IOSR Journal Of Pharmacy www.iosrphr.org
(e)-ISSN: 2250-3013, (p)-ISSN: 2319-4219
Volume 8, Issue 9 Version. I (September 2018), PP. 57-68

The Preventive Effects of N-Acetylcysteine on Tacrolimus Induced Nephrotoxicity

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Abstract: Tacrolimus exhibits its nephrotoxic effect in relation to its inhibition of one of the antioxidant enzymes named catalase. Glutathione peroxidase catalysesa similar reaction done by catalase. The factor which increases GPx's activity is Glutathione levels.Subsequently, Glutathione levelsare increased by N-Acetyl cysteine (NAC). Thus, it seems NAC can influence the prevention of Tacrolimus mediated nephrotoxicity. In this study,Sprague-Dawley ratsreceived1 mg/kg/day dose of tacrolimus in the control group while the three treatment groupsreceived 1mg/kg/day of tacrolimuswith NAC in 100,200 and 300 mg/kg/day doses respectivelyfor 28 days. Upon study completion, the biochemical markers of tacrolimus nephrotoxicity (urinary microalbumin,serum creatinine and BUN) showed dramatic increase in both the control group and in the three treatment groups. Urinary microalbumin levels showed significant decrease with 300 mg/kg NAC dosing ($p<0.01$). Furthermore, serum creatinine levels showed significant decrease in all three doses ($p<0.05$) while BUN showed significant decrease in NAC 100 and 200 mg/kg doses ($p<0.01$) and 300 mg/kg dose ($p<0.001$). In the control group on tacrolimus alone, an increase in interstitial fibrosis and tubular atrophy- two important markers of chronic tacrolimus nephrotoxicity- was observedwhereas in contrastthe NAC treatment groups revealed a significant decrease in interstitial fibrosis ($p<0.05$ in NAC 200 and 300) and tubular atrophy ($p<0.05$ for NAC 200 and 300). These findings confirm the nephroprotective effects of N-acetylcysteine in chronic tacrolimus nephrotoxicity which acts in a dose dependent manner.

Key words: *N-acetylcysteine; Tacrolimus nephrotoxicity; Glutathione peroxidase; Catalase; BUN; Microalbuminuria*

Date of Submission: 02-10-2018 Date of acceptance: 18-10-2018

I. INTRODUCTION

The miracle of allograft transplantation in medical sciencesarose in the previous century and today became a successful therapeutic intervention through the usage of immunosuppressant drugs to preventthe transplanted tissues' rejection. The introduction of calcineurin inhibitors cyclosporine and tacrolimus heralded a grate revolution in transplantation and made it a preferable therapeutic method for some end stage diseases (1-2).

Nowadays, tacrolimus is a recognized immunosuppressive drug after solid organ transplantations and has both short-term and long-term advantages over conventional drugs including association with less frequent rejection, hypertension and hypercholesterolemia compared with cyclosporine (3). Also,in some cases with acute cellular rejection notresponding to cyclosporine, it has been used to rescue the allograft (4).The mechanism of action of tacrolimus and cyclosporine is through a Second Messenger Agent (calcineurin) inhibition of T-lymphocytes activation that plays an important role in inflammatory processes and tissue rejection. It's clear both drugs decrease organ rejection and increase survival rates in patients making them commonly used in clinic. However, both drugs exhibit nephrotoxic effects seen by increasing their

concentration in blood (5), therefore using these drugs in high concentrations is avoided. By decreasing their blood concentrations,their therapeutic effect would be decreased and in the case of transplant patients, mortality rates and organ rejection rateswouldthereafter be increased (6).These drug types are among the narrow therapeutic index range group of drugs. In accordance to these causes in patients using these drugs, takingblood samples and maintaining blood concentrations at optimum levels (tacrolimus 4-20 ng/ml – cyclosporine 100-300 µg/ml) is necessary (7). By comparing Tacrolimus with cyclosporine, the pharmacokinetic consistence and nephrotoxic effect of Tacrolimus is lower than Cyclosporine and correspondingly the use of Tacrolimus as an immunosuppressive agent in organ transplantation is more offered, but as mentioned before,its nephrotoxic effect is very significant. The nephrotoxic effect of Tacrolimus is through Catalase inhibition that is one of the antioxidant systems which converts hydrogen peroxide to water and oxygen(8). Catalase however is not the only way for hydrogen peroxide degradation (9).Glutathione peroxidase performs this reaction as well (8).Consequentially,what has beenobserved is the nephrotoxicity caused by Tacrolimus through Catalase inhibition being diminished through theincrease of Glutathione Peroxidase (GPx) activity. The factor increasing GPx's activity is Glutathione levels(8,9). Glutathionesubsequently is increased by N-Acetylcysteine (NAC) (10).The increasing effect of Glutathione by NAC is well known and according to this effect,NAC is being used in the treatment of acetaminophen toxicity (11).That is why it has been hypothesizedNAC administration can decrease the nephrotoxic effect of tacrolimus.

