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In-Vitro Interaction of Paraquat with Some Amino Acids

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Abstract: In this study, the interaction paraquat of (1,1' dimethyl 4,4' dipyridylium dichloride), which is a herbicide are employed on a large scale in the agricultural struggle, with some amino acids was investigated,, *in vitro*. The interactions between the employed doses of 1,5 and 10 ppm of paraquat and different amino acids were observed by thin layer chromatography.

During the experiments with or without incubation on the mixtures of paraquat and amino acids, it was observed that the paraquat was bound with the amino acids, and as a result of which the Rf values decreased when compared to those of the control amino acids and differences occured in the absorption peaks.

The obtained results indicate that paraquat can penetrate into mammalian systems through indirect methods and different toxic effects may also be influential in in-vitro conditions, that is without being metabolized.

Key words: Paraquat, Amino Acids.

Paraquat'ın Bazı Amino Asitler Üzerine In-vitro Etkisi

Özet:Bu çalışmada, tarımda yaygın olarak kullanılan ve bir herbisit olan paraquat'ın (1,1' dimethyl 4,4' dipyridylium dichloride), kullanılmıştır. Paraquat'ın 1, 5 ve 10 ppm'lik dozlarının bazı amino asitlerle etkileşim İnce Tabaka Kromatografisi Yöntemi ile incelenmiştir..

İnkübe edilen ve edilmeyen paraquat ve amino asit karışımlarında paraquat + amino asit karışımları üzerinde yapılan deneyler sırasında paraquat'ın amino asitlerle bağlanabildiği gözlendi ve bunun sonucu

olarak da kontrol amino asit Rf değerlerine göre karışım Rf değerlerinde düşüş ile absorbsiyon piklerinde farklılıkların olduğu gözlenmiştir.

Elde edilen sonuçlar, dolaylı yollarla memeli sistemlerine giren ve buralarda değişik toksik etkiler gösteren paraquat'ın *in-vitro* şartlarda da yani metabolize olmadan da etkili olabileceğini göstermektedir.
Anahtar kelimeler: Paraquat, Amino asitler.

Introduction

Mankind has developed several methods to obtain better nourishment. The residue arising from the application of herbicides is being left to the environment without any control mechanisms. Environment pollution udoubtly effects living systems and indirectly the future of the world. One of the most important of these pollutants is pesticides. Pesticides supply important benefits for destroying and/or controlling harmful organisms. In addition to this, when used irregularly or in uncontrolled conditions, they cause important health and environmental problems [1].

Paraquat (1,1' dimethyl 4,4' dipyridylium dichloride) which is used on a large scale in Turkey is included in a group of non-specific pesticides and has serious toxic effects on mammalian systems [2,3]. Since this substance accumulates and stays in pulmoner tissues, certain lesions have been observed in human lungs. Apart from this, pesticides are known to cause damage to kidney and liver [4]. According to Matsuoka et al. (1993), stability of paraquat in human serum is much higher than diquat, and when compared with each other it accumulates at higher amounts in lungs than the adipose tissue [5]. Based on the reports of Saenz et al. (1993) and İbrahim (1990) concerning the effects of paraquat on algae, it is determined to have inhibitory effect on the growth and metabolic activity of algae, leading to shrink the cell size, and finally to inhibit the chlorophyll a, b, carotenoids, carbohydrate and protein contents [6, 7]. According to a study carried out by Marzio et al. (1993) on the effects of paraquat on asethylcholine esterase activity (AchE) of *Bryconamericus iheringii*, a kind of fresh water fish, AchE activity was inhibited both *in-vivo* and *in-vitro* conditions. After a 16 day application at a dose of 15 mg Pq/L to the fishes, 100 % mortality occured [8]. Hirai et al. (1992) showed that paraquat is reported to cause structural disorders on the mitochondria of rats [9]. During the past 25 years, in which period paraquat has been used, Many Cases of poisoning and death caused by the pesticide have been reported. In Japan, 500-1200 people die owing to this substance every year. Therefore, the Japanese government has

forbidden the commercial products in which these substances are used [10]. After this improvement, the investigations related to paraquat have gained momentum.

