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EFFECT OF VITAMIN *A* ON CYCLOSPORINE NEPHROTOXICITY IN UNINEPHRECTOMIZED RATS

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ABSTRACT

The influence of vitamin A (1.5 mg kg^{-1} body weight) administration for two weeks on cyclosporine A (CsA, 25 mg kg^{-1} body weight) induced renal dysfunction has been investigated in uninephrectomized and sham nephrectomized rats. Single kidney weight was significantly increased after uninephrectomy and CsA treatment. In addition CsA induced degenerative changes in renal tubule in uninephrectomized rats. The drug also significantly increased, serum total proteins, creatinine and plasma renin activity as well as urinary excretion of N-acetyl-β-D-glucosaminidase and 6-keto prostaglandin $F_{1\alpha}$. On the other hand, urine flow rate and creatinine clearance have been significantly reduced by CsA. Meanwhile no-significant change was observed on urinary prostaglandin E_2. Administration of vitamin A in combination with CsA reduced all abnormalities induced by CsA on Kidney structure and functions. In conclusion vitamin A could be considered as an attenuating factor for CsA induced nephrotoxicity.

INTRODUCTION

Cyclosporine A (CsA), a fungal cyclic polypeptide, has been shown to suppress both humoral and cell mediated immunity by affecting early steps of T-cell activation[1,2]. It is one of the most extensively used immunosuppressive drugs in organ transplantation[3,4]. CsA has also been used in treatment of various apparently immune-mediated diseases such as diabetes mellitus, multiple sclerosis and others, with a varying degree of success[5-7].

One major problem experienced following CsA treatment in humans and animals is the drug induced nephrotoxicity[8-10].

Early studies[2,11] showed that, fish oil containing high concentrations of polyunsaturated fatty acids, reduces CsA-nephrotoxicity in rats and since fish oil is very rich in vitamin A. The aim of the pesent study was to investigate the effects of CsA alone and in combination with vitamin A on some kidney functions and histological changes in uninephrectomized and sham-nephrectomized rats.

MATERIAL AND METHODS

Animals and drugs:

Six groups of age-matched male albino rats weighing 180 ± 20 g were maintained on purina rat chow and tap water ad libitum. CsA, (Sandoz Pharmaceuticals. New Jersey, USA) was prepared by dissolving in olive oil to give a solution containing 5 to 10 mg ml^{-1} of CsA. Vitamin A alcohol, was obtained from (Servo, Gmbh, Germany) and diluted with olive oil.

Uninephrectomy (UN):

Under ether anesthesia a right subcostal incision was made, the right kidney was quickly isolated, the vascular pedicle clamped, and a 3.0 silk ligature tied proximal to the clamp. The kidney was then removed with care to leave the adrenal gland intact. Absorbable chromic suture was used to close wound.

Sham nephrectomy (SN):

This was performed in a similar manner except that the kidney was simply manipulated and left intact.

Work design:

Two weeks after the surgery both UN and SN rats were divided into three groups, each consisted of 8 animals. The first group was assigned to CsA treatment in a daily IP dose of 25 mg kg^{-1} body weight, for two weeks. The second group received combined doses of CsA (25 mg kg^{-1} body weight, IP) and vitamin A (1.5 mg kg^{-1} body weight, IM) for two weeks. The third group of UN and SN rats was treated with olive oil vechicle for the same duration of study period and served as control.

At the end of the experiment urine was collected using metabolic cages over a 24 hours period and fasting samples were withdrawn from retrobular venous plexus for serum and plasma determinations. Animals were anesthetized with urethane (2 mg kg^{-1} body weight, IP) and sacrificed by cervical dislocation. Left kidney was bisected sagitally and accurately weighed.

Histology:

Coronal sections of the kidneys were fixed in 4% buffered formaldehyde, embedded in paraffin, cut at 3-5 um and stained with hematoxylin and eosin (H & E).