II. METHOD AND MATERIAL

Drugs

Tacrolimus (FK506) was kindly provided by Astellas Pharmaceutical Co., Ltd (Killorglin, Ireland) asProgaf ampules (5mg/ml) whileN-acetylcysteine was provided by Idol Pharmaceutical Co., Ltd (Istanbul, Turkey) as ampulesof 300mg/3ml.

Animals and experimental procedures

This study was performed at the Faculty of Medicine, Marmara University, Istanbul, Turkey. Healthy adult maleSprague Dawley rats (n=36) weighing between 250-350 g were obtained from the Animal Care and Research Centre of Marmara University. Before study initiation, ethical approval wasobtainedwith the serial number of 75.2010.mar from the ethics committee of Marmara University, Faculty of Medicine. The rats were kept in standard rat cages, under standard environmental conditions and were fed with normal granulated food and had free access to water. They were divided in five groups as follows; the control group (only received 1 ml/day saline 0.9% NaCl by SC injection, n=8), TAC group (injected with 1mg/kg/day tacrolimus SC, n=8), NAC-100 (injected with 1mg/kg/day tacrolimus + 100 mg/kg/day NAC SC, n=8), NAC-200 group (injected with 1mg/kg/day tacrolimus + 200 mg/kg/day NAC SC, n=8) and NAC-300 group (injected with 1mg/kg/day tacrolimus + 300 mg/kg/day NAC SC, n=8) for 28 days. Rats were monitored daily for weight gain. In the 28th day of experiment after the last injection, rats were taken to metabolic cages to collect the urinary samples and they were deprived from food but not water for 16 hours before sacrifice. In the end stage of research, rats were sacrificed by decapitation under urethaneanaesthesia (1.2g/kg, IP).Blood samples were collected by cardiac puncture and their kidneys were obtained by bilateral nephrectomy.Blood serum was isolated by centrifuge and were maintained at -20 °C for serum creatinine and BUN levels analysis. Other blood samples werekept in EDTA containing tubes for measuring tacrolimus concentration. Urinary samples were maintained at -20 °C for micro albuminuria and urinary creatinine levels analysis. Kidneys were weighed immediately after nephrectomy and were fixed informalinsolution natural buffer(10%) for histopathological studies.

III. RESULTS

Statistical analysis

Statistical analysis was performed using One-Way ANOVA, Dunnet and Tukey post-hoc tests for parametric variables and Chi-square, Fisher's exact post hoc test for non-parametric variables using GraphPadPrism softwareversion 5.03. The results were expressed in mean ± SD and statistical significance was set for $p< 0.05$.

Tacrolimus concentration in whole blood samples:

Tacrolimus concentration in whole blood samples,which was collected in EDTA containing tubes,was detected with Quantitative Microsphere System (QMS) immunoassay kits by Thermo Scientific CDX90 model immunoassay set. The detectable levels of tacrolimus by these kit is between 1-30 ng/mL. Blood levels of tacrolimus were determined over a therapeutic range (20ng/ml)in toxic range in all groups (23,22 ± 4.50 for TAC group and 21,52 ± 3.56, 22,86 ± 3.42 and 22,80 ± 4.41 ng/ml for NAC 100, NAC 200 and NAC 300 respectively) and there were no significant differences between groups ($p>0.05$, fig 1).

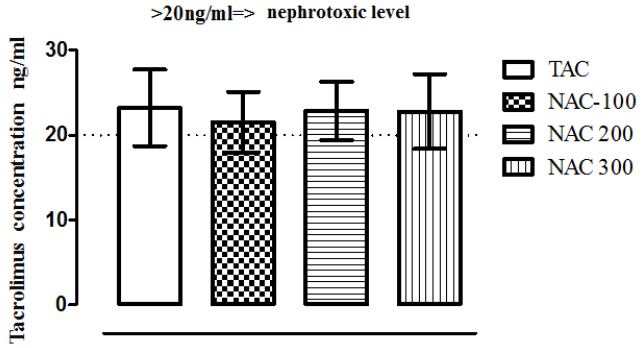

Fig 1.*Comparison of Tacrolimus concentrations (ng/ml)amongst the groups*

Body weight changes:
Body weight changes were shown asa percentage and were calculated by the following formula:

$$\text{Percent of weight change} = \frac{\text{Body weight in 28th day (g) - Body weight in 1th day (g)}}{\text{Body weight in 1th day (g)}} \times 100$$

Weight gain was represented as Positive values whereas weight loss was represented as negative values. However according to fig (2), there was a significant decrease in body weight change percentage in the TAC group (-6.43±1.75 g) compared to NAC 100 (-0.70±0.43 g), NAC200 (0.41±0.70 g) andNAC 300 (1.16±0.87 g) groups ($p<0.01$, $p<0.001$ and $p<0.001$ respectively).

