100 g of edible portion). Carrots and sweet potatoes are especially high in β-carotene. Orange vegetables are rich source of β-carotene (carotenoids). Green leafy vegetables such as brussels sprouts, kale, cabbage spinach and broccoli are ascetically high in β-carotene. Tomatoes contain lycopene that is rare in other common vegetables. The major carotenoids in these vegetables are the oxygenated carotenoids (xanthophylls). Lutein is the main oxygenated carotenoid in mustard greens, kale, parsley and spinach. By cooking, carotenoids in vegetables are destroyed to some extent, and among various carotenoids, the oxygenated carotenoids are destroyed to a greater extent than β-carotene. Some nutrients from vegetables do not stay in the food for a long time or can make reaction with the extra food components causing unwanted special effects.

Regarding to the route of management, effectiveness of antioxidant complexes mainly rely upon its integrity, bioavailability. Actually, a minor ratio of molecules is absorbed orally, due to low solubility, low permeability and insufficient gastric residence time. Their uncertainty during food processing, storage and distribution even in gastro-intestinal region (enzymes, pH, and availability of other nutrients) restrict actions and possible health benefits of these compounds [19].

The normal intake of these naturally occurring compounds is delicate due to its sensitive nature to environmental circumstances, including biological, organic and physical circumstances.

Unluckily, these compounds oxidize very fast prominent to progressive appearance of somewhat different color and sometime also annoying odors/aroma. A variety of compounds from naturally plant sources are remarkable for their functions. Furthermore, many of them contain a hostile taste should be masked prior to their merger in other food stuffs and also in verbal remedies. Therefore, the management of these compounds has need to be formulation of end products capable in maintaining its structural reliability until consumed or to cover its taste, increase its water solubility and bio-availability, and deliver it exactly toward a physiological aim [20].

Amongst the already current and balanced procedures and techniques, micro-encapsulation is a fascinating means. The proper use of encapsulated products instead of free compounds is foremost source for several works.

Recently, several microencapsulation techniques are available on both scales [20]. The microencapsulated produces are broadly used in the pharmaceutical, nutrition, and cosmetic sectors, also in other many sectors such as industrial chemicals, personal care, veterinary medicine, sensor industries biotechnology, biomedical industries.

2. Microencapsulation

Microencapsulation is a technique by which liquids, solids, or even gaseous active ingredients are enclosed within a second microscopic materials forming thin coating of wall materials for the purpose of shielding the active compounds from the surrounding environment [21]. Micro particles, with a size range between 1 micron and 1 millimeter. The active ingredients are chosen as the core material whereas the surrounding material forms the shell or protective wall. A widespread interest has shown in this technology as it has been employed in a diverse range of fields from pharmaceuticals to chemicals and from printing to cosmetics sectors.

This process dates back to 1950s when Green and Schleicher for the first time in history produced microencapsulated dyes by complex coacervation method of gum arabic and gelatin, for the manufacture of carbonless copy paper. Till today, this carbonless copying paper is one of the greatest important yields to utilize microencapsulation technology, and still producing commercially [22, 23].

A variety of methods are available for microencapsulation process. Generally they are classified into two basic groups' physical and chemical methods. The physical method further subdivided

into physicochemical methods. The first category consists of those methods in which starting materials are only polymers and no chemical reactions take places while only shape fabrication occurs. While the second group contains those techniques where initial materials are pre-polymers, monomers where chemical reactions occur along with microsphere formation. The most common and well used encapsulation techniques are as follow:

- Air suspension
- Centrifugal process
- Coacervation phase
- Pan coating
- Polymerization
- Solvent evaporation techniques
- Spray drying and congealing

2.1 Air Suspension

Professor Dale E. Wruster from the department of pharmacy and the University of Wisconsin invented this coating process [24]. Apparatus for air suspension includes air distribution plate, control panel, coating chamber and nozzle for film coating (Figure 1). Particles are suspended on an upward stirring air stream within the coating chamber. Coating materials are applied in coating zone by spraying to moving core particles. This cycle is carried out till the desired product thickness is obtained. The products can be dried by air stream during encapsulation [25].

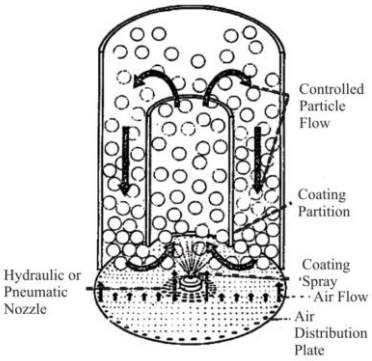

Figure 1. The Wruster process [24]

2.2 Centrifugal Process

This process was developed by the Southwest Research Institute (SWRI). The Processing system consist of the movement level of the core and coating shells , rotational speed of cylinder, the concentration, surface tension and viscosity of the core materials. This process is capable of microencapsulating solids and solutions of different size arrays, with varied coat walls. The encapsulated products can be provided as slurry in the dry powder or hardening media. The production rate of this process was recorded from 50 to 75 pounds per hour [26].

2.3 Coacervation Phase

Encapsulation by coacervation phase is usually accredited to The National Cash Register Corporation and the patents of B.K. Green et al. The process consists of normally three steps:

I. Development of three immiscible phases (core material, liquid manufacturing phases and a coating shell phase).
II. Deposition of core constituents into liquid polymer coating.
III. Rigidization of coating material usually by desolvation techniques or thermal process in order to form microcapsule products [27].

