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The Detection of Fractal Dimension with Protective Effects of Lycopene and Catechin Carbon Tetrachloride-Treated Rat's Liver

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ABSTRACT: Our aim is to investigate the detection of fractal dimension (Box-counting dimensions) with protective effects of lycopene and catechin in carbon tetrachloride-treated rat's liver. Fractal dimension measure intensity and complexity of subject. The rats were divided into six groups as liquid oil, lycopene, catechin, carbon tetrachloride plus lycopene, carbon tetrachloride plus catechin and carbon tetrachloride. The tissue samples were obtained at end of 2İst day. The liver tissue distortions were evaluated using hematoxylin and eosin staining. The regions of hepatocyte were separated into Small Square under light microscopy. Then, counted at least one nucleus with intersection of one square. Fractal dimension were determined by using this formula; $\ln(N(n))/\ln(2^n)$. Notably, there was no difference between control and catechin-treated rats on fractal dimension values. But, a lycopene value was bigger than control and catechin administrated rats. In carbon tetrachloride-treated rat, fractal dimension is less. It was increased in lycopene plus carbon tetrachloride and catechin plus carbon tetrachloride with compare to carbon tetrachloride group. The findings confirmed, if hepatocyte is less, fractal dimension will decrease On other hand, carbon tetrachloride decreased fractal dimension due to less hepatocyte. It was increased in fractal dimension due to high hepatocyte in catechin and lycopene treated rats, because of their protective effects. This study is combination of medicine and mathematics, as fractal dimension. It was calculated, toxicity and protective effects of antioxidant materials could be determined in this way.
KEY WORDS: CCl4, Lycopene, catechin, Fractal dimension

ÖZET: Amacımız karbon tetraklorür uygulanmış sıçan karaciğerinde likopen ve kateşinin koruyucu etkilerinin fraktal boyut hesaplaması kullanılarak ortaya konmasıdır. Fraktal boyut nesnenin yoğunluğunu ve karmaşıklığını ölçer. Sıçanlar; sıvı yağ, likopen, kateşin, karbon tetraklorür + likopen, karbon tetraklorür + kateşin ve karbon tetraklorür olmak üzere altı gruba ayrıldı. Doku örnekleri 21. gün sonunda elde edildi. Karaciğer dokusundaki harabiyet, hematoksilen ve eozin boyama ile değerlendirildi. Hepatosit bölgeleri ışık mikroskobu altında küçük karelere ayrıldı. Sonra en az bir nükleusun bir kareyle kesiştiği alan sayıldı. Fraktal boyut $\ln(N(n))/\ln(2^n)$ formülle hesaplandı. Özellikle, fraktal boyut değerleri açısından kontrol ve kateşin ile tedavi edilen gruplar arasında bir fark yoktu. Ama likopen değerleri kontrol ve kateşin uygulanan sıçanlarda daha yüksek bulundu. Karbon tetraklorür uygulanmış sıçanlarda fraktal boyut düşüktü. Karbon tetraklorür uygulanmış gruba göre karbon tetraklorür + likopen ve karbon tetraklorür + kateşin gruplarında fraktal boyutta artış gözlendi. Bulgular hepatosit miktarındaki azalmaın, fraktal boyutun azalmaya neden olacağını göstermiştir. Diğer taraftan, karbon tetraklorür hepatositlerin az olması nedeniyle fraktal boyutu azaltmıştır. Kateşin ve likopen uygulanan gruplarda yüksek hepatosit miktarına bağlı olarak fraktal boyutta artış görüldü. Bu çalışma fraktal boyut kullanılarak tıp ve matematiği birleştirmektedir. Bu hesaplamalar doğrultusunda, antioksidan maddelerin toksisite ve koruyucu etkisi tespit edilebilir.
ANAHTAR KELİME: CCl4, likopen, kateşin, fraktal boyut