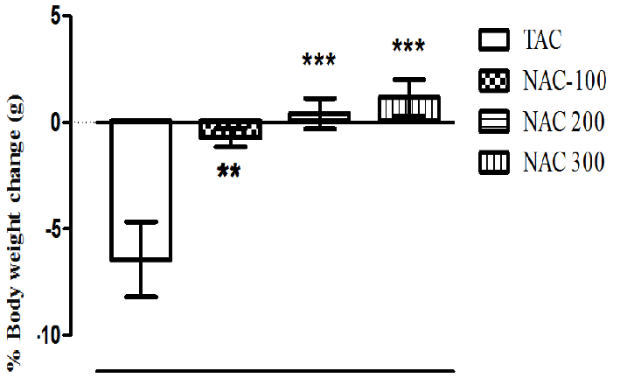

Fig 2.*Comparison of Weight change percentage values between the groups, Positive Values represent weight gain while Negative values represent weight loss*

Kidney weight changes:
According to results, there was a moderate decrease in kidney weight in TAC group (1.29 ± 0.03) compared with control group (1.48 ± 0.05) and NAC-100,200 and 300 groups (1.61 ± 0.08; 1.51 ± 0.11 and 1.50 ± 0.10 respectively), but this reduction was not statistically significant ($p > 0.05$, fig3).

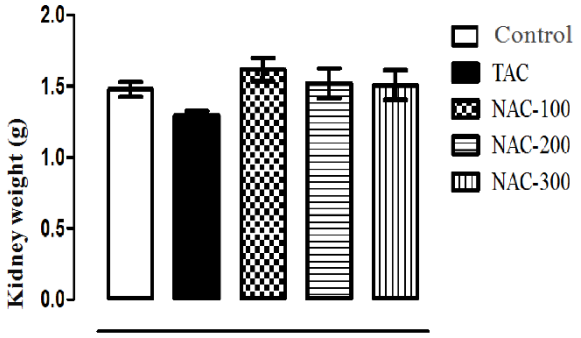

Fig 3*.Comparison of Kidney weight (g) between the groups*

Biochemical parameters:

Urinary microalbumin levels were measured byAbbott Architect 16000 model set with Abbott The Multigent Microalbumin Assay kits. This is a turbidimetric immunoassay method and polyclonal antibodies were used in detecting albumin molecules. Microalbumin levels between 5-500 µg/mL are detectable by this method.

Because24-hour urine collection was not available for assessing urinary microalbumin levels,in spot urinary samples creatinine and microalbumin levels weremeasured and the proportion of microalbumin/creatinine was evaluated. The values for microalbumin/creatinine (µg/mg) in spot Urinerepresent the following:<30 normal; 30-299 microalbuminuria; ≥300 macro (clinical) albuminuria.

There was an increase in urinary microalbumin (Urinary albumin/urinary creatinine µg/mg) levels in TAC group (44.98 ± 7.75 µg/mg) comparing NAC100, 200 and 300 groups (28.17 ± 3.40; 25.80 ± 4.56 and 15.06 ± 3.69 µg/mg, respectively). But only in NAC-300 group there was a significant difference in urinary microalbumin comparing with TAC group (15.06 ± 3.69 and 44.98 ± 7.75 µg/mg respectively; $p<0.01$, fig 4). Also in TAC group, the urinary microalbumin level is beyond normal average levels (0-30 µg/mg) and it refers to the existence of microalbuminuria in rats thatreceived tacrolimus without N-acetylcysteine treatment.

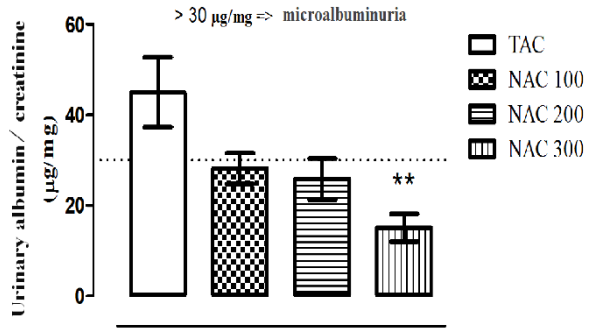

Fig 4*. Comparison of Microalbuminuria (µg/mg) levels among the groups*

Serum creatinine levels:

Serum creatinine levels were measured usingBeckman Coulter Modified Jaffe, Kinetic Test kits by Beckman Coulter AU 5800 model set. Measurable levels of creatinine by this method are between 0.06 - 25 mg/dL. Also, Urinary creatinine levels were measured through theOlympus AU 400 cihazinda Thermo Scientific DRI method withCreatinine-Detect Test kits.Measurable levels of creatinine by this method are between 0.78-420 mg/dL.

According to experiment results, serum creatinine levels in all groups were between normal ranges (0.2-0.8 mg/dl), but there wasa significant increase (fig 5) in serum creatinine levels in TAC group in comparison with NAC100,200 and 300 groups (0.71 ± 0.16; 0.38 ± 0.1; 0.36 ± 0.1 and 0.36 ± 0.1 respectively; $p<0.05$).

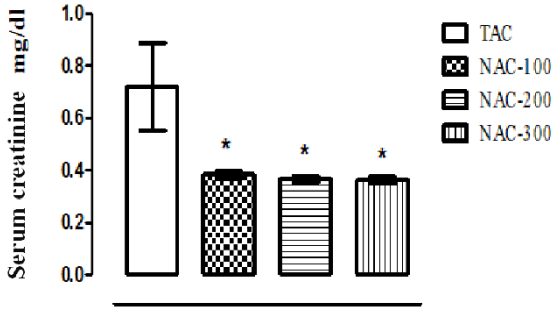

***Fig 5**. Comparison of Serum creatinine (mg/dl) levels between the groups*

Changes in BUN levels:

Serum urea nitrogen levels were measured with Beckman Coulter UREA (serum/plasma) GLDH (Glutamate Dehydrogenase), Reagent Assay kits in Beckman Coulter (AU 5800 model). Levels between 5-300 mg/dL are detectable by this method.