Biochemical determinations:

Analytical procedures were used for determinations of serum total proteins[12], creatinine[13] and blood urea nitrogen[14] (BUN). Plasma renin activity (PRA) was determined by radioimmunoassay[15]. Analysis of urine sample including the activity of lysosomal marker, N-acetyl-β-D-glucosaminidase (NAG)[16], prostaglandin E_2 (PGE_2) and 6-keto-prostaglandin

$F_{1\alpha}$[17](6-keto-$PGF_{1\alpha}$). Creatinine clearance was calculated in the usual manner.

Statistical analysis was performed by Student's t-test at 0.05 probability.

RESULTS

As shown in Table 1, left kidney weight was markedly increased in both UN control and CsA treated rats.

Histological studies revealed that, CsA induced nephrotoxicity in UN rats in the form of degenerative changes in the tubule associated with diffuse and focal lymphophatic infiltration when compared with control **(Figs. 1, 2)**. Kidney sections of UN rats receiving vitamin A in combination with CsA showed less nephrotoxicity in the form of mild tubular degeneration **(Fig. 3)**.

Table 1: Kidney weights of uninephrectomized, and sham nephrectonized rats treated with CsA (25 mg kg^{-1} BW.) or CsA (25 mg kg^{-1} BW.) plus vitamin A (1.5 mg kg^{-1} BW.) for two weeks.

Group	Kidney weight (g)	
	Right (R)	Left (L)
UN:		
Control	1.41 ± 0.04	1.70* ± 0.09
CsA	1.45 ± 0.03	1.70* ± 0.04
CsA + vitamin A	1.55 ± 0.08	1.71 ± 0.07
SN:		
Control	1.34 ± 0.10	1.40 ± 0.04
CsA	1.40 ± 0.07	1.32 ± 0.06
CsA + vitamin A	1.50 ± 0.03	1.43 ± 0.021

Values expressed as mean of eight observations ± SEM
Right kidneys removed at start of protocol and left kidneys removed after two weeks of treatment.
* P<0.05 left kidney vs right kidney.

Administration of CsA induced a significant elevation in serum total proteins, creatinine and PRA in UN and SN rats (Table 2 & Fig. 5). No significant effect was observed on BUN (Table 2).

Regarding urine analysis, CsA was significantly reduced creatinine clearance and urine flow rate (Table 3). On the other hand, urinary excretion of NAG and 6-keto $PGF_{1\alpha}$ were significantly increased (Figs 4,6). No significant changes were observed on urea and urinary PGE_2 excretion (Table 3 & Fig. 7).

Treatment of UN and SN rats with CsA in combination with vitamin A did not induce any significant changes in all parameters under study except, a significant increase in urinary excretion of NAG (Fig. 4).

DISCUSSION

CsA is well known to induce vasospasm of renal microcirculation[9,18]. This in turn leads to ischemia of tubulointersitial compartment with resultant tubule atrophy and reduction of glomerular filtration rate (GFR)[19]. The evidence of compensatory renal hypertrophy observed in our results in both control UN and UN rats treated with CsA, points out that the vasodilatory influence which depends upon an increase in renal plasma flow (RPF) and GFR[18,20] must partially overcome CsA induced vasospasm and hypofiltration.

The present study demonstrates that, accompanying the compensatory hypertrophy of single kidneys in uninephrectomized CsA treated rats there is a degenerative change in the tubule. These findings are in accordance with previous studies[19,21,22]. Since it has been reported that the damage to the straight segment of the proximal tubule is the most pronounced observation, while no structural changes are present in the remainder of the nephron, including the glomerulus[23], the structural damage to proximal tubule may result in an impairment of tubular functions.

Our data also indicated that administration of CsA to rats resulted in a statistically significant increase in serum total proteins and creatinine levels. By contrast creatinine clearance and urine flow rate were significantly decreased. The findings confirm that CsA induce renal impairment, as already described in humans and animals studies[8-10].

From another point of view, urinary enzymes have been used as sensitive markers of specific tubular damage in both animals[24] and man[25]. Regarding our data, NAG, a microsomal tubular enzyme showed a significant increase after CsA treatment in both UN and SN rats. These results are in agreement with those obtained by Whiting et al.[26] who reported that, CsA induced a structural renal damage in rats proximal tubule and was accompanied by increased urine level of NAG. Moreover, increases appear before either functional or structural deterioration is present. The increased NAG enzymuria may, therefore, be related to increased lysosomal / outophagic activity within the tubular cell.