1. Introduction

Fractal theory supply an effective method of quantitatively describing complicated nuclear shapes in in normal and malignant hepatocyte. The fractal dimension determined for a two-dimensional digitized image of human hepatic nuclei (1). The other hand, the carbon tetrachloride acts as solvent which is used in petrolium, insects drug, candle, rubber, furniture, ready-make clothing industries and cooler agent at heat transfer (2). The carbon tetrachloride treatments caused classical damage in the rat liver at 24 hour (3) and the expected relationship between carbon tetrachloride dose and carcinogenic response in the liver (4). Lycopene and catechin are the major carotenoids that have antioxidant activity (5, 6, 7, 8, 9) against the effects of carbon tetrachloride treatments (10, 11). the carotenoids are important biological compounds that fruits and vegetables contain the various carotenoids (12). Epidemiological studies have also demonstrated that the increased consumption of lycopene-rich foods, such as tomatoes and tomato-based products, is associated with risk of decreased cancer (12). Tea (Camellia sinensis) is one of most popular beverages in the worldwide. It defined that among the various types of tea, green tea contains a relatively high level of polyphenols, which consist of flavanol monomers, also referred to as catechins (13, 14). The green tea is proposed to be the main preventive mechanism against several diseases including cancer, which have pathogenic oxidation events as one causative background in common (15). With the aim of determining the fractal dimension, lycopene and catechin were investigated experimentally with respect to their protective effects against carbon tetrachloride-induced rat liver on nuclei dimension of the heaptocyte.

2. Material Methods

The rats were divided into six groups as control, lycopene, catechin, carbon tetrachloride (CCL4)+lycopene, carbon tetrachloride+catechin and carbon tetrachloride. The tissue samples were obtained at end of 21st day. The liver tissue distortions were evaluated using hematoxylin and eosin staining. The regions of hepatocyte were separated into small squares under light microscopy. Then, they were counted at least one nucleus with intersection of one square. Fractal dimension were determined by using this formula; $\ln(N(n))/\ln(2^n)$. Three sections for every rat liver were photographed. The figures of fractals were created from these images using a computer program (fractal-generating software), in the pattern clear image showing only the nucleus of the cell as black points. Borders of every figure were divided into 2^n unit parts for n=l,2….,9 and to get small squares. Using box-counting method, minimum square number N(n), intersecting with at least one cell nucleus, for every step was calculated. It was found ($\ln(2^n)$, $\ln(N(n))$ points for every n value to form Graphic 1. As seen in graphic 1, the points that have high deviation for first five steps were excluded. Inclination of line, nearest to the points found for n=6,7,8,9, was calculated with the smallest squares method and fractal dimension value was determined. The calculated means for every group were given in the tables, applying this method to all figures. Nucleus figures and variation graphics were given together for every group.

Statistical Analysis

Statistical analysis was performed using IBM SPSS Statistics version 21. Quantitative data were presented as mean ± standard deviation (SD). Comparisons between groups were performed using One-way ANOVA. A p value <0.05 statistically significant.

3. Results

Three sections for every rat liver were photographed (Figure 1). The figures of fractals were created from these images, these clear images showing only the nucleus of the cell as black points (Figure 2). Each figure were divided into 2^n unit parts (Figure 3). The fractal dimension of groups were given in Table 1 and The every n value calculated for each step according to $\ln(2^n)$, $\ln(N(n))$ points for to form Graphic 1. The nearest to the points found for n=6,7,8,9, was calculated with the smallest squares method and fractal dimension value was determined (Graphic 2). The fractal dimension values for every chemical agent administered to rats were presented in Graphic 3. Mean values of groups for fractal dimension were given Table 2.

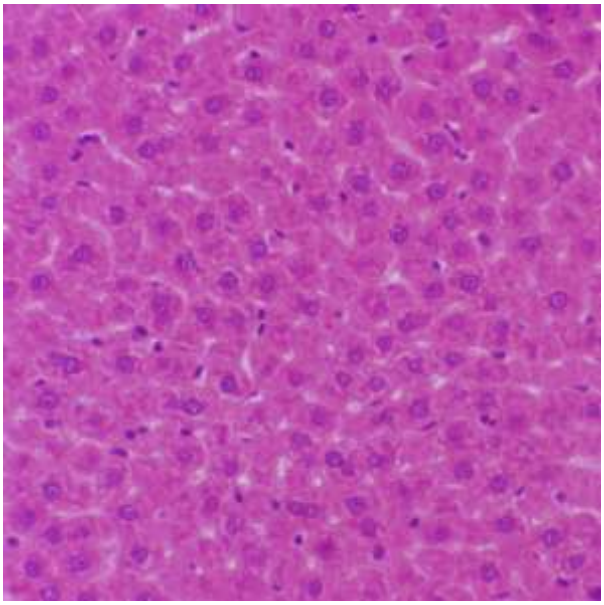


Figure 1: *Three sections for every rat liver were photographed as 1024X1024 pixels.*