Detected serum urea nitrogen (BUN) levels wereabove the normal range (20 mg/dl) in all experimental groups (53.37 ± 8.97 mg/dl for TAC, 29.90 ± 1.32 mg/dl for NAC-100, 28.19 ± 1.46 mg/dl for NAC-200) except NAC 300(18.70 ± 1.64 mg/dl).According to results, there was a significant difference in BUN levels (fig 6) of TAC group compared with NAC100, 200 and 300 groups ($p<0.01$, $p<0.01$ and $p<0.001$ respectively).

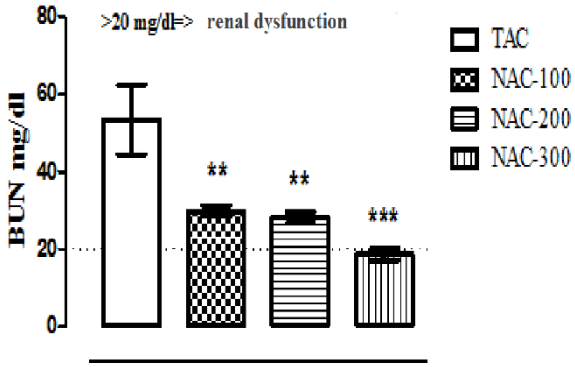

***Fig 6.** Comparison of BUN (mg/dl) levels between the groups*

Pathologic results:

Histopathologic changes in groups were observed in a moderate manner thusall histopathologic investigations were evaluated as + / - results. For this category of nonparametric results, Chi-square Fisher's exact non-parametric statistical test was applied and all test groups (NAC-100, NAC-200 and NAC-300) were compared with control group (TAC).According to histopathologicalfindings,what was shown was an increase in interstitial fibrosis and tubular atrophy in control group (TAC) - which are two important markers of chronic tacrolimus nephrotoxicity – while in contrast, NAC treatment groups showed a significant decrease in interstitial fibrosis ($p<0.05$ in NAC-200 and 300) and tubular atrophy ($p<0.05$ for NAC-200 and 300) depending on NAC dosage (see table 1, Fig7 and Fig 8). However, there was no significant difference in arteriolar hyalinosis and tubular micro-calcifications between control group (TAC) and test groups (NC-100, NAC-200 and NAC-300) statistically (see table 1, Fig9 and Fig 10).

Table 1. Comparison of histopathological changes in test groups (NAC-100, NAC-200 and NAC-300) with control group (TAC)

Type of change	Groups (n= 8)	Number of (+) results	Number of (-) results	Odds ratio	P value
Interstitial fibrosis	**TAC**	**7**	**1**	**_**	**_**
	NAC-100	**3**	**5**	**11.6**	**0.11**
	NAC-200	**2**	**6**	**21**	**0.04†**
	NAC-300	**1**	**7**	**49**	**0.01†**
Tubular atrophy	**TAC**	**7**	**1**	**_**	**_**
	NAC-100	**4**	**4**	**7**	**0.28**
	NAC-200	**2**	**6**	**21**	**0.04†**
	NAC-300	**2**	**6**	**21**	**0.04†**
Arteriolar hyalinosis	**TAC**	**4**	**4**	**_**	**_**
	NAC-100	**2**	**6**	**3**	**0.6**
	NAC-200	**2**	**6**	**7**	**0.28**
	NAC-300	**2**	**6**	**7**	**0.28**
Tubular micro-calcifications	**TAC**	**3**	**5**	**_**	**_**
	NAC-100	**2**	**6**	**1.8**	**1**
	NAC-200	**1**	**7**	**3**	**0.56**
	NAC-300	**3**	**5**	**1**	**1**

TAC: tacrolimus, NAC: N-acetylcysteine, †: significantly different from the control at p value < 0.05.