Our data showed that plasma renin activity was increased in rats given CsA . This finding is in agreement with other investigators[27,28]. The mechanism by which CsA stimulates renin is still unclear. It has been reported that CsA directly stimulates intrarenal release of renin[28] whereas the data of Murray et al[29] suggested that renin release in CsA treated animals is mediated by a stimulation of sympathetic nervous system via the renal nerve.

Short term studies in the rat suggest that activation of the vasopressor renin-angiotensin system by CsA play an initiating role in the genesis of CsA associated nephropathy. In addition, it increases the availability of angiotensin II which mediates the renal vasoconstriction and leads to reduction in the GFR[29].

Table 2: Serum total proteins, creatinine (Cr) and blood urea nitrogen (BUN) in uninephrectomized, UN and sham nephrectomized, SN rats treated with CsA (25 mg kg^{-1} BW.) or CsA (25 mg kg^{-1} BW.) plus vitamin A (1.5 mg kg^{-1} BW.) for two weeks.

Groups	Total proteins ($g\ dL^{-1}$)	Cr ($mg\ dL^{-1}$)	BUN ($g\ dL^{-1}$)
UN:			
Control	5.03 ± 0.48	0.75 ± 0.06	27.11 ± 3.81
CsA	6.84* ± 0.55	0.94* ± 0.05	33.34 ± 5.00
CsA + vitamin A	5.70 ± 0.43	0.82 ± 0.09	31.51 ± 4.07
SN:			
Control	4.75 ± 0.32	0.62 ± 0.051	24.82 ± 3.59
CsA	6.28* ± 0.62	0.83* ± 0.07	31.29 ± 2.81
CsA + vitamin A	5.19 ± 0.51	0.65 ± 0.05	28.90 ± 2.47

Values expressed as mean of eight observations ± SEM
* $P < 0.05$ groups Vs their respective control.

Table 3 : Creatinine clearance (Cr_r), urine flow rate (UFR) and urea excretion in uninephrectomized, UN and sham nephrectomized, SN rats treated with CsA (25 mg kg^{-1} body weight) or CsA (25 mg kg^{-1} body weight) plus vitamin A (1.5 mg kg^{-1} body weight) for two weeks.

Groups	Cr_r ($ml\ min^{-1}$)	UFR ($ml\ day^{-1}$)	Urea excretion ($m\ mol\ day^{-1}$)
UN:			
Control	0.93 ± 0.07	9.54 ± 1.13	8.70 ± 0.89
CsA	0.72* ± 0.05	6.63* ± 0.51	6.85 ± 0.46
CsA + vitamin A	0.86 ± 0.071	8.00 ± 0.72	7.52 ± 0.63
SN:			
Control	0.88 ± 0.05	10.31 ± 1.01	7.41 ± 0.55
CsA	0.75* ± 0.03	7.40* ± 6.69	6.38 ± 0.70
CsA + vitamin A	0.80 ± 0.07	8.56 ± 1.00	6.50 ± 0.24

Values expressed as mean of eight observations ± SEM
* $P < 0.05$ groups Vs their respective control.

Administration of CsA to UN and SN rats resulted in a marked increased in urinary excretion of 6-keto-$PGF_{1\alpha}$ while no significant effect was observed in PGE_2 excretion. Increased urinary excretion of 6-keto-$PGF_{1\alpha}$ has been also seen in other conditions characterized by renal hypoperfusion and increased renin secretion,[29,30].

Since the glomerulus is a dynamic structure capable of regulating GFR by interaction between vasoconstrictor peptides, such as angiotensin II and the vasodilatory Pgs[31]. Changes in the renal arachidonate metabolism may affect the renal vascular resistance and impair the renal function. CsA has been reported to increase PGE_2 synthesis in human peripheral blood monocytes[32]. Moreover, previous studies have shown a decreased PGI_2 stimulating factor activity in plasma from rabbits treated with CsA[33]. Since it has been demonstrated that normal glomerular tissue synthesizes PGs[34]. Failure to detect any significant modification in the glomerular synthesis of PGE_2 in animals receiving CsA suggests that CsA does not alter the glomerular metabolism of vasodilatory PGs_2.