In this study, the interaction of the paraquat, which is still employed in our country, with some amino acids was explored *in-vitro* by thin layer chromatography (TLC).

Material And Methods

In our study, 25 g of silica gel G-60 was used for five glass plates, 20x20 cm in dimensions and 4 mm in depth. Silica gel was homogenated in 60 ml of double distilled water and spread onto glass plate, 0.25 mm in depth by a spreader. Plates were prepared by activating them at room temperature for 24 hours. The amino acids employed in this experiment (Arg, Phe, Glu, His, Met, Thr, Trp, Tyr) were prepared at a concentration of 10 mg/mL while the concentration values of the paraquat were prepared as 1.0, 5.0 and 10.00 ppm. These doses were used according to W.H.O [3]. Each concentration of paraquat was mixed with every amino acid (1:1) and, non-incubated paraquat-amino acid mixtures and mixtures incubated for 24 hours were used in the experiment. Incubation was at room temperature. Three (3) μL of amino acids, paraquat and mixtures were individually applied to the double plates. For running process, plates were put in a mixture prepared with EtOH (96 %) and double distilled water at a proportion of 70:30 for 4.5 hours. At the end of this period, the running levels of the solvent were marked and plates were dried. The double plates contained the same sample. Which one of the plates, However, was coloured with ninhidrine, the other plate was recovered from the marked place at a 1.0 cm in diameter. After a five minutes centrifugation of the scraped parts with three mL of 0.1 N HCI at 3000 rpm, supernatant was read out at 200-300 nm wave length using the Shimadzu 160 UV spectrophotometer [11]. For calculating the Rf values of the samples, the experiments were repeated 6 (min.) to 10 times (max.). Variant analysis was applied for evaluating the data [12] and “Multiple Range Test” was carried out for the importance control of the difference between the averages [13].

Results

For all the experimental studying periods, Rf values of the amino acids and mixtures on the plates stained with ninhidrine after TLC, were calculated. The obtained results and the statistical data applied to them are given in Table 1. The spectra of the recovered samples taken from the parallel plates were calculated and maximum absorbance and wave length values are shown in Table 2.

Maximum absorbance value of paraquat was determined as 258.1 nm in the spectrum range applied between 200 and 300 nm (Figure 1).

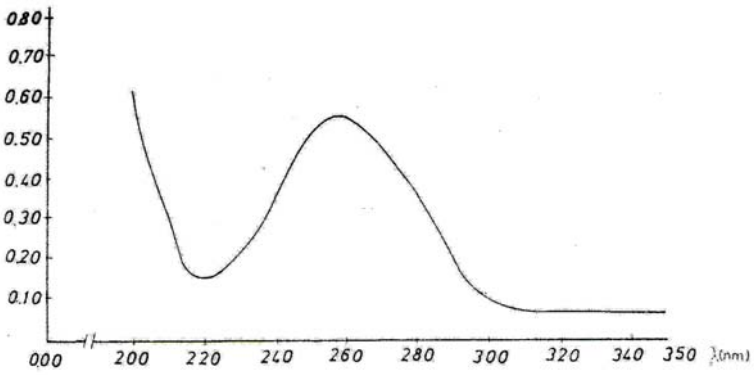

Figure 1. The absorbance graphic of paraquat

Rf values of Arg and Met were determined as 14.58 and 84.78 nm, respectively. Rf values of the 1.5 and 10 ppm dose of these amino acids and paraquat (both incubated and non-incubated) were different from the Rf values of the amino acids (Table 1). The spectra of the Arg recovered from TLC by scraping was determined as 201.4 nm at maximum (Table 2). While there were no differences at the maximum absorbance wave length of the TLC scraping obtained from the non-incubated amino acid and paraquat mixture, a decrease was determined at the absorbance. While the spectra of the Arg-paraquat mixture obtained after a 24 hour incubation was reduced, a second cast iron was determined at 218 nm (Figure 2). There were no changes at the maximum absorbance wave length of both incubated and non-incubated spectrums of Met, while some absorbance differences were recorded (Figure 3).