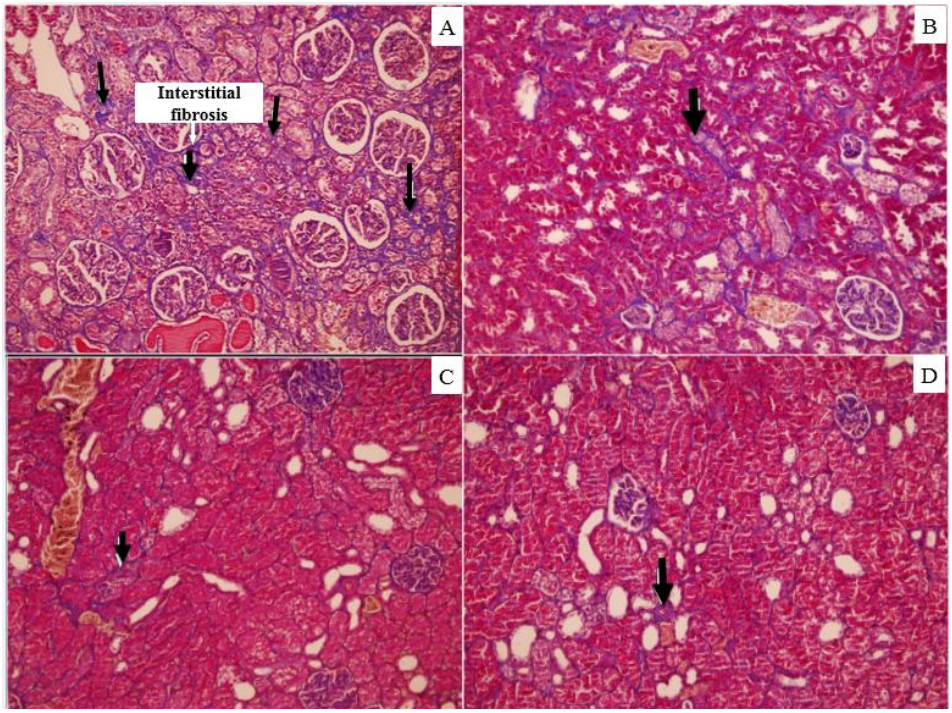

Fig 7*: Comparison of Interstitial Fibrosis between the groups, A represents TAC control group, B, C and D represent NAC 100, 200 and 300 respectively*

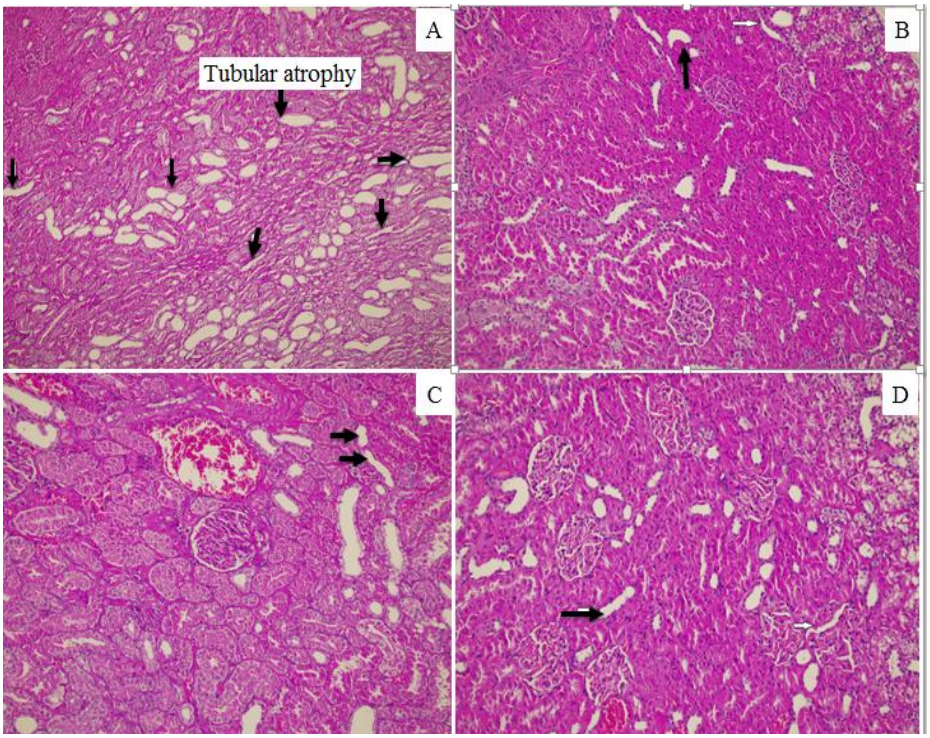

Fig 8: *Comparison of Tubular Atrophy between the groups, A represents TAC control group, B, C and D represent NAC 100, 200 and 300 respectively*