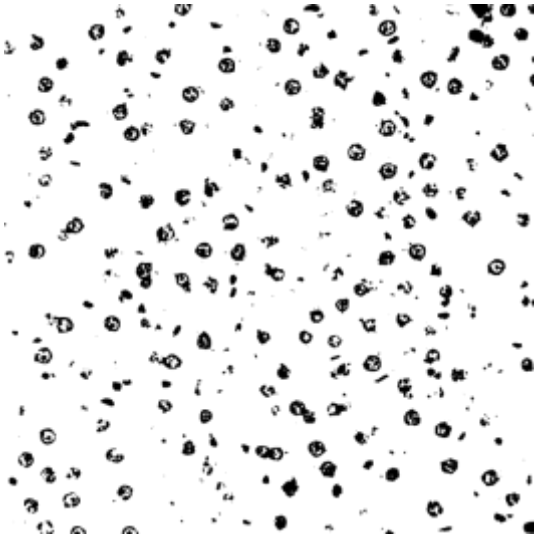


Figure 2: *The clear image showing only the nucleus of the cell as black points.*

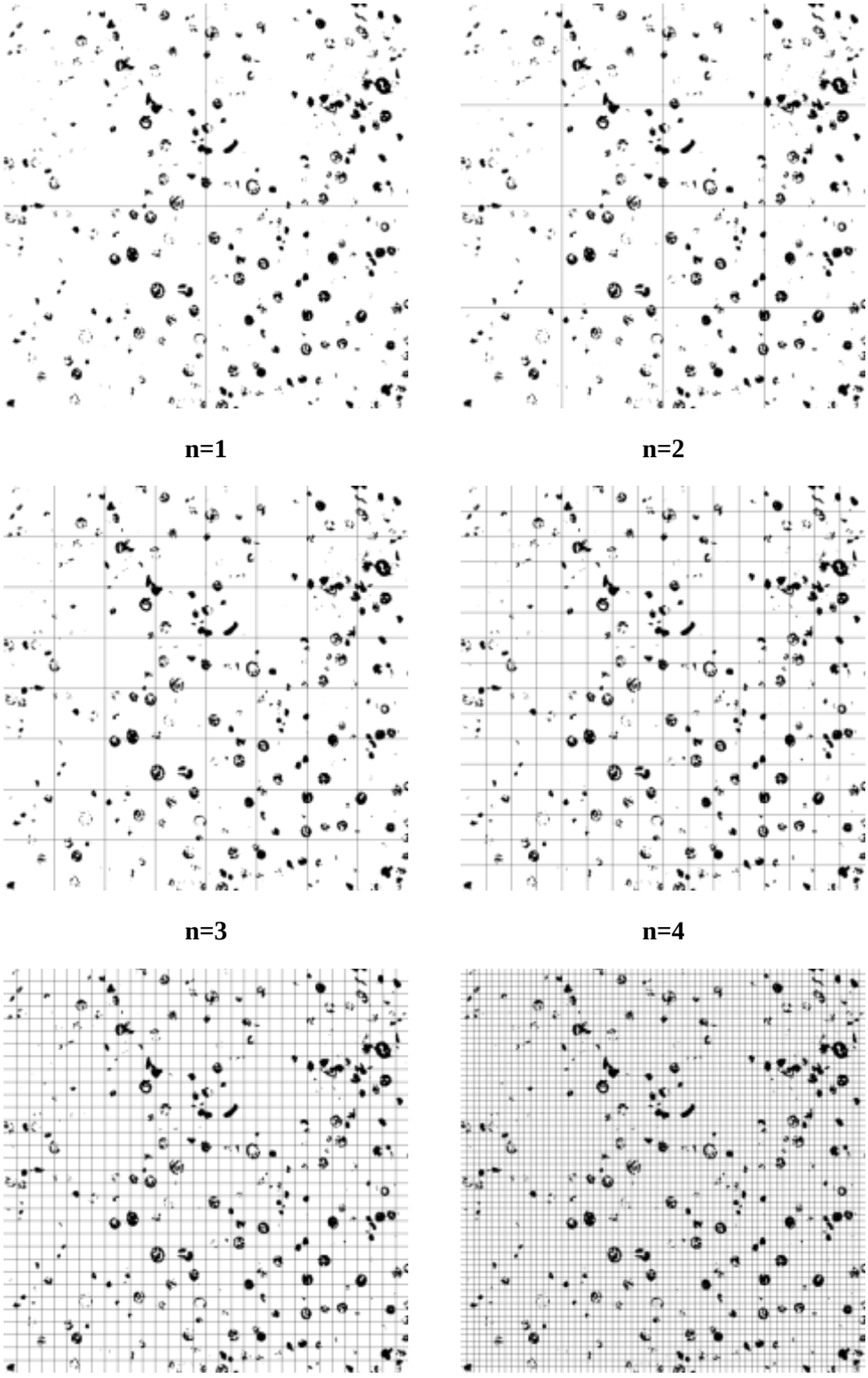
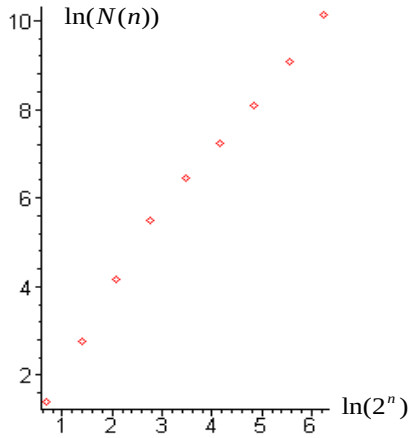


