Egypt. J. Exp. Biol. (Zool.), 9(1): 141 – 150 (2013)

RESEARCH ARTICLE

Amany Abd El-monem Tohamy
Warda A. Khalifa
Manal F. El Khadragy
Adel K. Ibrahim

GENOTOXIC AND MOLECULAR STUDIES ON CYCLOSPORINE A-AZITHROMYCINE INTERACTION AND THE PROTECTIVE EFFECT OF GARLIC DURING ADMINISTRATION

ABSTRACT:

The experiment was conducted to study the effects of both Cyclosporine A- (CsA) and Azithromycine (AZM), and to demonstrate the interaction of combination of CsA with AZM and the protective properties of garlic against each drug of them, besides Cyclosporine - Azithromycine interaction. The study was planned to clarify changes in chromosomal aberrations (CsA), expression of tumor necrosis factor alpha (TNF-α) and biochemical changes in different organs of Male Sprague-Dawley rats during period of oral administration of drug or drugs. The alterations after oral administration of animals with doses equal to 25 mg/kg of CsA, 500 mg/kg of AZM and 300 mg/kg of garlic were estimated after one and seven days. Non significant changes were observed in all types of structural and numerical chromosomal aberrations after one day of oral administration of the used drugs. After seven days of oral administration of CsA alone, CsA + garlic, CsA + AZM, and CsA + AZM + garlic, a significant increase in structural and numerical chromosomal aberrations were noticed. No significant changes in structural and numerical CAs were noticed after seven days of oral administration of AZM or AZM + garlic. The mean values of TNF-α gene expression, when measured with real time PCR assay, revealed that its expression in the three control groups were higher than the groups received the drug alone, in case of AZM and AZM+ CSA, while the group received CSA alone was the lowest group in TNF-α expression. Biochemical results showed significant changes in serum AST, ALT, GGT, LDH, ALP, globulin, BUN, creatinine and glucose after oral administration of CsA. In conclusion, CsA was genotoxic on chromosomes and TNF-α gene, AZM administration alone or with CsA showed safety protection and minimal interaction while garlic may improve the hazard effect of CsA.

KEY WORDS:

Cyclosporine A, Azithromycine, Garlic, Genotoxicity, TNF-α expression, Biochemical changes

CORRESPONDENCE:
Amany Abd El-monem Tohamy
Zoology department, Faculty of Science, Helwan University, Egypt
E-mail: amanytohamy2005@yahoo.com

Warda Aly Khalifa*
Manal Fawzy El Khadragy*
Adel Khalil Ibrahim**
* Zoology department, Faculty of Science, Helwan University, Egypt
**Clinical pathology department, faculty of veterinary medicine, Cairo university, Egypt

ARTICLE CODE: 17.01.13

INTRODUCTION:

Drug-Drug interactions (DDI) are important cause of drug related health problems and this includes significant morbidity and mortality. DDI can lead to a variety of adverse events, and it has been suggested that preventable adverse events are the eighth cause of death among patients (Goldstein *et al.*, 2005).

Cyclosporine A (CsA) is a frequently used immunosuppressive agent in transplant medicine to prevent graft rejection (Mascarell and Truffa-Bachi, 2003; Hagar, 2004; Zhang *et al.*, 2007). In addition, it was used in the treatment of wide spectrum of autoimmune diseases such as psoriasis (Hesselink *et al.*, 2004; Kurus *et al.*, 2008), rheumatoid arthritis, inflammatory bowel disease, and asthma (Andrés *et al.*, 2005). CsA is usually administered orally or intravenously (i.v.). Oral preparations may contain corn, castor, or

olive oil in ethanol while i.v. preparations contain 33% ethanol and castor oil vehicle (Corcoran, 2006). The clinical use of CsA presents inconvenient adverse side effects such as hepatotoxicity and nephrotoxicity (Andrés *et al.*, 2001). CsA primary mode of immunosuppressive action appears to be the regulation of T cell function by inhibiting the expression of cytokines such as IL-2 and affects of the expression of TNF-α (Ciesielski *et al.*, 1997; Leitner *et al.*, 2011). In rats treated with CsA, TNF-α levels were lower than in rats received no preventative treatment with a mechanism linking CsA with the prevention of cell death in addition to inhibition of T cell proliferation (Burke *et al.*, 1994; Ciesielski *et al.*, 1997). It is well documented that TNF-α mRNA gene transcription is bloked by CsA (McCaffrey *et al.*, 1994). This drug is extensively metabolized in the liver by CYP3A enzymes (Mehvar and Chimalakonda, 2004). Pharmacokinetics of CsA is characterized by a low clearance and a high volume of distribution in rat tissues (Tanaka *et al.*, 2000).

Azithromycin (AZM) is the first clinically developed antibiotic in a new subclass of the macrolides. It is composed of 15-cyclic lactone antibiotic and semisynthetic erythromycin derivative (Van Bambeke *et al.*, 1996). It exhibits some advantages over erythromycin including better oral bioavailability, higher tissue concentrations, and fewer side effects. AZM is active against some Gram-positive and Gram-negative organisms and plays its role by binding to the 50S subunit of the bacterial ribosome (Imamura *et al.*, 2005). This action influences the microbial protein synthesis by preventing transpeptidation and translocation processes. Thus, AZM has been used to treat respiratory infections, skin and soft tissue infections and some sexually transmitted diseases (Avramov *et al.*, 2006).

AZM are relatively free of interactions with CYP3A4 *in vitro*. AZM have been shown to inhibit the production of interleukin-1, granulocyte/monocyte colony stimulating factor and TNF-α from mononuclear cells and other cells (Culić *et al.*, 2002).

Garlic has been used for centuries as a herbal medicine in treating abscesses, cough, poisoning, parasites, worms, digestive and circulatory problems, snake bites (Fallon *et al.*, 1998), hemorrhoids, abdominal pain, loss of appetite and pneumonia (Aouadi *et al.*, 2000). Garlic was known as an effective material in decreasing of high blood pressure and cholesterol, (Stevinson *et al.*, 2000; Steiner, 2001). It can also inhibit LDL oxidation (Tohidi and Rahbani, 2000), platelet aggregation and adhesion (Rahman and Billington, 2000; Banerjee *et al.*, 2001; Steiner, 2001), cardiovascular diseases (El-Demerdash *et al.*, 2005; Khar *et al.*, 2011) and can increase nitric oxide production (Morihara *et al.*, 2002). Because of these beneficial effects of garlic, treatment with garlic extracts was found to improve the activation of natural killer cells, the function of T-lymphocytes and the level of interleukin – 2 (Sumiyoshi, 1997).

The aim of the present study was planned to evaluate the effects of Cyclosporine A, Azithromycine, as well to demonestrate the interaction between both drugs on chromosomes, TNF-α gene expression and on organs functions of rats taking in consideration the protective actions of garlic against the effects of each drug alone or on the interaction between both drugs in combination.