2.4 Pan Coating

Relatively large particles encapsulation can be carries out by pan method and the product known as pellet. In this practice of encapsulation, the coating is practiced as an atomized spray or a solution to preferred solid core material in coating pan. Warm air is conceded over coated materials to eliminate the coating solvent. Sometimes the final solvent removal is carried out in drying oven [26].

2.5 Polymerization

It is a relatively new microencapsulation technology to form protective microcapsules. Polymerization reaction in this technique occurs at liquid-gas, solid-liquid, or liquid-liquid, solid-gas interface. This method is mostly employed for nanoparticles and also applicable for particle size up to 15 um. [28, 29].

2.6 Solvent Evaporation Techniques

The polymer dissolved in volatile organic solvent such as chloroform or dichloromethane, into which the core materials also dissolved [30]. The active compound present polymer solution is injected into a continuous aqueous phase containing a surfactant (Figure 2). The polymers precipitate to form nanoparticles as it is insoluble in the mixture of water and solvent. The collected particles are washed after the removal of the removal of solvent and then freeze-dried [31, 32].

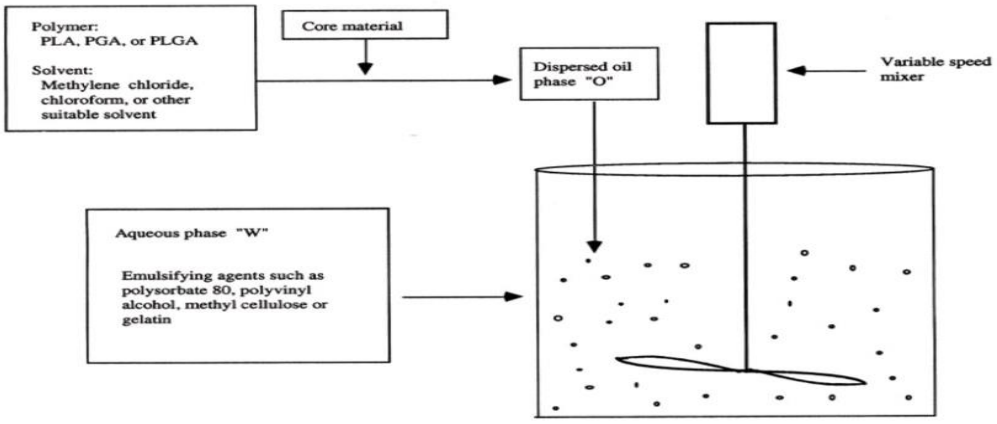

Figure 2. Solvent Extraction [27]

2.7 Spray Drying and Congealing

This technique is one of the oldest and low cost commercial processes commonly available for encapsulation of oil and flavors and fragrances (Figure 3). Usually in this procedure an emulsion is prepared by distributing the core ingredients. The resultant emulsified materials are atomized into a spray of droplets by pushing the slurry through rotating disc into the heated section of a spray drier chamber. Yielding dried capsules by evaporation of water portion from emulsion. This technique was used for the encapsulation of lycopene inside the gelatin microcapsules [33, 34].

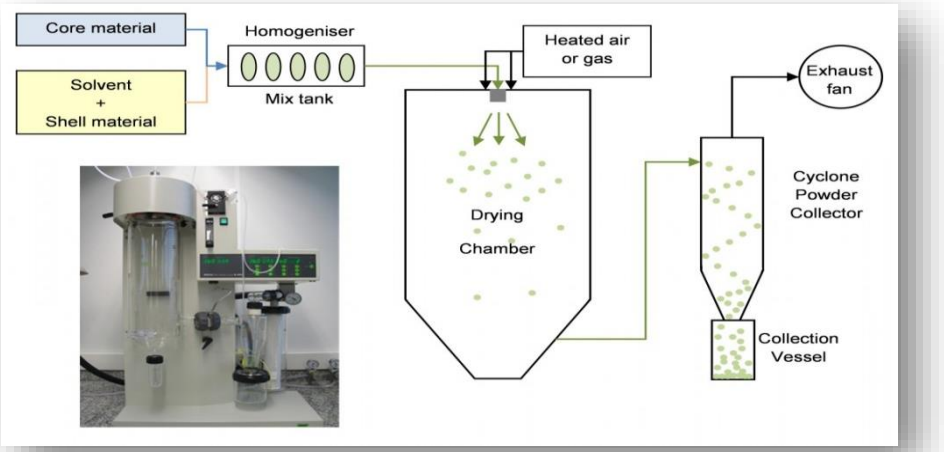

Figure 3. Spraying drying

3. Conclusion

Antioxidants are the powerful most active bio-compounds synthesized by various plants. Some of the problems, such as rare solubility and stability, weak bio-availability, have to be resolved in order to make these bio-compounds more available to growing demands in various industries, like food, health, cosmetics, bio-technology and nutrition fields. In this review, various micro-encapsulation methods that are realistic to various bio-compounds from vegetables sources showed us that the micro-encapsulation technology is really interesting and new technology in this area in order to potentialize their activity according to growing demand. Different studies found in this regard shown that technology of encapsulation provided a significant protection to various bio-active compounds against extreme conditions like as thermal degradation and oxidation, also playing a vital role to increase the shelf life of the micro-encapsulated active compounds. In addition, it was also shown in various studies that this technology is used to mask an unwanted smell, flavor and taste of active ingredients, as well as, to alter the physical properties of initial materials to improve the bioavailability of the compounds.

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None.

Conflicts of Interest

There is no conflict of interest

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