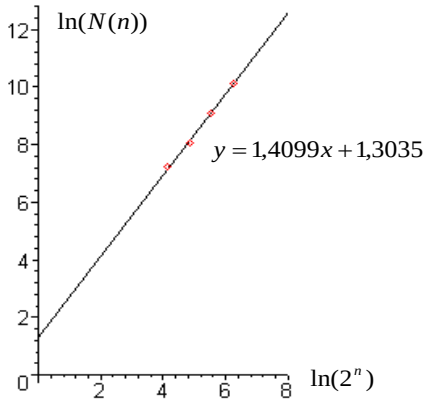
Figure 3: *Borders of every figure were divided into 2n unit parts for n=l,2....,9 and to get small squares*

Tablo 1
The fractal dimension of groups.

Groups	No of the rat	Fractal dimension of Picture 1	Fractal dimension of Picture 2	Fractal dimension of Picture 3
Control	1	1,3983	1,3885	1,4193
Control	2	1,3970	1,4173	1,4370
Control	3	1,4190	1,4283	1,4324
Control	4	1,4099	1,4229	1,4159
Control	5	1,3906	1,3688	1,3516
Lycopene	1	1,3388	1,3708	1,3880
Lycopene	2	1,3775	1,3457	1,3836
Lycopene	3	1,3946	1,3340	1,3848
Lycopene	4	1,3870	1,3753	1,3832
Lycopene	5	1,3937	1,3726	1,3940
Lycopene	1	1,3388	1,3708	1,3880
Catechin	1	1,4395	1,3828	1,3755
Catechin	2	1,4049	1,3484	1,4145
Catechin	3	1,4379	1,3901	1,3824
Catechin	4	1,3945	1,4057	1,3984
Catechin	5	1,4123	1,3995	1,3921
Catechin	1	1,4395	1,3828	1,3755
CCl_4	1	1,2758	1,2781	1,3594
CCl_4	2	1,3013	1,2872	1,3107
CCl_4	3	1,2846	1,3288	1,2935
CCl_4	4	1,3173	1,2547	1,2678
CCl_4	5	1,2978	1,2616	1,2478
CCl_4	1	1,2758	1,2781	1,3594
Lycopen + CCl_4	1	1,3388	1,3319	1,3967
Lycopen + CCl_4	2	1,3669	1,3733	1,3538
Lycopen + CCl_4	3	1,4020	1,3762	1,4183
Lycopen + CCl_4	4	1,4261	1,3770	1,3876
Lycopen + CCl_4	5	1,3969	1,3025	1,3627
Lycopen + CCl_4	1	1,3388	1,3319	1,3967
CCl_4+Catechin	1	1,3755	1,4175	1,3756
CCl_4+Catechin	2	1,4114	1,3925	1,3945
CCl_4+Catechin	3	1,3870	1,4060	1,3886
CCl_4+Catechin	4	1,4140	1,3831	1,3946
CCl_4+Catechin	5	1,4034	1,3984	1,3960
CCl_4+Catechin	1	1,3755	1,4175	1,3756



Graphic 1. *Determination of ln(N(n)) points for every n value to form graphic.*



Graphic 2. *The calculations with the smallest squares method and fractal dimension value was determined.*

Tablo 2
Mean values of groups for fractal dimension.

