

Hepatotoxic effect of chronic exposure of Tacrolimus in male Albino ratsAli, I. Al-Ameedi¹, Falah, M. K. Al-Rekabi¹ and Ahmed, Sh. J. Al-Rikabi²¹Department of Physiology and Pharmacology, ²Department of Anatomy, Histology and Embriology, College of Veterinary Medicine, Baghdad University, Iraq.E-mail: ali.alameedy89@yahoo.com

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Summary

This study was carried out to investigate the hepatotoxicity of chronic administration of an immunosuppressant Tacrolimus in male albino rats. Thirty male albino rats were randomly divided into 3 equal groups, the first one (T1) received therapeutic dose (70 µg/kg.BW) of Tacrolimus. The second group (T2) received double dose (140 µg/kg.BW) of Tacrolimus, while the third group received distilled water for the same period and considered as control (C). Alanine aminotransferase, Aspartate aminotransferases, Alkaline Phosphatase, serum potassium and serum calcium showed significant increase in both treated groups T1 and T2 as compared with control, proportional to the dose of Tacrolimus. There were histopathological changes seen in liver at the end of exposure manifested by necrosis, hemorrhage, hyperplasia and fibrosis. It can be concluded that Tacrolimus has hepatotoxic effects when used for a long period

Keywords: Hepatotoxic, Chronic exposure, Tacrolimus, Albino rat.**Introduction**

Tacrolimus is a macrolide immunosuppressant obtained from *Streptomyces tsukubaensis* (1). It inhibits T-lymphocyte activation, the exact mechanism of action is not known (2). Tacrolimus binds to an intracellular protein FKBP-12, forming a complex with calcium, calmodulin and calcineurin which inhibits phosphorylation activity (3). This prevents the effect of dephosphorylation of nuclear factor of T-lymphocytes which is necessary to initiate gene transcription of interleukin IL-2 synthesis. Thus the net outcome inhibits T-lymphocyte activation (2). Tacrolimus uses include prophylaxis of transplant rejection in liver, kidney or heart allograft recipients. (0.15-0.20 mg/kg.BW) orally (4), treatment of Eczema and dermatitis in concentration (0.1%-0.3%) topically (5). Also in treatment of many autoimmune diseases (6). Tacrolimus is primarily metabolized by enzymes of the cytochrome P-450 3A subfamily (7). The main reactions tacrolimus undergoes during metabolism are demethylation and (or) hydroxylation (8). Tacrolimus is found producing cholelithiasis in dogs and rats (9 and 10) and cholestasis in rats at 3-5 mg/kg.BW (11). Tacrolimus study showed hepatotoxicity and cholestatic jaundice due to the active metabolite that produces (12). This study is conducted to evaluate the chronic exposure of

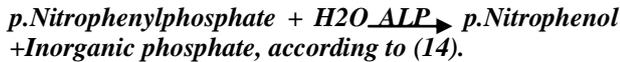
available pharmaceutical in our of tacrolimus (cprgraf)^R.

Materials and Methods

The study includes thirty male albino rats (aged 6-8 weeks and weight 250-300g), supplied from the animal house of the College of Veterinary Medicine, Baghdad University. They were housed and maintained in a conventional animal facility, with controlled conditions of temperature (20 ± 5°C). Standard pellet and diet were provided *ad libitum*. The animals were randomly divided into 3 groups of ten animals each following: 1st group (T1) received by gavage needle orally with (70 µg/kg B.W) of Tacrolimus daily for 90 days as therapeutic dose. 2nd group (T2) received Tacrolimus orally with (140 µg/kg.BW) as two fold dose. 3rd group (C) was received drinking water orally. Tacrolimus has been dosed orally by gastric gavage daily for 90 days with overnight fasting. This work was carried at approval of College of Veterinary Medicine of Baghdad University in accordance with international ethical standards of research of work with laboratory animals. Alanine aminotransferase, Aspartate aminotransferases and Alkaline Phosphatase were measured manually by using kits according to (13). Dependent on formation 2,4-Dinitro-Phenyl hydrazine (NAPH) to give colored hydrazones, while oxaloacetate produced by AST decarboxylates

spontaneously to pyruvate. While ALP activity assessed by IFCC recommendations, upon formation:

0.05 level in using statistical package for social sciences (SPSS), Version 10.



Results and Discussion

Liver was obtained at the end of exposure and fixed in 10% formosaline. Paraffin sections of thickness of 3-4 μm were prepared and stained with hematoxylin and eosin (H and E) for histopathological examination under light microscopy. Statistical analysis was applied by two ways ANOVA with LSD test and the mean difference is significant at the

The results of liver injury enzymes (U/L) revealed a significant increase (P<0.05) in serum activity of (ALP, ALT and AST) of both T1 that received therapeutic dose and T2 which received double dose of Tacrolimus groups compared with the control one which dosed distilled water (Table, 1) the animals of both treated groups showed increase in serum activity of all liver injury enzymes in a dose dependent manner.