MATERIAL AND METHODS:

Experimental Animals:

A total of 170 Male Sprague Dawley rats (*Rattus norvegicus*), approximately 2 months old, weighing 70-100 g were obtained from Egyptian Institution of Serum and Vaccine (Helwan, Cairo, Egypt). The rats were housed in specially designed cages and kept in the laboratory under the normal light-dark rhythm, food and water supplement for at least one week before the start of the experiment.

Animal Groups:

Nine groups composed of 10 rat per each were used. Group one animals were kept without medication while the second group received only the vehicle olive oil (2.5 ml), the third group received CsA, fourth group received AZM, fifth group received garlic, sixth group received both CsA and AZM, seventh group received CsA and garlic, eighth group received AZM and garlic, while finally group nine received CsA, AZM and garlic.

Drugs:

CsA, AZM and garlic were administrated for one and seven days by oral route in a dose equivalent to 25 mg/kg body weight in olive oil, 500mg/kg body weight in double distilled water or 300 mg/kg body weight in distilled water, respectively.

Biochemical studies:

Blood samples were collected at the end of experiments. Sera from all samples were separated for measurement of glucose, total proteins, blood urea nitrogen (BUN), creatinine, aspartate aminotransferase (AST), aspartate aminotransferase (ALT), gama glutamyl transferase (GGT), lactate dehydrogenase (LDH), alkaline phosphatase (ALP), and albumin, by kits supplied by Stainbio Laboratory, Taxas, USA.

Chromosomal analysis:

Five animals of each group were sacrificed after one day of the treatment and

another five animals in the same group were sacrificed after seven days of the treatment by head decapitation at the appropriate sampling time, Colchicine was given at the dose of 1 mg/kg BW i.p. two hours before sacrifice. Chromosomal preparations for scoring both structural and numerical aberrations from bone marrow cells of rat were made according to the method described by (Helbig *et al.*, 1995) with some modifications. Slides were stained with Giemsa and well spread metaphases were analyzed for chromosomal aberrations. Fifty metaphase spreads per animal were examined microscopically for chromosomal aberrations. Only cells with well spread chromosomes were selected for scoring. The mitotic index (MI) is determined as the number of dividing cells in 1000 cells.

RNA Isolation and Real-Time Quantitative RT-PCR:

Using guanidinium thiocyanate-phenol-chloroform extraction method (Stratagene, Germany).to obtain total RNA was isolated from 5×10^3 cell obtained from each tested group. Synthesis of cDNA depended on total extracted RNA using a reverse transcription was performed with random hexamers (Applied Biosystems, USA), MULV reverse transcriptase (Promega, USA), dNTPs (Promega,USA) and 1µl RNase inhibitors (Promega,USA), at 42°C for 50 min, followed by denaturation at 99°C for 5 min. Addition of 1 µL RNase inhibitor (all from Invitrogen-Life Technologies) for another 20 minutes at 37°C. Quantitative real-time PCR was performed on a sequence-detection system (LightCycler 480 II; Roche, Switzerland) using heat-activated Taq DNA polymerase (LightCycler 480 SYBR Green I Master), according to the manufacturer's protocol. Primers were directed to study TNFα (TNF-α sense, 5'-AGAACT CAGGCGGTGTCC-3'. TNF-α antisense, 3'-GATTCCTGTGGGGACTCCCT-5 (484 bp). Endogenous control for expression was based on using β-actin (β-actin sense, 5'- AAC CCT AAG GCCAACCGTGAAAAG-3' β-actin antisense, 3'-ATACAACGGGATCTGAAGCTCG-5' (540 bp). Cycling conditions were as follows: 95°C for 10 min, followed by 40 cycles of 95°C for 5 s and 60°C for 1 min. The amplified mRNA was presented as relative quantities measured by n-fold increase of the amplification treated groups compared to control group. Amplification of β actin mRNA was used as a positive control to estimate the efficiency of the experimental conditions.

Statistical Analysis:

Statistical analysis was carried out by using one way analysis of variance (ANOVA) followed by Post hoc test (Dunkan) using the Statistical Package for the Social Sciences (SPSS) version 10. Histograms of cytogenetic data were drawn using Excel 2007 (Microsoft, USA).

RESULTS:

Chromosomal aberrations (CAs) assay:

Structural chromosomal aberrations observed in the present study were in the form of ring chromosome (Fig.1), and centromeric attenuations (Fig. 2). Numerical chromosomal aberrations were observed in the form of endomitosis (Fig. 3) and polyploidy (Fig.4).

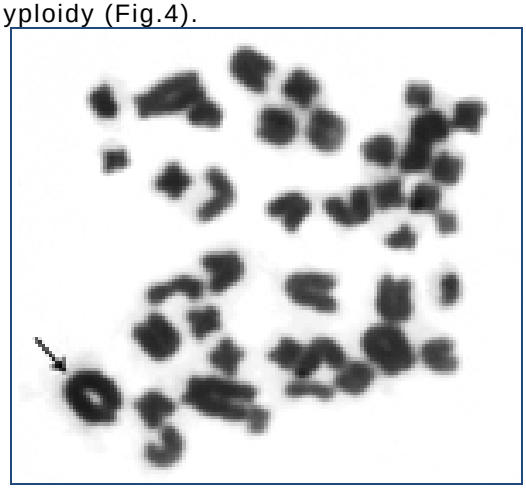

Fig. 1. A photomicrograph of a metaphase spread from rat bone marrow cells showing ring chromosome.

Fig. 2. A photomicrograph of a metaphase spread from rat bone marrow cells showing centromeric attenuation.

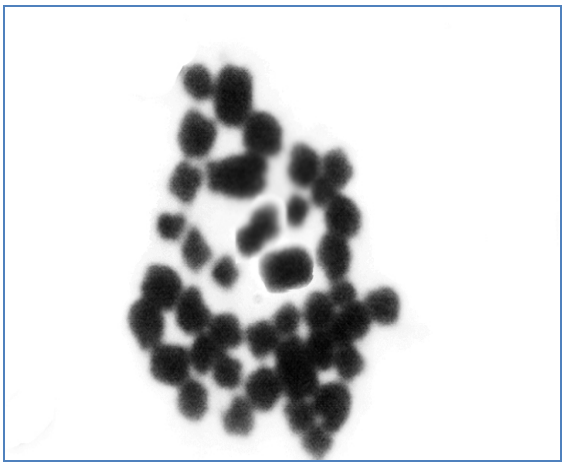

Fig. 3. A photomicrograph of a metaphase spread from rat bone marrow cells showing endomitosis.