Groups	Fractal	Multiple Comparisons Dependent Variable: fractames Tukey HSD					
		A	B	C	D	E	F
A (Control)	1.4064±.02201						
B (CCl4)	1.2911±.01573	<0.001					
C (Catechin)	1.3986±.00551	0.961	<0.001				
D (Catechin+CCl4)	1.3958±.00419	0.870	<0.001	1.000			
E (Lycopene)	1.3746±.00902	0.028	<0.001	0.153	0.257		
F (Lycopene+CCl4)	1.3740±.02213	0.024	<0.001	0.137	0.234	0.234	

4. Discussion

Fractal analysis measures the complexity of geometric structures. The fractal dimension offers a rapid means of assessing cell shape analysis(16). Many analytical methods have been proposed to determine the fractal dimension of natural objects. A fractal object tends to fill up space, and its dimension value is not an integer. For example, a complex curve will cover a large area of the plane with a dimension lying between 1 and 2 (the fractal dimension of the von Koch snowflake curve is 1.2). The 'monster curves' described by mathematicians tend to fill the whole plane and have a high fractal dimension (the Peano curve is 1.67). In the same way, a very rough surface will occupy a large volume with a dimension between 2 and 3. (17). Previous studies suggest that there is less interobserver variability with fractal analysis by image analysis than with the semiquantitative score(18). The fractal dimension was previously been used in the diagnostic process of various types of cancers, both for identifying tumoral tissue, for stadialization or as a prognostic factor. Goutzanis L et al. recently demonstrated that FD is an independent prognostic factor of survival in oral cancer patients . He reported that FD values are significantly different for each TNM stage, thus validating the method as a tool for cancer stadialization(19). Uppal et al showed the correlations of fractal dimensions of cell contours with the latent factors(20). Previous studies supported the idea that fractal image analysis can indeed be used as aquantitative, semi–automated computer-aided technique used for image analysis of histological samples(21). Olefirenko et al studied the fractal [corrected] and morphometrical analysis of images obtained by life-time photo- and videorecording of microhemocirculatory changes in the rats in health, experimental cirrhosis, and variants of its treatment was carried out. They showed the differences in the fractional dimensions of the studied organs were significant and correlated with morphological values, which suggests fractal [corrected] analysis for the diagnosis and prediction of hepatic tissue status in vivo(22). In a previous study FD was used as a prognostic factor for laryngeal carcinoma, in an other study Goutzanis and his team proved that increased FD for cellular elements is inversely correlated with survival in oral cancer(23,24). Also some studies also theorized on the usage of FD in cancer prognosis(25,26). Gheonea et al reported that nuclear and vascular FDs calculated from histological images are good quantifiers for morphological aspects of liver parenchyma and can therefore fit perfectly as input variables in a perceptron feed-forward hidden-layer ANN system(27). Epigallactocathechin has been proposed to possess various properties including the ability to induce anti angiogenesis, apoptosis, cell cycle regulation, and antimicrobial activity(28). Also lycopene has been reported to possess the interference of cell proliferation, inhibition of cell cycle progression, modulation of signal transducing pathways and upregulation of cancer detoxification(29). Our findings showed that the nuclei administered CCL4 were deformed and had decreased fractal dimension value. When catechin and lycopene were administered to the same type nuclei fractal dimension value seemed to the value of control group. But it was found that catechin has more advantage when compared to lycopene for its effect to nuclei structure. In catechin+CCL4 administrated group, fractal dimension value approached to the fractal dimension value of catechin group. This result occurred because of the transformation of deformed nuclei to their healthy form. Similar condition was shown for lycopene + CCL4 group. This study is combination of medicine and mathematics, as fractal dimension. It was calculated, toxicity and protective effects of antioxidant materials could be determined in this way.

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