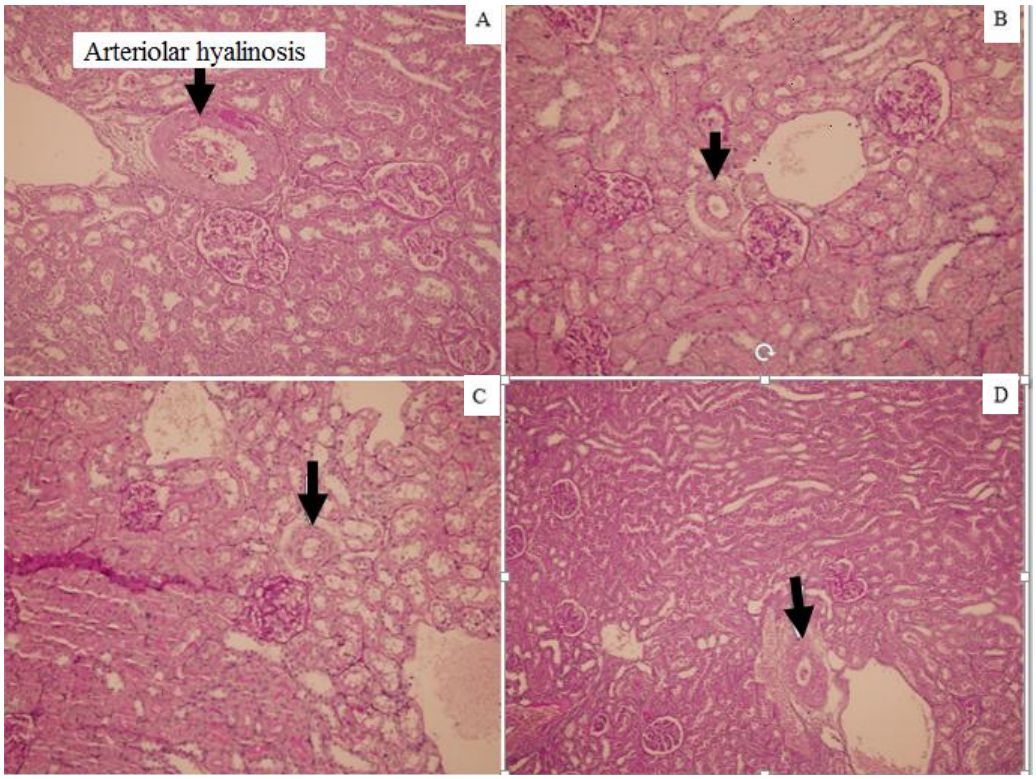

Fig 9: *Comparison of Arterial Hyalinosis between the groups, A represents TAC control group, B, C and D represent NAC 100, 200 and 300 respectively, no statistical significance*

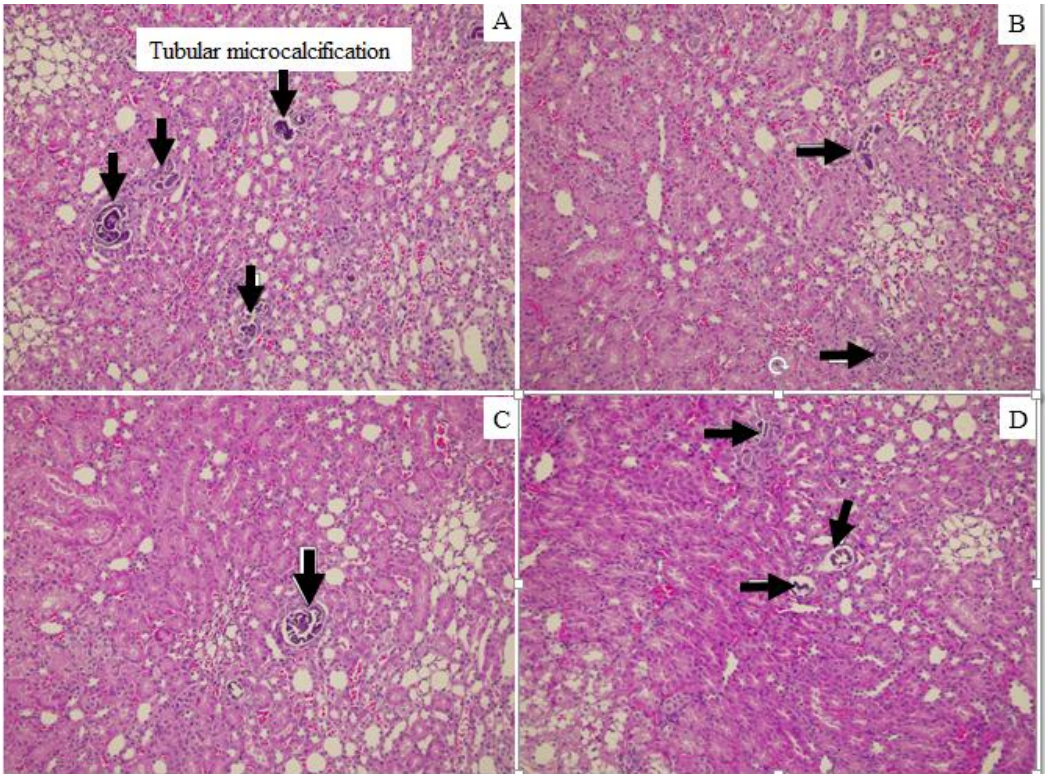

***Fig 10**: Comparison of Tubular micro-calcifications between the groups, A represents TAC control group, B, C and D represent NAC 100, 200 and 300 respectively, no statistical significance*

IV. DISCUSSION:

Immunosuppressant drugs such as tacrolimus and cyclosporine-A which are calcineurin inhibitors have markedly improved the clinical outcome of solid organ transplants. Tacrolimus is 10-100 times more potent than cyclosporine-A in suppressing T-cell activation in allograft tissue rejection mechanisms but, it inducessignificant side effects on kidney function. Oxidative stress induced by tacrolimus administration is believed to cause oxidative renal damage in tubules, interstitial tissue and arterioles such as tubular atrophy, interstitial fibrosis and arteriolar hyalinosis. Tubular injury is most commonly seen in proximal tubules which are dependenton oxidative phosphorylation for energy production and therefore are more sensitive to oxidative stress (9).