Relative Rf values of Phe, Glu, His, Thr, Trp and Tyr amino acids were determined as 94.2. 84.1, 62.49, 88.77, 94.58 and 98.14, respectively (Table 1). While there were no changes at the relative Rf values obtained from the non-incubated mixtures of Phe, Thr and Trp, some reductions were observed for Glu, His and Tyr.

These reductions were accepted as important reductions at the level of 0.05 probability (Table 1). Between the relative Rf values of the 24 hours incubated mixtures, while there were no changes established for only His, important decreases were fixed for Phe, Glu, Thr, Trp and Tyr from the statistical point of view. No strappings were found at the maximum absorbance wave length of the samples obtained from the TLC scrapes of both incubated and non-incubated mixtures of Phe, His, Tyr and Trp (Figures 4, 5, 6 and 7). On the other hand, some strappings were determined for Glu and Thr (Figures 8 and 9). For Thr, three different cast irons were determined in the experiment without incubating with paraquat. Maximum absorbance wave length was recorded as 221.5 nm after a 24 hours incubating period (Table 2).

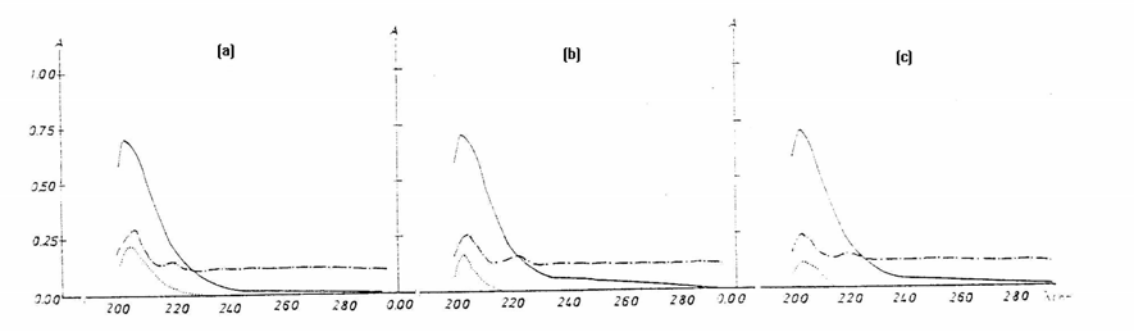

Figure 2. The absorbance graphics of a-) 1ppm b-) 5 ppm and c-) 10 ppm paraquat interactions from TLC scrapings ___ Arg, ---- incubated ….. non- incubated