The presented results strongly suggest an attenuation of CsA induced renal dysfunction by administration of vitamin A to UN and SN rats. The mechanism by which vitamin A induced this attenuation is not explained. Elzinga et al.[2,11] in an experimental rat model showed that fish oil which is very rich vitamin A reduced CsA nephrotoxicity. In those studies, it was concluded that the beneficial effect of fish oil was mainly due to the intrarenal inhibition of thromboxane A_2 (TxA_2), a potent vasoconstrictor CsA increases the production of TxA_2 in animal model[2]. In particular, eicosapentaenoic acid appears to be able to successfully compete with arachidonic acid in the cyclo-oxygenase pathway, thus limiting the production of TxA_2[35]. Complete inhibition of TxA_2 production in rats with a thromboxane synthetase inhibitor[36] did not return GFR to control level, suggesting that other mechanisms are also involved in the loss of renal function induced by CsA.

The results of the present study strongly suggested that vitamin A can favourably modifyed CsA associated renal dysfunction. Further, studies are warranted both to validate and to explain the findings.

Fig (1) :Unilateral nephrectomy (Control + Vehicle). Section of the kidney shows unremarkable glomerular and tubular changes x 90.

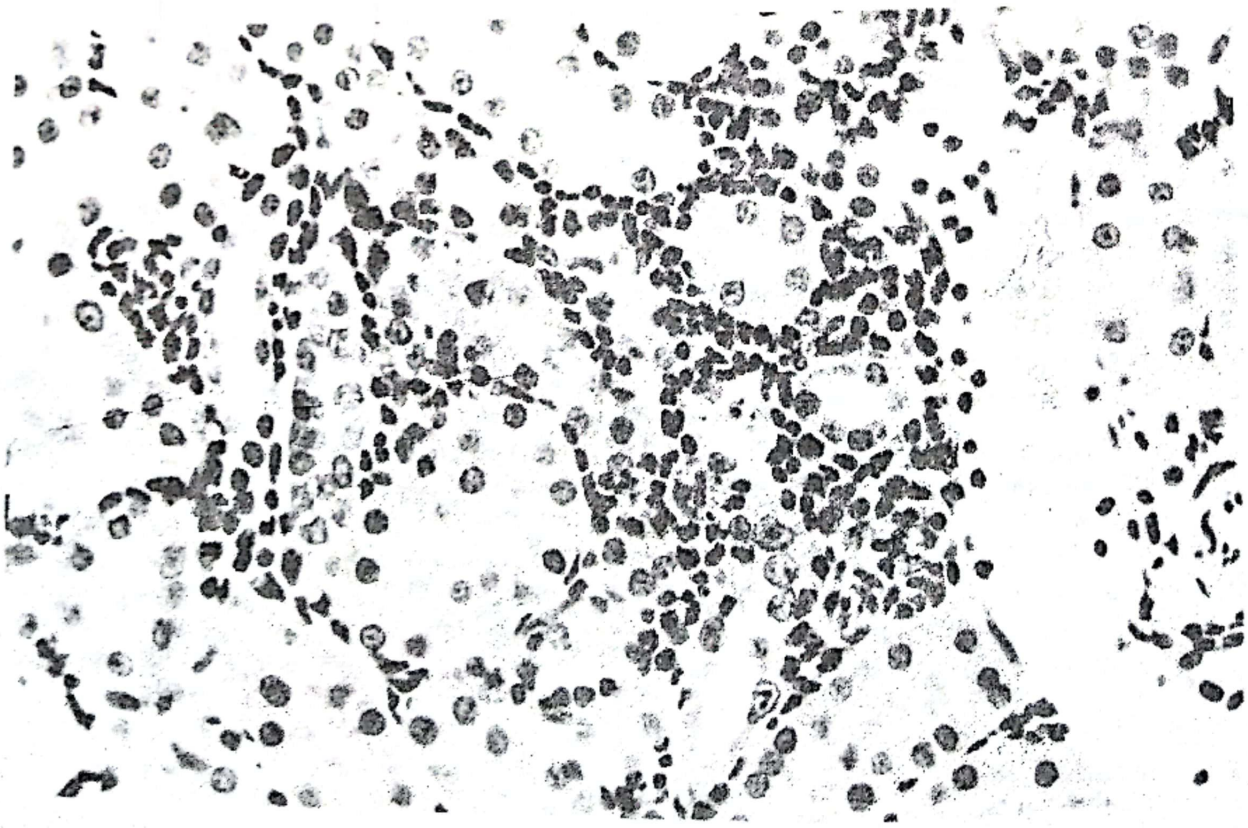

Fig (2) : Unilateral nephrectomy (Cyclosporine). Section shows peritubular and perivascular lymphmatic infiltrate x 100.

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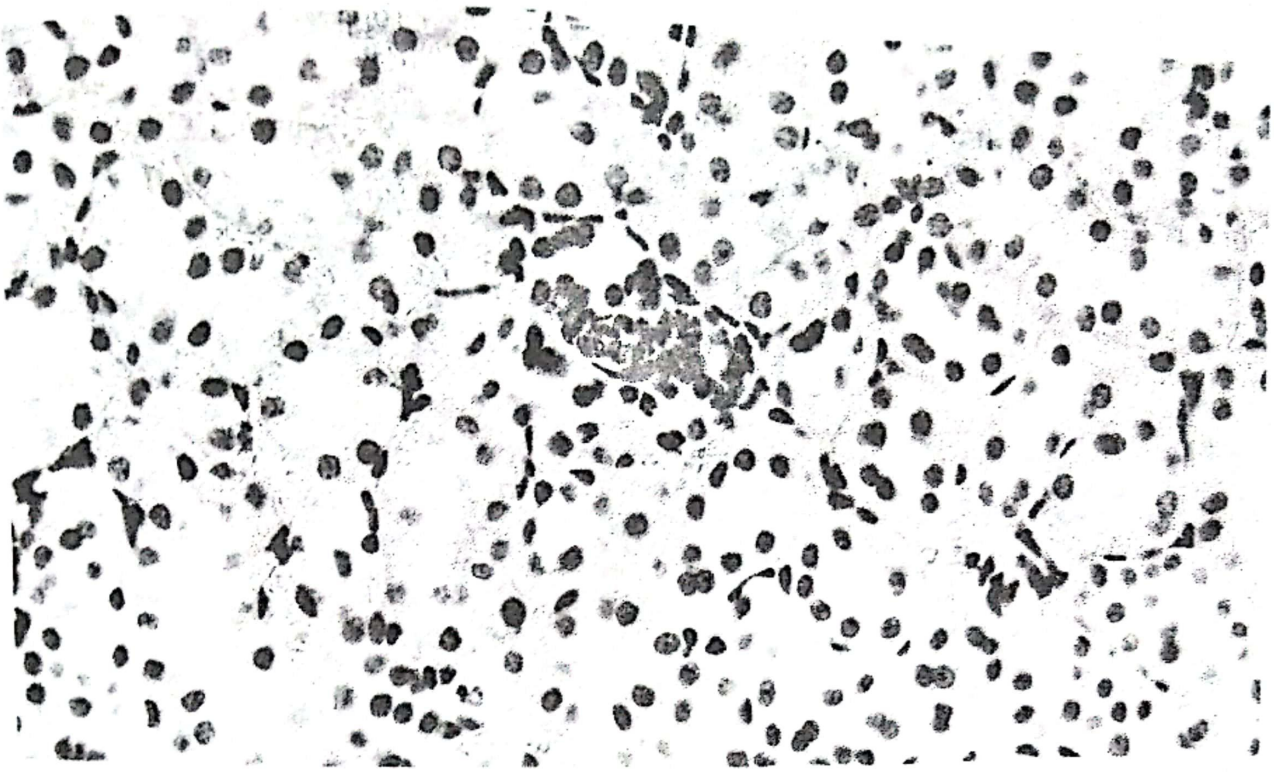

Fig (3) : Unilateral nephrectomy (Cyclsporine+ Vitamin A). Section shows focal lymphocytic collections between tubules x 110.