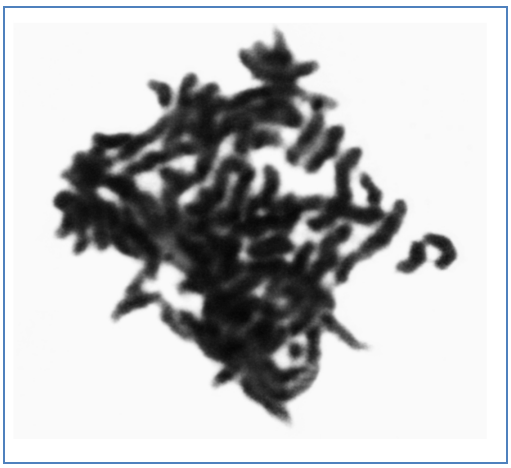

Fig. 4. A photomicrograph of a metaphase spread from rat bone marrow cells showing polyploidy.

CAs assay after one day of oral administration:

Oral administration of either garlic or olive oil alone to normal rats induced non significant changes in the mean values of all observed types of structural and numerical chromosomal aberrations when compared to that of the normal control group. Similarly, the results of group 3 received CsA alone and group 7 received CsA plus garlic showed non significant change in the mean values of all types of structural and numerical CAs when compared to normal control (group 1), vehicle control (olive oil) (group 2) and garlic (group 5) only (Table 1; Figs 5 & 6).

Table 1. Chromosomal aberrations induced in rat bone marrow cells after one day and seven days of oral administration of different treatments alone or combined.

Groups	Number of structural chromosomal aberration						Number of numerical chromosomal aberration					
	Centromeric attenuation		Ring chromosomes		TSA		Polyploidy		Endomitosis		TNA	
	One day	Seven days	One day	Seven days	One day	Seven days	One day	Seven days	One day	Seven days	One day	Seven days
Control	1.2 ± 0.447	1.6 ± 0.447	0	0	1.2 ± 0.849	1.6 ± 1.131	1.4 ± 0.548	1.4 ± 0.548	0	0.2 ± 0.447	1.4 ± 0.989	1.6 ± 0.849
Olive oil	1.4 ± 0.548	1.2 ± 0.836	0	0	1.4 ± 0.989	1.2 ± 0.849	1.6 ± 0.548	1.2 ± 0.447	0.2 ± 0.447	0	1.8 ± 0.989	1.2 ± 0.849
CsA	1.4 ± 0.447	2.4 ± 0.707 a b c	0.2 ± 0.447	1 ± 0 a b c	1.6 ± 0.849	3.4 ± 0.989 a b c	1.4 ± 0.894	2.4± 0.548 a b c	0	1.6 ± 0.548 a b c	1.4 ± 0.989	4 ± 0.566 a b c
AZM	0.8 ± 0.448	1 ± 0	0	0	0.8 ± 0.566	1 ± 0.707	1.4 ± 0.894	1 ± 0	0.2 ± 0.447	0	1.6 ± 0.849	1 ± 0.707
Garlic	1.2 ± 0.837	1.2 ± 0.447	0	0	1.2 ± 0.849	1.2 ± 0.849	1.2 ± 0.837	1.6 ± 0.548	0	0	1.2 ± 0.849	1.6 ± 1.131
CsA + ZMA	1.2 ± 0.837	2.2 ± 0.548 a b c	0	0.6± 0.548a b c	1.2 ± 0.849	2.8 ± 1.131 a b c	1 ± 0	2.6 ± 0.548 a b c	0	1 ± 0.707a b c	1 ± 0.707	3.6 ± 1.131 a b c
CsA + Garlic	1.6 ± 0.548	2.6 ± 0.548 a b c	0	0.6 ± 0.894 a b c	1.6 ± 1.131	3.2 ± 1.414 a b c	1.2 ± 0.837	2.8 ± 0.837 a b c	0.4 ± 0.548	1.4 ± 0.894 a b c	1.6 ± 0.566	4.2 ± 0.989 a b c
AZM + Garlic	1 ± 0	1 ± 0.707	0	0	1 ± 0.707	1 ± 0.707	1±0	1.2 ± 0.447	0	0	1 ± 0.707	1.2 ± 0.849
CsA + AZM +Garlic	1.2 ± 0.447	2 ± 0 a b c	0	0.8± 0.837 a b c	1 ± 0.849	2.8 ± 0.849 a b c	0.8 ± 0.447	2.2 ± 0.894 a b c	0.2 ± 0.447	1 ± 0 a b c	1.2 ± 0.424	3.2 ± 0.849 a b c

• Data were expressed as Mean± Standard Deviation (M±SD).

a: Significant change at P<0.05 with respect to normal control group.

b: Significant change at P<0.05 with respect to vehicle control (olive oil) group.

c: Significant change at P<0.05 with respect to Garlic group.

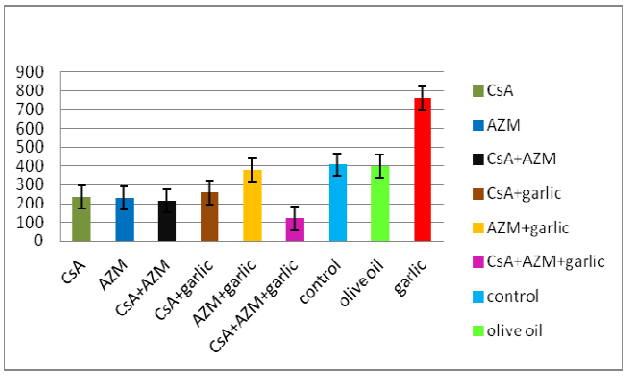

Fig. 5. Mean values of one day expression of TNF-α by using RT-PCR assay in different treated groups.

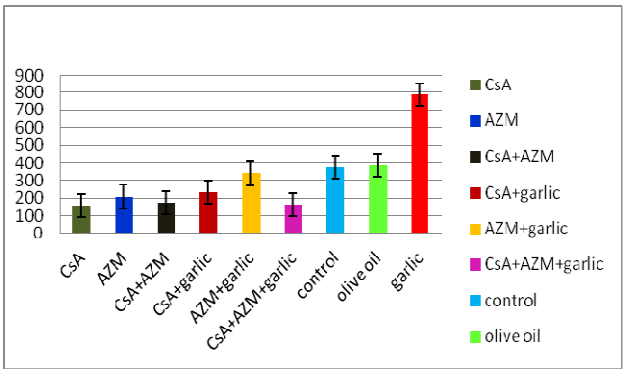

Fig. 6. Mean values of seven days expression of TNF-α by using RT-PC assay in different treated groups.

CAs assay after seven days of oral administration:

As indicated from table 1, oral administration of either garlic or olive oil alone to normal rats induced a non significant decrease in the mean values of all observed types of structural and numerical chromosomal aberrations when compared to that of normal control group. On the opposite side, the results of group 3 received CsA alone and group 7 received CsA plus garlic induced a significant increase ($P<0.05$) in the mean numbers of ring chromosomes, centromeric attenuation, total structural CAs, polyploidy, endomitosis and total numerical chromosomal aberrations as compared to normal control (group 1), vehicle control (olive oil) (group 2), and garlic alone (group 5).