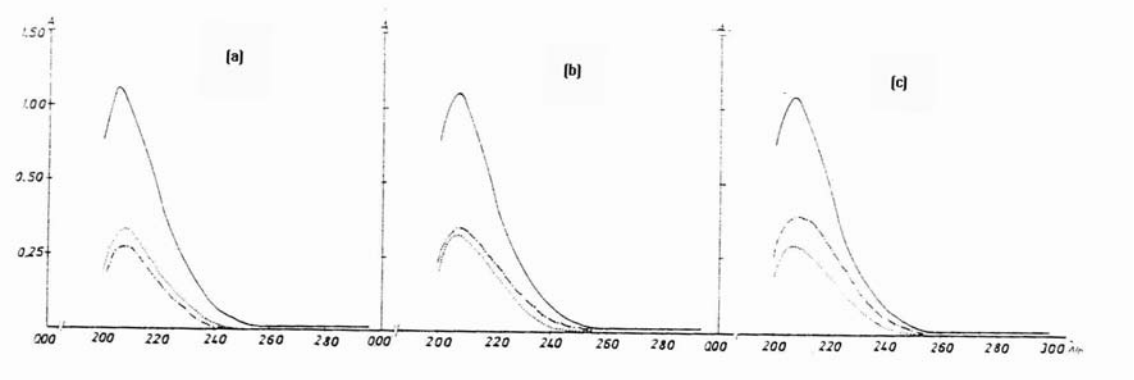

Figure 3. The absorbance graphics of a-) 1ppm b-) 5 ppm and c-) 10 ppm paraquat interactions from TLC scrapings ___ Met, ---- incubated ….. non- incubated

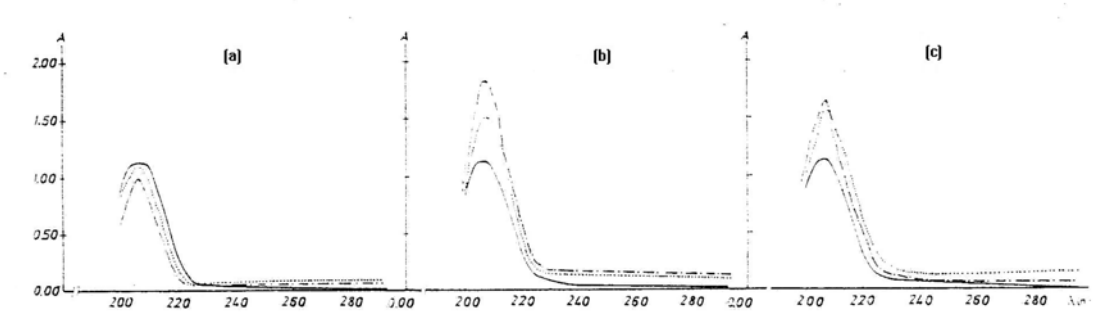

Figure 4. The absorbance graphics of a-) 1ppm b-) 5 ppm and c-) 10 ppm paraquat interactions from TLC scrapings ___ Phe, ---- incubated ….. non- incubated

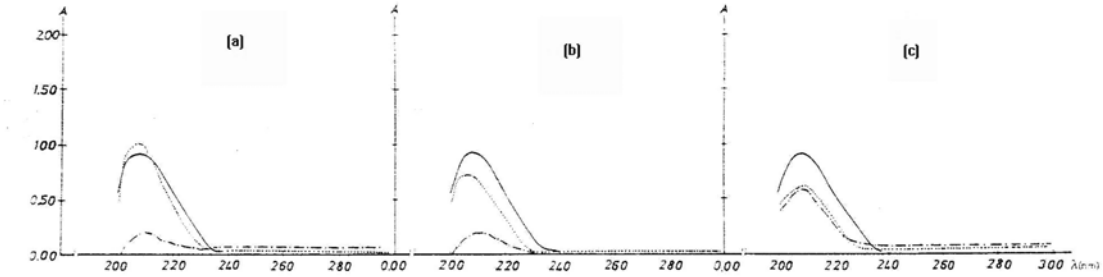

Figure 5. The absorbance graphics of a-) 1ppm b-) 5 ppm and c-) 10 ppm paraquat interactions from TLC scrapings ___ His,---- incubated ….. non- incubated