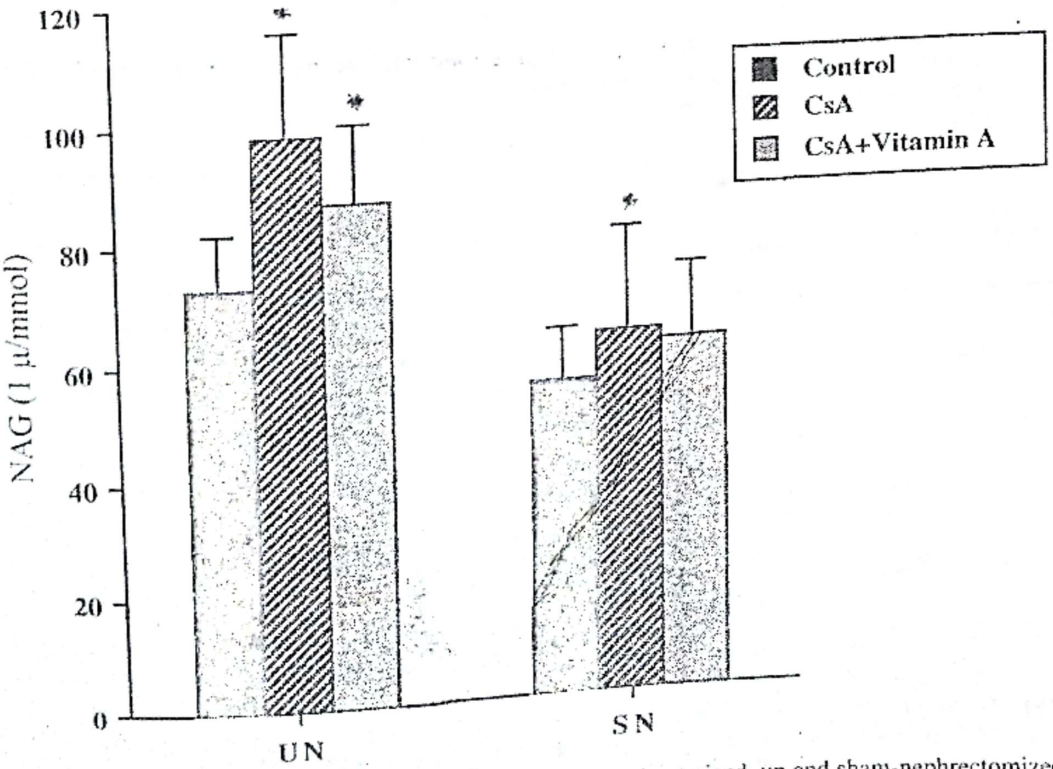

Fig (4) : Urinary N- acetyl-βD- glucosaminidase(NAG) in uninephrectmized, un and sham-nephrectomized SN ratstreated with CsA or CsA± vitamin A for two weeks Results are mean ±SEM (n=8) represents significant difference from control groups at P<0.05.

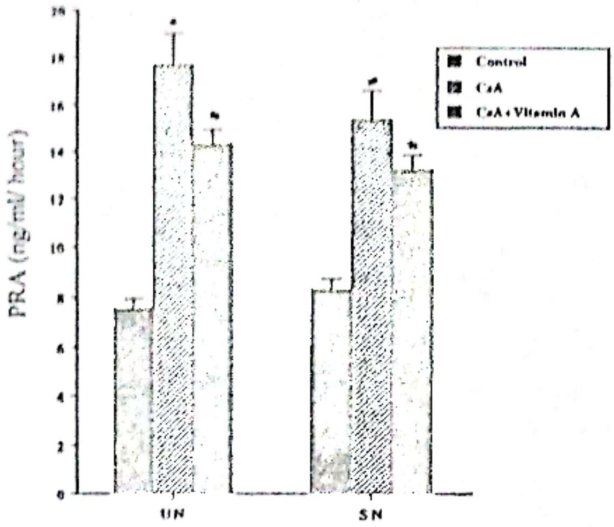

Fig (5) : Plasma renin activity (PRA) in uninephrectomized UN and sliam-nephrectomized SN rats treated with CsA or CsA ± vitamin A for two weeks Results are mean ± SEM (n=8).
* represents significant difference from control groups at P<0.05