Mitotic Index (MI):

Table 2 represents the percentage of mitotic indices recorded after one and seven days of oral administration of different treatments alone or combined together. A non significant change in the percentage of mitotic indices in bone marrow cells was recorded after one and seven days oral administration of CsA alone, CsA plus garlic as compared with that of the normal control, vehicle control (olive oil), and garlic groups.

Table 2. Mitotic activity percentage recorded in rat bone marrow cells after one day and seven days of oral adminstration of different treatments alone or combined.

No. of animals / Treatments	Number of dividing cells / 1000 cell / animal													
	One day							seven days						
	I	II	III	IV	V	M ± SD	MI%	I	II	III	IV	V	M ± SD	MI%
Control	21	14	15	16	20	17.2 ± 3.114	1.72%	21	14	15	16	20	17.2 ± 3.114	1.72%
Olive oil	17	15	16	17	18	16.6 ± 1.140	1.66%	17	20	14	17	16	16.8 ± 2.168	1.68%
Garlic	15	16	19	21	14	17 ± 2.915	1.70%	15	20	16	17	18	17.2 ± 1.924	1.72%
CsA	18	15	17	20	15	17 ± 2.121	1.70%	16	13	20	18	18	17 ± 2.646	1.70%
AZM	19	14	16	16	17	16.4 ± 1.817	1.64%	13	18	15	18	20	16.8 ± 2.775	1.68%
CsA + AZM	16	20	10	18	17	16.2 ± 3.768	1.62%	15	13	20	16	19	16.6 ± 2.880	1.66%
CsA + garlic	18	17	17	14	16	16.4 ± 1.517	1.64%	13	17	15	20	18	16.6 ± 2.702	1.66%
AZM + garlic	17	16	18	17	18	17.2 ± 0.837	1.72%	19	13	16	17	19	16.8 ± 2.489	1.68%
CsA + AZM + garlic	16	15	18	14	17	16 ± 1.581	1.60%	13	18	16	20	16	16.6 ± 2.608	1.66%

Molecular results:

The expression levels of TNF-α (Figs 5&6) was highest in the group received garlic alone as compared with the values of the control group or olive oil only while group received CsA only showed great reduction in the expressed TNF-α in both one day and seven days of oral administration.

DISCUSSION:

In the present study, oral administration of 25 mg/kg of CsA alone for seven days showed genotoxic effects. That was determined by several cytogenetic parameters such as a statistically significant increase in the total chromosomal aberrations and in the number of abnormal metaphases.

These results are in line with the study reported by Palanduz *et al.* (1999) who showed that CsA has a genotoxic potential on human lymphocytes. Some previous studies reported that CsA did not induce genetic damage in a number of test systems (e.g., gene mutations in prokaryotes, gene mutations and chromosomal aberrations in cultured mammalian cells, chromosomal aberrations and micronuclei in rodent bone marrow cells (Zwanenburg and Cordier, 1994). Other studies reported that CsA causes sister-chromatid exchanges in human lymphocytes *in vitro*, unscheduled DNA synthesis and chromosomal aberrations in the peripheral blood lymphocytes of kidney transplant patients treated with CsA and prednisolone (IARC, 1990). The induction of genetic damage by CsA may be due to the oxidative stress caused by CsA that leads to the structural and functional damages in the tissues (Türk *et al.*, 2007; Shakiba *et al.*, 2009). It was suggested that CsA increases reactive oxygen species (ROS) that is hallmark of oxidative stress (Shakiba *et al.*, 2009). The excess production of ROS by CsA may be explained by its ability to produce alterations in mitochondria by blocking the permeability transition pore (Nicolli *et al.*, 1996), originating an increase in mitochondrial Ca^{2+} concentration (Fournier *et al.*, 1999), and alterations in mitochondrial electron transport chain. Nevertheless, the ROS generation may not be exclusive to the mitochondrial level, since CsA is metabolized by cytochrome P-450 3A that can also generate ROS (Serino *et al.*, 1994). High ROS levels may thus induce mitochondrial DNA (mtDNA) damage and results in nuclear DNA fragmentation and increased apoptosis (Venkatesh *et al.*, 2010; Kumar *et al.*, 2012). By time, the presence of mitochondrial dysfunctions may exert cellular detrimental effects leading to imbalances in energy production, modulation of apoptosis, or redox maintenance (Wallace, 1994).

In the present study, oral administration of garlic alone for one and seven days had no significant increase in the numbers aberrant cells or decreased in the mitotic index in bone marrow cells of rats when compeard to normal control group. On the other hand, oral administration of garlic combined with CsA and/or AZM after one and seven days did not give any significant reduction in the number of aberrant cells as compared to the normal control group. The obtained results agree with the studies which suggested that administration of garlic extract daily for at least 30 days was protecting against the clastogenic effect (Das *et al.*, 1995; Sengupta *et al.*, 2002). Also, oral administration of olive oil alone did not change the mitotic index and did not induce chromosomal aberrations when was compared with the respective control group. These results agree with the study reported by (Evangelist *et al.*, 2004). Moreover, the present study showed that oral administration of AZM alone and AZM plus garlic showed no significant changes in all types of structural and numerical chromosomal aberrations after one and seven days. Amacher *et al.* (1993) made an assessment in male and female mice with a single oral dose of 200 mg/kg of AZM followed by sacrifice after 24 or 48 h. They concluded that AZM did not cause gene mutation in microbial or mammalian cells, or chromosomal aberrations in cultured human lymphocytes or in mouse bone marrow *in vivo*. The present study agrees with this conclusion. Furthermore, our results revealed that the significant increase in the incidence of chromosomal aberration in bone marrow cells in combination group of CsA + AZM (for one and seven days), was due to the effect of CsA only because AZM was shown previously to have remarkable side effects and does not affect levels of CsA (Tokgöz *et al.*, 2004). Here, a non significant change in the mitotic indices was detected of bone marrow cells in different groups after one and seven days of oral administration. This is in line with Tannuri *et al.* (2008), who observed that CsA was cytotoxic in growing animals, but did not change the mitotic index when compared with the control group.