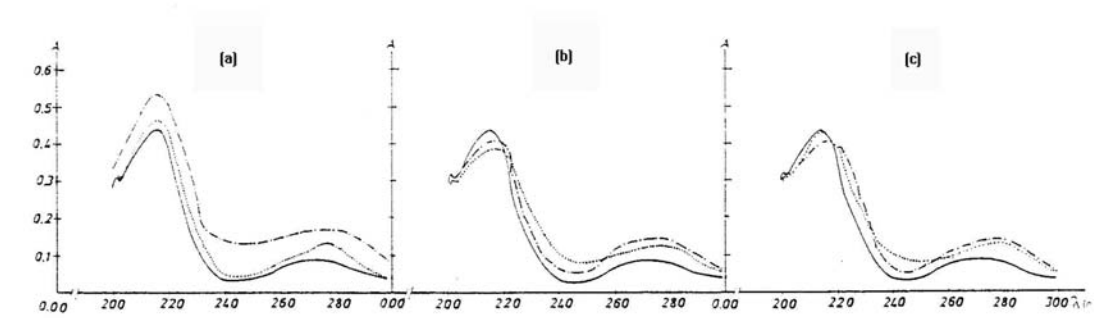

Figure 6. The absorbance graphics of a-) 1ppm b-) 5 ppm and c-) 10 ppm paraquat interactions from TLC scrapings ___ Tyr, ---- incubated ….. non- incubated

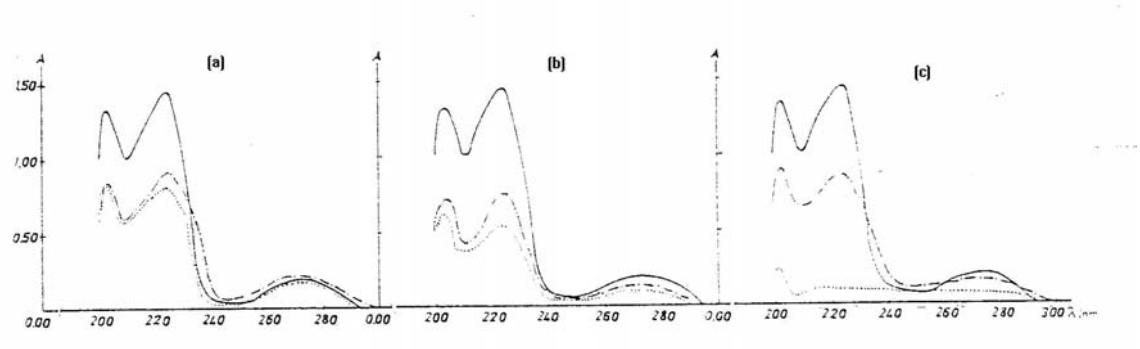

Figure 7. The absorbance graphics of a-) 1ppm b-) 5 ppm and c-) 10 ppm paraquat interactions from TLC scrapings ___ Trp, ---- incubated ….. non- incubated

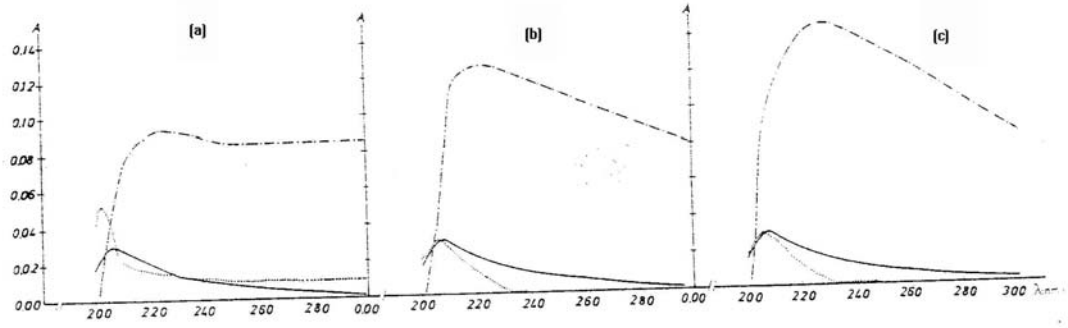

Figure 8 The absorbance graphics of a-) 1ppm b-) 5 ppm and c-) 10 ppm paraquat interactions from TLC scrapings ___Glu, ---- incubated ….. non- incubated