Table, 1: The effect of different doses of Tacrolimus given orally for 90 days on liver injury enzymes (ALP, ALT and AST) in mal albino rats.

Parameter	Alkaline phosphatase (U/L)		Alanine aminotransferase (U/L)		Aspartate aminotransferase (U/L)	
	ALP		ALT		AST	
	Zero time M ± S.E	90 days M ± S.E	Zero time M ± S.E	90 days M ± S.E	Zero time M ± S.E	90 days M ± S.E
C N=10	81.40± 5.10 a	90.20±3.42 C a	42.00±2.70 B b	51.60±1.70 C a	46.00±1.20 B a	51.60±1.60 C a
T1 N=10	75.00± 2.10 b	245.60±16.75 B a	43.60±2.37 B b	86.4±2.60 B a	54.80±2.10 A b	103.80±2.90 B a
T2 N=10	89.80±2.80 b	417.00±18.30 A a	54.20±2.03 A b	108.00 ±2.74 A a	56.20±2.20 A b	184.00±7.80 A a

L.S.D. Of ALP =23.4, L.S.D of ALT = 6.8, L.S.D of AST = 8.7 *Capital letters denote significant differences (P<0.05) between groups. *Small letters denote significant differences (P<0.05) within groups.

The liver has a variety of transaminases to synthesize and break down amino acids and to interconvert energy storage molecules. The concentrations of these enzymes in the serum are normally low. However, enzymes leak out into the blood stream (15). The elevation of the normal values due to the body's inability to excrete it through bile due to the congestion or obstruction of the biliary tract as confirmed by histopathological lesion noticed in Tacrolimus dosed rat liver which included vacuolar degeneration, slight fibrosis of the portal areas, hyperplastic nodules and extensive areas of parenchymatous hepatic necrosis and hemorrhage (Fig. 1, 2, 3 and 4), the severity is dose dependent. Katrin (16) reported impaired liver function, as a result of increase concentrations of Tacrolimus and its active metabolites indicating accumulation of metabolites, in particular second-generation metabolites such as didemethyl and didemethylhydroxy Tacrolimus (16). The

common adverse effect of using Tacrolimus is elevation of ALP (17). ALT is not commonly found outside the liver. AST too is most commonly found in the liver, but also in significant amounts in heart and skeletal muscle. In fact, AST is another liver enzyme that aids in producing proteins. It used as a part of diagnosing heart attacks (18). ALT plays an important role in amino acid metabolism and gluconeogenesis (19). The chronic exposure of Tacrolimus lead to accumulation of Tacrolimus and its metabolites in the hepatic tissue then impaired normal function of liver and elevation of liver enzyme activity in serum (16). The elevated AST and ALT may indicate inflammation or damaged cells in the liver. Inflamed or injured liver cells leak higher than normal amounts of certain chemicals, including liver enzymes, into the bloodstream, which can result in elevated liver enzymes on blood tests (20). This toxic effect was confirmed by the histopathological

changes included vacuolar degeneration, slight fibrosis of the portal areas, hyperplastic nodules and extensive areas of parenchymatous hepatic necrosis and hemorrhage (Fig. 1, 2, 3 and 4), the changes of liver injury enzyme in our study are in agreement with (21 and 22) who recorded an increase in ALT, AST levels in treated allograft patient with therapeutic dose of Tacrolimus within 90 days comparing with (0 and 45) days of transplantation. Also another researcher recorded increase in AST, ALP and ALT in transplantation of liver in dog treated with therapeutic dose of Tacrolimus (23). The result revealed significant increase ($P<0.05$) in serum calcium and potassium of both T1 and T2 groups compared with the control one. The increasing of serum calcium and potassium were significantly ($P<0.05$) and proportional to the dose of Tacrolimus also there are significant increase ($P<0.05$) of 90 days of treatment with Tacrolimus comparing with pretreatment (Table, 2).

Table, 2: The effect of different doses of Tacrolimus given orally for 90 days on serum calcium and potassium in male albino rats.