The biochemical results revealed that oral administration of CsA caused increasing livels of AST, ALT, GGT, LDH, and ALP. These results agree with Hagar (2004). Previous studies established that ROS production and oxidative stress situation are involved in CsA hepatotoxicity (Wolf *et al.*, 1997; Andrés *et al.*, 2001). Additional details were mentioned by Wolf *et al.* (1997) who reported that in primary rat hepatocyte culture. CsA increases reactive oxygen species (ROS) and malondialdehyde (MDA) production that are hallmarks of oxidative stress that. Also oral administration of CsA + garlic caused increased serum levels of AST, ALT, GGT, LDH, and ALP. These results were consistent with a study which suggested a reduction in the activity of one or more antioxidant systems due to direct toxic effect of CsA. CsA increased level of lipid peroxidation and oxidative stress and liver toxicity consequently (Türk *et al.*, 2007). Lipid peroxidation is a chemical process initiated by free radicals (Ozaras *et al.*, 2003) and an important mechanism in liver damage (Barón and Muriel, 1999).

Also oral administration of CsA+AZM showed significant increase in serum AST, ALT, GGT, LDH, and ALP. This is may be due to the damaging effect of CsA to hepatocyte membrane of CsA rather than the combination effect. On the other hand, oral administration of AZM alone showed significant increase in serum AST activities. Our results were similar to those obtained by Suriawinata and Min (2002), who observed that the AZM treatment led to a significant increase in the serum AST. Further, oral administration of AZM alone showed non significant changes in serum ALT, GGT, LDH, and ALP activities. This is similar to data discussed by Tokgöz *et al.* (2004) who noted that after AZM therapy, serum ALT, GGT, LDH, and ALP activities did not change significantly.

Oral administration of CsA alone showed non significant changes in serum total protein. The above results disagree with Rezzani *et al.* (2005), who observed that orall administration of CsA induce hepatotoxicity by inhibition of protein synthesis but with dose (7.5mg\kg) for 33 days that is different from our study. On the opposite side oral administration of CsA + garlic and CsA + AZM+ garlic showed significant increase in serum total protein. shaarawy *et al.* (2009) noted that administration of garlic prevent liver damage of rat due to its ability to reduced free radical induced oxidative liver damage. Also results revealed that oral administration of CsA alone, CsA + AZM, CsA + garlic and CsA + AZM + garlic showed non significant changes in serum albumin after one and seven days. Jeon and Kim (2011) reported that oral administration of CsA (100 mg\kg\day) for 21 days causes marked decreases in serum albumin. On the other hand oral administration of CsA alone CsA + garlic showed significant increase in serum globulin. Our results agree with Rezzani *et al.* (2005), who showed that CsA-induced hepatotoxicity is characterized by increase in serum globulin, decline in GSH, glutathione peroxidase and catalase concentrations also oral administration of CsA + AZM and CsA + AZM + garlic showed significant increase in serum globulin. This might attributed to the direct hepatotoxic effect of CsA rather than combination effect.

Data presented that oral administration of CsA alone showed significant increase in

serum creatinine and BUN after seven days. These observations agree with other publications who demonstrated significant changes in renal function parameters in both patient and experimental animals following by administration of CsA (Amudha *et al.*, 2007; Ishikawa and Homma, 2012). Alterations in renal haemodynamic, glomerular and/or tubular structures, including inflammation have all been reported to play an important role in CsA induced renal dysfunction (Wongmekiat *et al.,* 2008). The damage to the glomeruli and tubules in CsA mediated toxicity was facilitated through the expression of c-myc. This expression of c-myc in turn reflects the increased apoptotic death of the cells (Chakravarthi *et al.*, 2010). On the other hand, oral administration of AZM alone showed non significant changes in serum creatinine and BUN. Our results agree with that by (Tokgöz *et al.*, 2004), who demonstrated that after AZM therapy; serum creatinin and BUN did not change significantly. It may be used safely since it does not have remarkable side effect and does not affect on the level of creatinine (Wirnsberger *et al.*, 1998; Tokgöz *et al.*, 2004). Also oral administration of CsA + AZM showed significant increase in serum creatinine due to the reduction of renal perfusion induced by CsA than effect of combination.

Oral administration of CsA alone showed significant increase in serum glucose after seven days. Our results were agreed with (Böhmer *et a*l., 2010), who observed that CsA is involved in glucose homeostasis, and reinforcing reports that long-term CsA treatment affects glucose homeostasis, by decreasing serum insulin levels, increasing peripheral insulin resistance and serum glucose levels (Hjelmesaeth *et al.*, 2005; Penfornis and Kury-Paulin, 2006). The effect on insulin levels may result from compromised insulin production either via β-cell toxicity or via inhibition of DNA synthesis (Penfornis and Kury-Paulin, 2006). On the other hand, oral administration of CsA + AZM and CsA + AZM + garlic showed significant increase in serum glucose after seven days due to the reduction of renal perfusion induced by CsA than effect of combination, while oral administration of AZM alone showed a non significant change in serum glucose. From all biochemical parameters, the clear drug-drug interaction between CsA and AZM were little to be nearly absent this may be due to the difference between them in the metabolic pathway. There were no available publication studied the interaction between them at the level of biochemical parameters. In humans the immunosuppressive drug CsA has been used successfully in organ transplantation and in the treatment of autoimmune disorders. The drug, however, causes side effects that occur mainly in the kidney but also in the liver. Although, the mechanisms leading to the hepatic side effects are not fully understood, several reports have strongly suggested that reactive oxygen production is a common mechanism of drug toxicity (Wolf *et al.*, 1997) reported that CsA in primary rat hepatocyte cultures caused a concentration-dependent increase of free reactive oxygen species. CsA mediated reactive oxygen species formation. Therefore, garlic may have a protective effect on the deteriorated hepatic function resulting from oxygen free radicals in the CsA-induced hepatotoxicity. There were significant increases in ALT, AST, GGT levels in group, suggesting a significant hepatotoxicity due to oxygen free radicals. Garlic treatment significantly protected some parameters in hepatic function such as (ALT, GGT, LDH and globulin) , kidney function such as (creatinine) and free radical- mediated injury in the liver and kidney against CsA-induced changes. The results in this experiment suggest that garlic may be effective in preventing the CsA-induced hepatotoxicity and nephrotoxicity without changing plasma CsA levels in a rat model.

Regarding to figures 5 & 6, the results of RT-PCR assay revealed that the group recived CsA alone for one and seven days has lowest group in TNF-α gene expression. The present results agree with Ciesielski *et al.* (1997) who mentioned that in rats treated with CsA, TNF-α levels were lower than in rats receiving no preventative treatment linking CsA with the prevention of cell death in addition to inhibition of T cell proliferation. CsA act by forming a complex with immunophilin, which inhibits the Ca^{2+}/calmodulin-depended serin-threonine phosphatase calcineurin. Inactive calcineurin is unable to activate the nuclear factor of activated T cells (NFAT) (Eckstein *et al.,* 2005; Leitner *et al.,* 2011). TNF-α gene expretion was lower in the group recived AZM alone when compared with normal control group. This result is in line with Hall *et al.* (2002) and Tong *et al.* (2011) who observed that AZM administrated at lower doses than the minimum inhibitory concentration, reduced the serum levels of TNF-α, IL-6 and IL-8 and inhibited epithelial cell-mediated neutrophil survival by release of growth factor (GM-CSF). Inhibitory actions of AZM may be related to effects on the transcription factor, NF-KB, which is activated by a wide variety of inflammatory stimuli and mediates the expression of genes for many inflammatory mediators (Barnes and Karin 1997). According to the results of RT-PCR assay, the group that received garlic alone for one and seven days was the highest in TNF-α gene expression. The present results agree with Sumiyoshi (1997) who showed that treatment with garlic extracts improved the activation of natural killer cells, the function of T-lymphocytes and the level of IL-2. Also *in*

vitro and in vivo studies showed that aged garlic extracts stimulate immune functions (Chetty *et al.*, 2004).