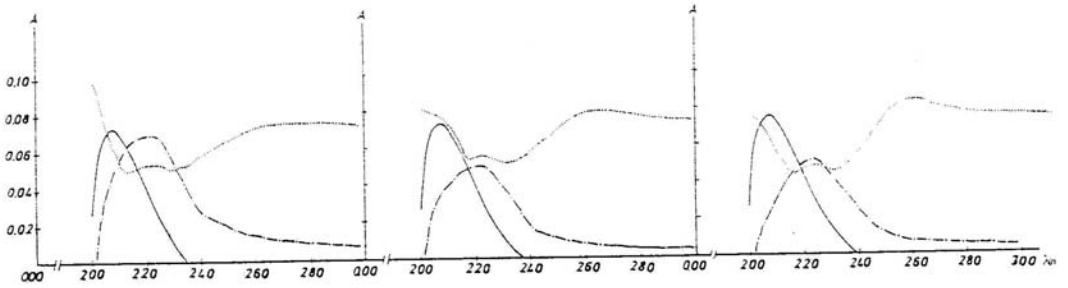

Figure 9. The absorbance graphics of a-) 1ppm b-) 5 ppm and c-) 10 ppm paraquat interactions from TLC scrapings ___ Thr, ---- incubated ….. non- incubated

Table 1: Rf values depending on doses of incubated and non-incubated mixtures♠

Amino acids	Paraquat Doses (ppm)	Amino Acid Mean±S.E.*	Paraquat Mean±S.E*	Non-incubated Mix Mean±S. E.*	24 hours incubated Mix Mean±S. E.*
Arg	1			33.56±0.13cx	59.58±0.72dx
	5	14.58±0.24a	91.66±0.00b	33.94±0.61cx	58.12±1.57dx
	10			32.49±0.48cx	54.16±2.40dx
Phe	1			94.99±0.70ax	78.80±0.30cx
	5	94.20±0.75a	91.66±0.00b	93 95±0.39ax	74.81±0.99cy
	10			95.62±0.20ax	78.25±0.78cx
Glu	1			47.78±0.73cx	68.81±1.04dx
	5	84.10±0.59a	91.66±0.00b	52.20±1.00cy	67.78±1.61dx
	10			53.38±0.87cy	69.99±0.33dx
His	1			47.91±3.60cx	62.91±0.53ax
	5	62.49±1.70a	91.66±0.00b	41.66±2.40cx	66.87±0.85ay
	10			45.58±2.55cx	61.66±1.14ax
Met	1			88.95±1.99bcx	86.99±2.11acx
	5	84.78±1.34a	91.66±0.00b	88.33±1.48cx	87.42±1.64cx
	10			84.99±1.48ax	90.83±0.47by
Thr	1			85.95±0.78ax	76.53±1.27bx
	5	88.77±3.39a	91.66±0.00a	86.58±1.38abx	71.15±1.10cx
	10			86.66±0.48ax	76.15±2.56bx
Trp	1			94.16±0.83ax	87.87±0.00cxy
	5	94.58±0.99a	91.66±0.00b	93.01±0.23ay	85.98±0.21cy
	10			93.12±0.52ax	90.52±1.53bx
Tyr	1			90.62±1.19bx	94.67±0.25cx
	5	98.14±0.7a	91.66±0.00b	83.74±3.93cy	93.35±1.19bx
	10			96.04±5.68cy	92.71±1.32bx

* Means with the same letters do not significantly differ at 0.05 level (abc and xyz).

S.E : Standard Error

♠Results are means of 6 (min), 10 (max) different experiments

Table 2: Maximum absorbance and wavelengths of the samples recovered from TLC by scrapings.