In a study done by Zhu and colleagues in 2004, they showed that the nephrotoxic effect of Tacrolimus is related to inhibitingthe activity ofCatalasewhich is one of the antioxidant systems that converts hydrogen peroxide to water and oxygen(8).

However, catalase is not the sole way for hydrogen peroxide degradation (9). Glutathione peroxidase performs this reaction as well (8).Consequentially,what has been observed isthat the nephrotoxicity caused by Tacrolimus through Catalase inhibition isbeing diminished through theincrease of Glutathione Peroxidase (GPx) activity. The factor increasing GPx's activity is Glutathione levels(8,9). Glutathione subsequently is increased by N-Acetylcysteine (NAC) (10).

In a study performed usingcyclosporine which has the same mechanism of action as tacrolimus, N-acetylcysteine administration was observed toattenuate cyclosporine induced nephrotoxicity in rats (12).

It has been shown that concomitant treatment of NAC along with tacrolimus caused significant increase in cell viability in porcine renal proximal tubular cell cultures comparedto cells with only tacrolimus administration.While Tacrolimus induced cytotoxicity by increasing hydrogen peroxide production which increased ROS activity, NAC - a glutathione precursor - negated the effect of tacrolimus on ROS activity (8).

According to these findings,this study was planned to examine the protective effect of various NAC doses on tacrolimus induced nephrotoxicity.

Tacrolimus shows its therapeutic effect as an immunosuppressive drug in 4-20 ng/ml whole blood concentrations 12 hours after its last administration dose in humans (13).Thus, in concentrations more than 20 ng/ml,the resulting nephrotoxic effect will be increasedconsiderably. Therefore,with the purpose of inducing nephrotoxicity, high doses of Tacrolimus were administrated to reach whole blood concentrations above 20 ng/ml (14).Afterwards, blood samples were collected 20-24 hour after the last Tacrolimus administration and

whole blood concentrations were observed to be more than 20 ng/ml in all experimental groups. This lead the Tacrolimus doses to be determined assufficiently toxic to induce nephrotoxic effects (see Fig 1).

In the present study, treatment with Tacrolimus alone resulted in a significant decrease in body weight while treatment with NAC did not affect the body weight in Tacrolimus treated rats (Fig 2). According to this result,the weight loss depends on the toxic dose of tacrolimus to be considered. There are many studies about the effect of tacrolimus administration in rats' body weight that found similar results to this study (15-16-17). Also, in a study done by Tariq and colleagues in 1999, their results showed there was no significant difference in body weight between NAC administered rats and control group rats and they concluded that NAC administration had no effect on body weight in rats similarly to the results presented in this paper (12). On the other hand, these results confirm NAC administration dependingon thedose can prevent the effect of Tacrolimus on weight loss. While the cause of weight loss induced by this drug is not explained clearly, some studies indicate that it has diabetogenic effects in humans and rats through inhibiting insulin gene expression in pancreatic beta cells (18-19-20). Fisac and colleagues in 2007 studied the relationship between the diabetogenic effect of Tacrolimus and weight loss in rats where they administered 0.1 mg/kg/day dosesfor 15 days in rats and after 9days, diabetic patternswereshown and weight loss was established. In the same study after they stopped Tacrolimus administration, weight loss was reversed with the disappearance ofdiabetic patterns(19). As previously known, weight loss is one of the symptoms that depends oninsulin lack in type-1 diabetes that is related to glucose metabolism impairment (21). Consequently, as the plan in this study included only NAC effects on Tacrolimus induced nephrotoxicity, no evaluation of its effect on Tacrolimus induced diabetes was performed and it is necessary to design new studies around that topic.

In this study,the addition of a new group acting as a fauxgroup (n=8), that received 1 ml/day SCnormal saline to demonstrate the kidney weight changesin the experimental groups. Results showed only one group receiving Tacrolimus aloneunderwent a decrease in kidney weightcompared to other groups, but this decrease was not statistically significant (see fig 3). In a study done by Tada and colleagues using Tacrolimus in rats, they reported similar result to thisstudy (22).The cause of decreasein kidney weight by chronic Tacrolimus administration is not clear but maybe is related to interstitial fibrosis and tubular atrophy in Tacrolimus induced nephrotoxicity, as some studies demonstrated significant decrease in kidney weight under these conditions (23-24).

As mentioned before, Tacrolimus nephrotoxicity is a significant concern and appears to progress over time when tacrolimus exposure is maintained (25). However, microalbuminuria has been used as an early marker of nephrotoxicity to diagnose small changes related to tubular epithelial cells injuries (26). In healthy rats, reference values for urinary microalbumin (urinary albumin/urinary creatinine) are between 0-30 µg/mg (27). In nephrotoxicity,values are higher than 30 µg/mg leading to a condition named microalbuminuria (25-28). According to this study's results, microalbuminuria was solely observed in the control group that took Tacrolimuswhereas in groups which received tacrolimus with different doses of NAC, microalbumin was within normal range and specifically in the group which received NAC 300 mg/kg/day dose, the microalbumin level showed significant decrease compared with control group (see fig 4).