From the present study we conclude that CsA was genotoxic on chromosomes and TNF-α gene, administration of CsA should be with great medical care for different organs function parameters, CsA render the immunity of experimental animals, so care for infection must be considered. Garlic may improve the hazard effect of CsA and AZM administration alone or with CsA is quite safe and minimal interaction take place.

REFERENCES:

Amacher DE, Ellis JH Jr, Joyce AJ, Muehlbauer PA, Turner GN, Wahrenburg MG, Holden HE, Ray VA. 1993. Preclinical toxicology studies with azithromycin: genetic toxicology evaluation. Mutat. Res., 300(2):79-90.

Amudha G, Josephine A, Mythili Y, Sundarapandiyan R, Varalakshmi P. 2007. Therapeutic efficacy of DL – α – lipoic acid on cyclosporine A induced renal alteration. Eur. J. Pharmacol., 571(2-3): 209-214.

Andrés D, Diez-fernandez C, Zaragoza A, Alvarez A, Cascales M. 2001. Induction of cell proliferation by cyclosporine A in primary cultures of rat hepatocytes. Biochem. Pharmacol., 61(4): 427-435.

Andrés D, Bautista M, Cascales M. 2005. Attenuation of cyclosporine A toxicity by sublethal that shock Role of catalase. Biochem. Pharmacol., 69(3): 493-501.

Aouadi R, Aouidet A, Elkadhi A, Ben Rayana C, Jaafoura H, Tritar B, Nagati K. 2000. Effect of fresh garlic (*Allium sativum*) on lipid metabolism in male rats. Nutr. Res., 20(2): 273–280.

Avramov ML, Petrovic SD, Mijin DZ, Zivkovic PM, Kosovic IM, Jovanovic MB. 2006. Studies on electrochemical oxidation of azithromycin and hemomycin at gold electrode in neutral electrolyte. Electrochim. Acta, 51(12): 2407-2416.

Banerjee SK, Maulik M, Manchanda SC, Dinda AK, Das TK, Maulik SK. 2001. Garlic- induced alteration in rat liver and Kidney morphology and associated changes in endogenous antioxidant status. Food Chem. Toxicol., 39(8): 793–797.

Barnes PJ, Karin M. 1997. Nuclear factor-KB-a pivotal transcription factor in chronic inflammatory diseases, N. Engl. J. Med., 336(15): 1066-1309.

Barón V, Muriel P. 1999. Role of glutathione, lipid peroxidation and antioxidants on acute bile-duct obstruction in the rat. Biochim. Biophys. Acta, 1472(1-2): 173-180.

Böhmer AE, Souza DG, Hansel G, Brum LM, Portela LV, Souza DO. 2010. Long-term cyclosporine treatment in non-transplanted rats and metabolic risk factors of vascular diseases. Chem. Biol. Interact., 185(1): 53-58.

Burke GW, Cirocco R, Markou M, Agramonte RF, Rabinovitch A, Miller J, Skyler JS. 1994. Effect of cyclosporine A on Serum tumor necrosis factor α in new-onset type 1 (insulin – dependent) diabetes mellitus. Diabetes and its complications, 8(1): 40–44.

Chakravarthi S, Haleagrahara N, Wen CF, Lee N, Thani PM. 2010. c-myc regulation and apoptosis in assessing the beneficial in cyclosporine induced nephrotoxicity. Res. J. Pharmacol., 4(1): 15-20.

Chetty KN, Calahan L, Oliver R, Chetty SN. 2004. Garlic induced alteration in liver mineral concentration in corn oil and olive oil fed rats. pathophysiology, 11(2): 129–131 .

Ciesielski CJ, Mei J, Neton JT, Piccinine LA. 1997. Effects of cyclosporine A and methotrexate on induction of tumour necrosis factor-alpha in rat cardiac allografts. Transpl. Immunol., 5(1): 25-34.

Corcoran TE. 2006. Inhaled delivery of aerosolized cyclosporine. Adv. Drug Deliv. Rev., 58(9-10): 1119-1127.

Culić O, Eraković V, Cepelak I, Barisić K, Brajsa K, Ferencić Z, Galović R, Glojnarić I, Manojlović Z, Munić V, Novak-Mircetić R, Pavicić-Beljak V, Sucić M, Veljaca M, Zanić-Grubisić T, Parnham MJ. 2002. Azithromycin modulates neutrophil function and circulating inflammatory mediators in healthy human subjects. Eur. J. Pharmacol., 450(3): 277-289.

Das TA, Choudhury R, Sharma A, Talukder G. 1995. Effects of crude garlic extract on mouse chromosomes in vivo. Food Chem. Toxicol., 34(1): 43-47.

Eckstein LA, Van Quill KR, Bui SK, Uusitalo MS, O'Brien JM. 2005. Cyclosporine A inhibits calcineurin / nuclear factor of activated T-Cells signaling and induces apoptosis in retinoblastoma cells. Invest. Ophthalmol. Vis. Sci., 46(3): 782-790.

El-Demerdash FM, Yousef MI, El-Naga NI. 2005. Biochemical study on the hypoglycemic effects of onion and garlic in alloxan – induced diabetic rats. Food Chem. Toxicol., 43(1): 57-63.

Evangelista CM, Antunes LM, Francescato HD, Bianchi ML. 2004. Effects of the olive, extra virgin olive and canola oils on cisplatin-induced clastogenesis in wistar rats. Food Chem. Toxicol., 42(8): 1291-1297.

Fallon MB, Abrams GA, Abdel-Razek TT, Dai J, Chen SJ, Chen YF, Luo B, Oparil S, Ku DD. 1998. Garlic prevents hypoxic pulmonary hypertension in rats. Am. J. Physiol., 275(2 Pt 1): 283–287.

Fournier N, Ducet, G, cravat A. 1999. Action of cyclosporine on mitochondrial calcium fluxes. J. Bioenerg. Biomembr, 19: 297-303.