Parameter	Calcium in serum mg/dl		potassium in serum mmol/l	
	Zero time	90 days	Zero time	90 days
	M ± S.E	M ± S.E	M ± S.E	M ± S.E
Groups				
C	10.56 ± 0.20	10.40 ± 0.30	1.80 ± 0.15	2.20 ± 0.20
N=10	a	C a	B a	C a
T1	10.50 ± 0.20	15.03 ± 0.40	2.20 ± 0.10	4.20 ± 0.30
N=10	b	B a	A b	B a
T2	10.40 ± 0.20	21.40 ± 0.90	1.50 ± 0.10	4.80 ± 2.10
N=10	b	A a	B b	A a

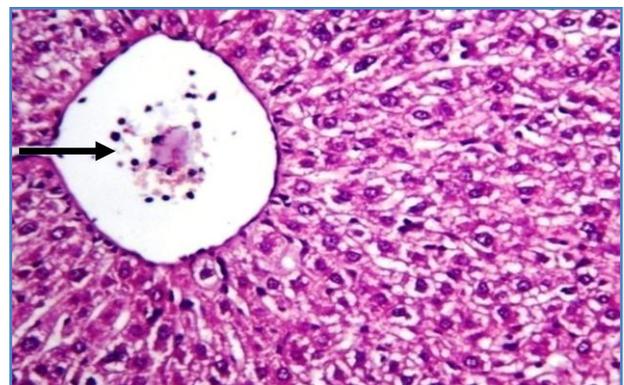
L.S.D of calcium= 1.2, L.S.D of potassium = 0.5

*Capital letters denote significant differences ($P<0.05$) between groups. *Small letters denote significant differences ($P<0.05$) within groups.

Calcium exists in 3 major forms in plasma, approximately 50% is in the free or ionized form, which is the physiologically important fraction, 40% is bound to albumin, and the remaining 10% is in soluble complexes with anions such as bicarbonate, phosphate, and lactate (24). Three sites of soft tissue calcification occur with hypercalcemia even in the absence of serum phosphate elevations. These are corneal and conjunctival calcification, chondrocalcinosis, bone and renal calcification (25). This increased in

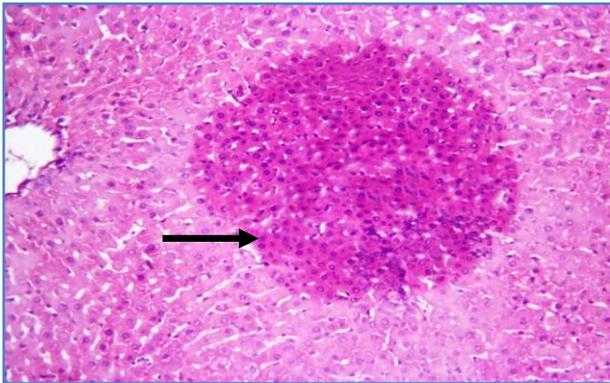
serum calcium is in agreement with Aydin Unal (26) who reported an increase in serum calcium concentration in Turkish renal transplant patients especially those treated with Tacrolimus also increase in glucose in the shorter post-transplant duration with risk factors for post-transplant hypomagnesemia.

An elevation in plasma potassium concentration may be due to the reduced efficiency of urinary potassium excretion is common in Tacrolimus treated patients, and can be caused by reduced renal excretion, In addition to acute and chronic renal failure, hypoaldosteronism, and massive tissue breakdown as in rhabdomyolysis (27). In a study of eight renal transplant patients with therapeutic doses founded that mean potassium level was higher on Tacrolimus than on cyclosporin under treatment with each drug (28). Another study recorded more toward hyperkalemia with Tacrolimus was noted in a study of bone marrow transplant patients (29). Tacrolimus may cause hyperkalemia by multiple mechanisms affecting potassium in the distal tubule (30). This toxic effect was confirmed by the histopathological changes included vacuolar degeneration, slight fibrosis of the portal areas, hyperplastic nodules and extensive areas of parenchymatous hepatic necrosis and hemorrhage (Fig. 1, 2, 3 and 4). Tissue microscopic sections of rats received therapeutic dose of Tacrolimus showed severe dilation and congestion of central veins and sinusoids. The hepatocytes undergo vacuolar degeneration with infiltration of inflammatory cells in the lumina of central veins (Fig. 1).

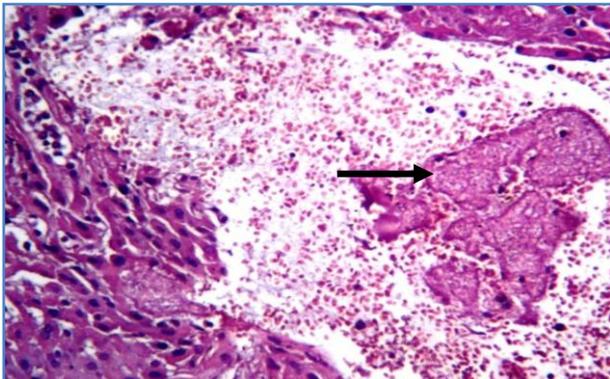


Figure, 1: Histopathological section of liver of therapeutic dose group showed dilation of central vein contain inflammatory cells with vacuola degeneration of hepatocyte (H and E 40 x).