Microalbuminuria has been seen in early stages of renal dysfunction followed by proximal tubular cells injury and decrease in GFR (29-30). Li and colleagues in 2009 reported that increased urinary microalbumin excretion is a biomarker of acute renal injury during theearly stage of Tacrolimus nephrotoxicity but in end stages,the serum creatinine and BUN levels were more important biomarkers showing sever renal dysfunction (25). Also, worthy of mentioningis a study performed by Spapen et al on rats where they concluded NAC (100 mg/kg/day) administration can significantly reduce urinary microalbumin excretion in acute sepsis. Theyattributedthis protective effect of NACto its effect inmaintaining endothelial integrity of kidney vessels by preventing capillary leakage (31).

Results obtainedshowthat serum creatinine levels in all groups are between reference values (0.2-0.8 mg/dl) (27) but there is significant decrease in serum creatinine levels in NAC (100,200 and 300 mg/kg/day) groups while in control group (TAC) it is close to the upper limit value (see fig 5). As commonly known, an increased serum creatinine level is one important biochemical marker for renal dysfunction(30). In addition, based onresearch results in chronic Tacrolimus nephrotoxicity,damage to renal tubules resulted in an increase of serum creatinine levels (9-30). Creatinine is excreted as result of glomerular filtration and tubular secretion in the kidneys and NAC further enhances creatinine clearance by increasing tubular secretion of creatinine (32).According to results obtained, NAC administration during Tacrolimus treatment can prevent increases in serum creatinine levels. Indeed,perhaps the basis for this effect is related to the protective role NAC plays against the nephrotoxic effect of Tacrolimus in the tubules.A study performedutilizing cyclosporine shows NAC treatment can improve serum creatinineenhancementduring cyclosporine induced nephrotoxicity (12).

In the present study, Tacrolimus administration alone for 28 days resulted in significant increase of BUN levels, suggesting a functionalkidneyimpairment. In addition, groups thatreceivedNAC 100 and NAC 200 mg/kg doses with Tacrolimus had BUN levels abovethe reference values (15-20 mg/dl) and only with NAC 300

mg/kg doses did BUN levels liewithin reference values. Noting that all treatment groups exhibited a significant decrease in BUN levelscompared with the control group (fig 6). Increased BUN levelsareconsidered as one of the importantbiochemical indicators of renal dysfunction associated with irreversible damage to tubular epithelial cells and apoptosis (9-30).Results in this studywere consistent with earlier investigations, which reported significant decrease of BUN levels in rats treated with NAC in Cyclosporine mediated nephrotoxicity (12). These resultsshow NAC treatment with the correct dose can improve BUN alteration during Tacrolimus induced nephrotoxicity thus exhibitinga protective effect.

In Tacrolimus induced nephrotoxicity depending on the administration period and even withtherapeutic doses, acute nephrotoxicity at early stages and chronic nephrotoxicity at later stages may occur (33). Based on previous research, interstitial fibrosis, tubular atrophy and arteriolar hyalinosis are common histopathological changes in CNI inhibitors induced chronic nephrotoxicity while irreversible tubular micro-calcificationshave been rarely seen (33-34-35).

In summary,the association between chronic nephrotoxic effectsresulting from administering high doses of long-term tacrolimusand the protective role of various doses of NAC against tacrolimus induced nephrotoxicity wasexamined in this study.According to the results obtained, histopathological studies revealed that tacrolimus administration caused a significant damage to tubular and interstitial compartments of the kidneys such as tubular atrophy and interstitial fibrosis. Additionally, results showed NAC administration in a dose dependent manner had a protective effect against Tacrolimus induced nephrotoxicity and reduced tubular atrophy and interstitial fibrosis occurrence in chronic Tacrolimus induced nephrotoxicity. However, NAC administration had no significant effect on arterial hyalinosis and tubular micro-calcifications.In fact, in this study Tacrolimus administration did not increase arterial hyalinosis and tubular micro-calcifications genesis statistically. Perhaps this was related to the short duration of Tacrolimus administration in the study.The protective effect of NAC in decreasing interstitial fibrosis has also been observed in another calcineurin inhibitor named Cyclosporinewhich induces chronic nephrotoxicity. This may be contributed to the protective effect of NAC due to its antioxidant and vasodilator effects (12). In another study, the protective effect of NAC on nephrotoxicity induced by ischemic reperfusion has been shown. In ischemic reperfusion induced nephrotoxicity, the development of fibrosis and atrophy in renal cells is related to oxidative stress followed by glutathione depletion in cells and that is where NAC has shown protective effects due toits precursor role through glutathione (36-37).

V. CONCLUSION:

These findings confirm the nephroprotective effects of N-acetylcysteine in chronic Tacrolimus induced nephrotoxicity in a dose dependent manner.

Contributors

AA, AK and RF designed and conceived the study. All authors contributed equally throughout the study. The manuscript was contributed to by all authors. RS edited the final version which was approved by all authors.

Declaration of interests

We declare no competing interests.

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Afsaneh Azhari. "The Preventive Effects of N-Acetylcysteine on Tacrolimus Induced Nephrotoxicity" IOSR Journal of Pharmacy (IOSRPHR), vol. 8, no. 09, 2018, pp. 57-68