Goldstien JN, Jaradeh IE, Jhawar P. 2005. ED drug-drug interactions: frequency and type, potential and actual, triage and discharge. Int. J. Emerg. Intensive care Med., 8(2): 1092-4051.+

Hagar HH. 2004. The protective effect of taurine against cyclosporine A-induced oxidative stress and hepatotoxicity in rats. Toxicol. Lett., 151(2): 335-343.

Hall IH, Schwab UE, Ward ES, Butts JD, Wolford ET, Ives TJ. 2002. Disposition and intracellular

activity of azithromycin in human THP-1 acute monocytes. Int. J. Antimicrob. Agents, 20(5): 348-360.

Helbig RH, Speit G. 1995. The pattern of adriamycin induced mutations in V-E5 Chinese hamster cells with chromosomal instability. Mutat. Res., 348(1): 1-6.

Hesselink DA, Baarsma GS, Kuijpers RW, van Hagen PM. 2004. Experience with cyclosporine in endogenous uveitis, posterior. Transplant. Proc., 36(25): 3725-3775.

Hjelmesaeth, J., Asberg, A., Muller, F., Hartmann, A. and Jenssen, T. 2005. New-onset posttransplantation diabetes mellitus: insulin resistance or insulinopenia? Impact of immunosuppressive drugs, cytomegalovirus and hepatitis C virus infection. Curr. Diabetes Rev., 1(1) 1–10.

IARC. 1990. Pharmaceutical drugs. International agency for research on cancer monographs on the evaluation of carcinogenic risk of chemicals to humans. IARC, 50. pp. 415.

Imamura Y, Higashiyama Y, Tomono K, Izumikawa K, Yanagihara K, Ohno H. 2005. Azithromycin exhibits bactericide effects on Pseudomonas aeruginosa through interaction with the outer membrane. Antimicrob. Agents Ch., 49: 1377-1380.

Ishikawa A, Homma Y. 2012. Beneficial effect of ubiquinol, the reduced form of coenzyme Q10, on cyclosporine nephrotoxicity. Int. Braz. J. Urol., 38(2): 230-234.

Jeon YJ, Kim YS. 2011. Cyclosporine A inhibits albumin synthesis in Huh7 cells. Korean J. Intern. Med., 26(3): 314-319.

Khar A, Banerjee K, Jadhav MR, Lawande KE. 2011. Evaluation of garlic ecotypes for allicin and other allyl thiosulphinates. Food Chem., 128(4): 988-996.

Kumar M, Pathak D, Venkatesh S, Kriplani A, Ammini AC, Dada R. 2012. Chromosomal abnormalities and oxidative stress in woman with premature ovarian failure (POF). Indian J. Med. Res., 135: 92-97.

Kurus M, Esrefoglu M, Karabulut AB, Sogutlu G, Kaya M, Otlu A. 2008. Oral L-arginine protects against cyclosporine-induced hepatotoxicity in rats. Exp. Toxicol. Pathol., 60(4-5): 411-419.

Leitner J, Drobits K, Pickl WF, Majdic O, Zlabinger G, Steinberger P. 2011. The effects of cyclosporine A and azathioprine on human T cells activated by different costimulatory signals. Immunol. Lett., 140(1-2): 74-80.

Mascarell L, Truffa-Bachi P. 2003. New aspects of cyclosporine A mode of action: from gene silencing to gene up-regulation. Mini Rev. Med. Chem., 3(3): 205-214.

McCaffrey PG, Goldfeld AE, Rao A. 1994. The role of NFATp in cyclosporine A-sensitive tumor necrosis factor–alpha gene transcription. J. Biol. Chem., 269(48) 30445 – 30450.

Mehvar R, Chimalakonda AP. 2004. Hepatic disposition of cyclosporine A in isolated perfused rat livers. Pharm. Pharm., 7(1): 47-54.

Morihara N, Sumioka I, Moriguchi T, Uda N, Kyo E. 2002. Aged garlic extract enhances production of nitric oxide. Life Sci., 71(5): 509-517.

Nicolli A, Basso E, Petronilli V, Wenger RM, Bernardi P. 1996. Interaction of cyclophilin with the mitochondrial inner membrane and regulation of the permeability transition pore and cyclosporine A-sensitive channel. J. Biol. Chem., 271(4): 2185-2192.

Ozaras, R., Tahan, V., Aydin, S., Uzun, H., Kaya, S. and Senturk, H. 2003. N-acetylcysteine attenuates alcohol-induced oxidative stress in the rat. World J. Gastroenterol., 9(1): 125-128.

Palanduz S, Sever MS, Oztürk S, Taşçioğlu C, Karan MA, Sönmez G, Cefle K, Güler K. 1999. Genotoxic potential of cyclosporine A in patients with renal transplantation. Cell Biol. Toxicol., 15(1):13–17.

Penfornis A, Kury-Paulin S. 2006. Immunosuppressive drug-induced diabetes. Diabetes Metab., 32(5 Pt 2): 539-546.

Rahman K, Billington D. 2000. Dietary supplementation with aged garlic extract inhibits ADP-induced platelet aggregation in humans. J. Nutr., 130(11): 2662-2665.

Rezzani R, Buffoli B, Rodella L, Stacchiotti A, Bianchi R. 2005. Protective role of melatonin in cyclosporine A-induced oxidative stress in rat liver. Int. Immunopharmacol., 5(9): 1397-1405.

Sengupta A, Ghosh S, Das S. 2002. Administration of garlic and tomato can protect from carcinogen induced clastogenicity. Mutat. Res., 22(7): 859-866.

Serino F, Grevel J, Napoli KL, Kahan BD, Strobel HW. 1994. Oxygen radical formatting by the cytochrome P450 system as cellular mechanism for cyclosporine toxicity. Transplant. Proc., 26(5): 2869-2870.

Shaarawy SM, Tohamy AA, Elgendy SM, Abd Elmageed ZY, Bahnasy A, Mohamed MS, Kandil E, Matrougui K. 2009. Protective effects of garlic and silmarin on NDEA–induced rats hepato-toxicity. Int. J. Biol. Sci., 5(6): 549–557.

Shakiba Y, Mostafaie A, Arshadi D, Sabayan B. 2009. Application of garlic organosulfur compounds in prevention of cyclosporine A-Induced hepatotoxicity. Iranian J. Med. Hypotheses Ideas, 3: 3.

Steiner MLW. 2001. Aged garlic extract, a modulator of cardiovascular risk factors: a dose-finding study on the effects of AGE on platelet functions. J. Nutr., 131(3s): 980S-984S.

Stevinson C, Pittler MH, Ernst E. 2000. Garlic for treating hypercholesterolemia. A meta-analysis of randomized clinical trials. Ann. Intern. Med., 133(6): 420-429.

Sumiyoshi H. 1997. New pharmacological activities of garlic and its constituents. Nippon Yakurigaku Zasshi, 110(Suppl 1): 93-97.