Furthermore many areas showed slight fibrosis of the portal areas. The hepatic parenchyma showed formation of hyperplastic nodules with hepatocytes has no arrangement and lacking the central veins, these nodules cause pressure atrophy to the adjacent hepatocytes (Fig. 2). While the liver of double dose group (T2) animals was showed extensive areas of parenchymatous hepatic necrosis and hemorrhage (Fig. 3).



Figure, 2: Liver of therapeutic dose group showed formation of hyperplastic nodule cause pressure atrophy to the adjacent hepatic parenchyma (H and E 40 x).



Figure, 3: Liver of double dose group showed extensive areas of necrosis and hemorrhage (H and E40 x).

Subcapsular and parenchymatous hemorrhage were also seen in other sections (Fig. 4). Moderate fibrosis of the portal area with slight infiltration of mononuclear cells and vacuolization of hepatocytes were also seen. The severity of pathological lesions in the liver, increasing within dose depending. The main route of Tacrolimus metabolism by CYP3A4 and CYP3A5 in the liver leading to produced several metabolites The main reactions Tacrolimus undergoes during metabolism are demethylation and (or) hydroxylation (8).

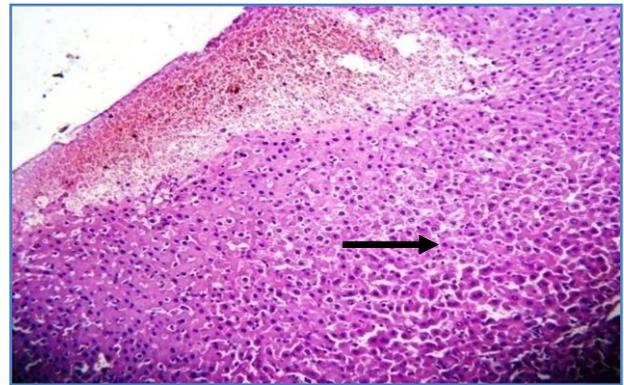


Fig. 4: Liver of double dose group showed subcapsular and parenchymatous hemorrhage.

The toxic effect of Tacrolimus that appear in histopathological sections in liver may be due to the accumulations of active metabolites of Tacrolimus for long time as a result of repeated exposure for 90 days, as well as the long half live of drug (36 hrs.). Hyperplasia is multi proliferations of cells its considered precancerous lesions (31). The results indicated that the free radical that generated from the inhibition of the nitric oxide synthetas by Tacrolimus (32). May be contribute with previous causes in formation of hepatic lesions.

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التأثيرات السمية الكبدية للتعرض المزمن لعقار التاكروليمس في ذكور الجرذان المهقاة

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الخلاصة

أجريت هذه الدراسة لتقييم السمية الكبدية بعد التعرض المزمن للمثبط المناعي عقار التاكروليمس في ثلاثين من ذكور الجرذان المهقاة من خلال قياس انزيمات أذى الكبد (الانين امينوترانسفيريز والاسبارتات امينوترانسفيريز والالكلالين فوسفاتيز) وبعض الكهارل (الكالسيوم والبوتاسيوم) والتغيرات النسجية المرضية للكبد. قُسمت الجرذان على ثلاث مجاميع متساوية حيث جرعت فمويًا بالعقار المذكور، المجموعة الأولى جرعت بجرعة علاجية من العقار 70 مايكروغرام لكل كيلوغرام من وزن الجسم في حين جرعت المجموعة الثانية بجرعة مضاعفة 140 مايكروغرام لكل كيلوغرام من وزن الجسم، أما المجموعة الثالثة فجرعت بالماء المقطر ولنفس الفترة (90 يوم) كمجموعة سيطرة. أظهرت النتائج زيادة معنوية في نشاط انزيمات الأذى الكبدية (الانين امينوترانسفيريز والاسبارتات امينوترانسفيريز والالكلالين فوسفاتيز) وكذلك مستوى الكالسيوم والبوتاسيوم في مصل الدم لكل من مجموعتي المعالجة بالعقار مقارنة بمجموعة السيطرة حيث تناسبت الزيادة طرديًا مع جرعة العقار، كما أظهرت نتائج الفحص النسجي المرضي تغيرات واضحة في نسيج الكبد للمجاميع المعالجة بالعقار تمثلت بفرط التنسج، تموت، ضمور ونزف مقارنة بمجموعة السيطرة.

الكلمات المفتاحية: السمية الكبدية، التعرض المزمن، التاكروليمس، الجرذان المهقاة.