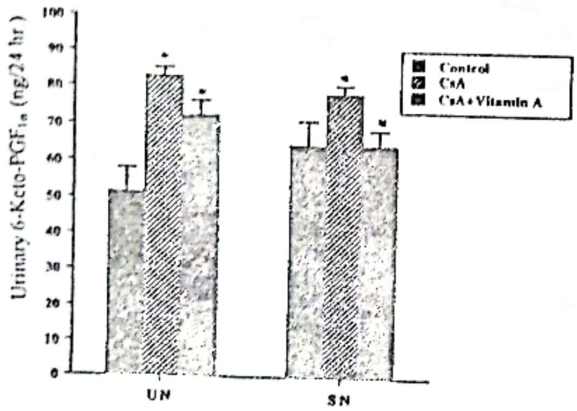

Fig (7) : Urinary prostaglandin E_2 (PGE_2) in uninephrectomized, Un; sham,- nephrectomized, SN rats treated with CsA or CsA + vitamin A for two weeks.
Results are mean ± SEM. (n=8)

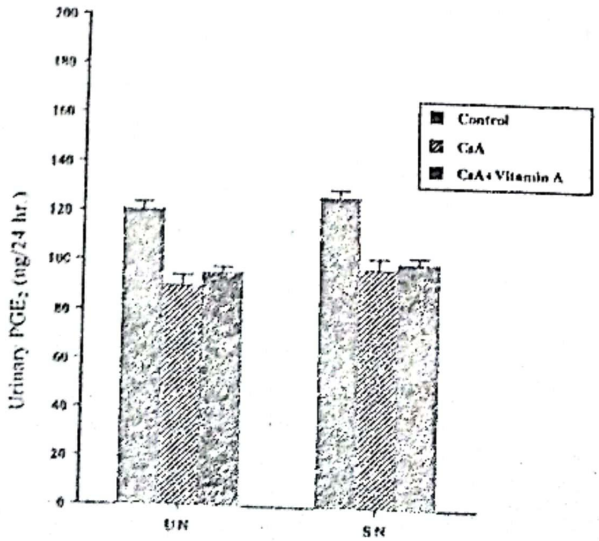

Fig (6) : Urinary 6-keto prostagland in $F_{1\alpha}$ in uninephrectomized, UN and sham-nephrectomized SN rats treated with CsA or CsA+ vitamin A for two weeks. Results are mean ± SEM. (n=8).
* represents significant difference from control groups at P<0.05

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تأثير فيتامين- أ على قدرة السيكلوسبورين على أحداث تأثير سمى فى الكلى فى فئران التجارب مستأصلة الكلية

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تم فى هذا البحث حقن كل من السيكلوسبورين (٢٥ مجم / كجم) وفيتامين- أ (١,٥ مجم/كجم) كلا على حده أو مجتمعين لمدة أسبوعين فى فئران التجارب مستأصلة الكلية أحدث استئصال الكلية اليمنى والعلاج بالسيكلوسبورين إلى إرتفاع ملحوظ فى وزن الكلية اليسرى للفئران مستأصلة الكلية، كذلك أدى حقن السيكلوسبورين إلى تكسير خلايا الكلية خصوصا فى الجزء الأنبوبى ورفع معدلات البروتين الكلى والكرياتنين فى مصل الدم وكذلك معدل الرنن فى البلازما. أحدث أيضا السيكلوسبورين زيادة فى نسبة أنزيم الجلوكوز امينيديز و ٦-كيتو بروستاجلا نذين F_1 فى البول وإنخفاض فى كمية البول ومقدار استخراج الكرياتنين. لم يحدث أى تأثير ملحوظ فى معدلات البولينا والبروستاجلاندين E_2. والخلاصة أن حقن الفئران بكل من فيتامين أ مع السيكلوسبورين أدى إلى خفض معدل التأثير السمى للسيكلوسبورين على وظائف ونسيج الكلية.