Suriawinata A, Min A. 2002. A 33-year-old woman with Jundice after azithromycin use. Semin. Liver Dis., 22(2): 207-210.

Tanaka C, Kawai R, Rowland M. 2000. Dose-dependent pharmacokinetics of cyclosporin A in rats: events in tissues. Drug. Metab. Dispos., 28(5): 582-589.

Tannuri AC, Tannuri U, Coelho MC, Mello ES, Santos NA. 2008. Effects of immunosuppressants on hepatocyte cell mitosis during liver regeneration in growing

animal models of partial hepatectomy. Transplant. Proc., 40(5):1641-1644.

Tohidi M, Rahbani M. 2000. Evaluation of the effect of garlic powder on blood pressure, serum lipids and lipoproteins. Pharm. J. Tabriz Univ. Med. Sci., 4: 16-20.

Tokgöz B, Sari HI, Yildiz O, Aslan S, Sipahioğlu M, Okten T, Oymak O, Utaş C. 2004. Effects of azithromycin on cyclosporine-induced gingival hyperplasia in renal transplant patients. Transplant. Proc., 36(9): 2699-2702.

Tong J, Liu ZC, Wang DX. 2011. Azithromycin acts as immunomodulatory agent to suppress the expression of TREM-1 in Bacillus Pyocyaneus-induced sepsis. Immunol. Lett., 138(2): 137-143.

Türk G, Ateşşahin A, Sönmez M, Yüce A, Ceribaşi AO. 2007. Lycopene protects against cyclosporine A-induced testicular toxicity in rats. Theriogenology, 67(4): 778-785.

Van Bambeke F, Montenez JP, Piret J, Tulkens PM, Courtoy PJ, Mingeot-Leclercq MP. 1996. Interaction of the macrolide azithromycin with phospholipids. I. Inhibition of lysosomal phospholipase A1 activity. 314(1-2): 203-214.

Venkatesh S, Kumar M, Sharma A, Kriplani A, Ammini AC, Talwar P, Agarwal A, Dada R. 2010. Oxidative stress and ATPase6 mutationis associated with primary ovarian insufficiency. Arch. Gynecol. Obstet., 282(3): 313-318.

Wallace DC. 1994. Mitochondrial DNA sequence variation in human evolution and disease. Proc. Natl Acad. Sci., 91: 8739-8746.

Wirnsberger GH, Pfragner R, Mauric A, Zach R, Bogiatzis A, Holzer H. 1998. Effect of antibiotic treatment with azithromycin on cyclosporine A-induced gingival hyperplasia among renal transplant recipients. Transplant. Proc., 30(5): 2117-2129.

Wolf A, Trendelenburg CF, Diez-Fernandez C, Prieto P, Houy S, Trommer WE, Cordier A. 1997. Cyclosporine A-induced oxidative stress in rat hepatocytes. J. Pharmacol. Exp. Ther., 280(3): 1328-1334.

Wongmekiat O, Leelarugrayub N, Thamprasert K. 2008. Beneficial effect of shallot (*Allium ascalonicum* L.) extract on cyclosporine nephrotoxicity in rats. Food Chem. Toxicol., 46(5): 1844–1850.

Zwanenburg TS, Cordier A. 1994. No cyclosporine-induced chromosomal aberrations in human peripheral blood lymphocytes in vitro. Mutat Res., 320(3): 217-221.

دراسة وراثية خلوية وجزيئية لتفاعل السيكلوسبورين والازيثروميسين مع اختبار حماية التاثي بواسطة الثوم خلال استخدام هذه االادوية

أماني عبد المنعم تهامي*، وردة على خليفة*، منال فوزي الخضرجي*، عادل خليل إبراهيم**

* قسم علم الحيوان، كلية العلوم، جامعة حلوان

** قسم الباثولوجيا الإكلينيكية، كلية الطب البيطري، جامعة القاهرة

يهدف هذا البحث إلى دراسة تأثير كلا من السيكلوسبورين (أ) والازيثروميسين وكذلك التفاعل بينهما ودراسة الخصائص الوقائية للثوم ضد كل دواء منهما على حدا وعلي التفاعل الدوائي لهما معا. خطة هذه الدراسة تناولت التغير في الكروموسومات وتعبير جين عامل نخر الورم ألفا والتغيرات البيوكيمائية في مختلف وظائف الأعضاء لذكور الجرذان خلال فترة تناول هذه الأدوية منفردة أو مع بعضها عن طريق الفم لمدة يوم وسبعة أيام. هذه الأدوية تم إعطائها بجرعة تساوي25مجم/كجم للسيكلوسبورين و 500 ملجم/ كجم ازيثروميسين و300 ملجم/ كجم للثوم لمدة يوم وسبعة أيام. أظهرت النتائج الوراثية الخلوية وجود تغيرات كروموسومية تركيبية على هيئة انفصال للسنترومیرات وكروموسومات دائرية وكذلك تغيرات كروموسومية عددية في شكل تعدد الصيغة الصبغية وتضاعف في عدد الكروموسومات بعد سبعة أيام من إعطاء السيكلوسبورين منفردا والسيكلوسبورين + الثوم ، والسكلوسبورين + الازيثروميسين والسيكلوسبورين + الازيثروميسين + الثوم . بينما لم تظهر إي تغيرات كروموسومية تركيبية أوعددية بعد سبعة أيام من إعطاء الازيثروميسين و الازيثروميسين + الثوم . بالإضافة إلى ذلك لم يتم ملاحظة أي تغيرات ملحوظة كروموسومية تركيبية أوعددية بعد يوم واحد من إعطاء الأدوية. عندما تم قياس قيم المتوسط لتعبير جين عامل نخر الورم آلفا بواسطة تقنية (RT-PCR) تبين أن التعبير الجيني لعامل نخر الورم آلفا في المجموعات الضابطة كان أكثر تعبيراً من المجموعة التي أستقبلت الازيثروميسين و المجموعة التي استقبلت السيكلوسبورين + الازيثروميسين بينما مجموعة السيكلوسبورين كانت أقل مجموعة في تعبير جين عامل نخر الورم . أظهرت النتائج البيوكيمائية وجود تغيرات ملحوظة في مستوى AST ، ALT ،GGT، LDH ، BUN، ALP، والجلوكوز بعد تناول السيكلوسبورين . من خلال هذه الدراسة استنتجنا أن السيكلوسبورين كان له تأثير سمي وراثي علي الكروموسومات وعامل نخر الورم ألفا ، أخذ الازيثروميسين منفرداً أو مع السيكلوسبورين أمن إلي حد ما لأن التفاعل بينهما أقل ما يكون بالإضافة إلى ذلك وجد أن الثوم من المحتمل أن يحسن التأثير الخطير للسيكلوسبورين .

المحكمون:

أ.د.

أ.د.