Amino Acids	Paraquat Doses (ppm)	Amino Acids, λ(nm)	Max.Abs	Non-incubated Mix λ(nm)	Max.Abs	24 hours incubated Mix λ(nm)	Max.Abs
Arg	1			202.0	0.215	207.5 218.0	0.229 0.100
	5	201.4	0.713	202.0	0.153	202.1 217.9	0.264 0.099
	10			202.0	0.090	202.7 218.0	0.206 0.110
Phe	1	205.0	1.172	206.3	1.102	207.0	1.023
	5			206.1	1.547	206.7	1.877
	10			206.3	1.570	207.6	1.669
Glu	1	208.8	0.031	202.0	0.052	222.7	0.095
	5			202.0	0.033	221.1	0.125
	10			202.0	0.033	223.1	0.141
His	1	210.4	0.941	210.9	1.043	212.1	0.191
	5			210.6	0.738	210.6	0.199
	10			210.4	0.615	210.8	0.609
Met	1	205.6	1.331	206.0	0.365	207.3	0.258
	5			206.0	0.355	206.9	0.379
	10			206.0	0.314	206.9	0.392
Thr	1			200.0 223.9 260.0	0.098 0.052 0.075	221.5	0.071
	5	207.1	0.074	200.0 224.6 264.4	0.083 0.056 0.079	21.5	0.048
	10			200.0 223.7 258.5	0.074 0.048 0.081	221.5	0.053
Trp	1			218.5 278.3	0.463 0.131	218.4 278.1	0.554 0.166
	5	217.2 278.0	0:440 0.080	218.0 279.4	0.384 0.124	217.5 278.8	0.403 0.135
	10			217.2 279.2	0.431 0.134	217.8 277.5	0.400 0.146
Tyr	1			202.2 223.7	0.856 0.827	202.2 223.2	0.846 0.895
	5	202.4 223.7	1.304 1.464	201.8 223.9	0.630 0.530	202.2 223.1	0.732 0.733
	10			201.2 225.3	0.210 0.082	202 2 223.6	0.895 0.892

Discussion

Since some incubated and non-incubated samples reduced or increased, some amino acids had strappings at their maximum absorabance wave length. In addition to this, formation of some new cast irons made us think that paraquat may react with amino acids. Hence the aminoacids are the molecular units of proteins, these reactions may also effect the structure and functions of proteins. This event may lead to some disorders in metabolism. Although *in-vivo* and *in-vitro* conditions are different from each other, staying [4] and accumulating ability of paraquat in liver, kidney and lungs may also effect amino acids *in-vitro*. Nauchi et al. (1993) showed that liver Mg^{++}-ATPase activity had been reduced 20%, and additional vitamin E prevented this reduction in rats [14]. In another study, paraquat had activated or inhibited the brain AchE activity depend on dose and presence of oxygen [8].

Except Phe, Thr and Trp, important changes were observed in the relative Rf values of the non-incubated amino acids from the statistical point of view. Except Met, relative Rf values were changed after incubation (Table 1). Some changes have also been observed at the absorbance of these amino acids in addition to new cast irons (Table 2).

These kind of interactions for amino acids may also effect the histon proteins and DNA interaction. Moreover, inhibition of DNA and protein synthesis were observed Ahmed et al. (1994) [15].

Paraquat shows remarkable toxicity to living systems and leads to some disorders in metabolism. However, living systems operate detoxification mechanisms to remove this toxic substance from the body. Amino acids have an important role in this system. Amino acids detoxificate toxic substances by conjugating with them. From this point of view, our study approaches the problem from an important aspect. In a study carried out with rats, paraquat and sistin were given together to the rats. As a result, the levels of paraquat were reduced in lung, liver, kidney and gastrointestinal systems. For the faeces and urine, an increase was observed in the level of paraquat [16]. However, it is claimed that the toxic effect of paraquat has arisen from the superoxide radicals that formed as a result of interaction between paraquat and molecular oxygen [9]. Formation of super oxide radicals lead to the formation of more toxic reduced oxygen forms (e.g. H_2O_2). These radicals destroy the cells by reacting with membrane phospholipids.

In this study carried out *in-vitro*, paraquat was reacted with some amino acids. However, we need some further investigations to determine the new compounds that arise from the interaction between paraquat and amino acids.

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