Pharmaceutical Sciences
September 2017, 23, 172-181
doi: 10.15171/PS.2017.26
http://journals.tbzmed.ac.ir/PHARM

Research Article

Prospects of N-Acetylcysteine and Melatonin as Treatments for Tramadol-Induced Renal Toxicity in Albino Rats

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Article Info

Article History:
Received: 16 October 2016
Accepted: 20 June 2017
ePublished: 30 September 2017

Keywords:
-Tramadol
-Antioxidants
-Kidney
-Toxicity
-Rats

ABSTRACT

Background: Tramadol (TD) has played an important role in the treatment of pain. However, renal toxicity due to TD abuse is a serious clinical challenge. This study assessed the effects of n-acetylcysteine (NAC) and melatonin (MT) on TD-induced renal toxicity in albino rats.

Methods: Rats were randomized into groups and treated with MT (10mg/kg/day), NAC (10mg/kg/day) and TD (15, 30, and 45mg/kg/day) respectively. Rats were pretreated with MT (10mg/kg/day) and NAC (10mg/kg/day) prior to treatment with TD (15, 30, and 45mg/kg/day) intraperitonialy for 7days respectively. Rats were sacrificed, serum extracted and evaluated for creatinine, urea and uric acid. The kidneys were evaluated for malondialdehyde (MDA), superoxide dismutase (SOD), catalase, (CAT), and glutathione (GSH) levels.

Results: Treatment with MT and NAC did not produce significant ($P>0.05$) effects on serum creatinine, urea, uric acid and kidney MDA, SOD, CAT, and GSH levels when compare to saline control. In contrast, serum creatinine, urea, uric acid and kidney MDA levels were increased while kidney SOD, CAT, and GSH levels were decreased significantly ($P<0.05$) and in a dose-dependent manner in TD-treated rats. Kidneys of TD-treated rats showed varying degrees of damage which were dose-dependent. However, in all evaluated parameters, TD-induced alterations were abrogated in NAC and MT pretreated rats. Abrogations were most evident in rats pretreated with combined doses of NAC and MT.

Conclusion: The present study showed prospects of n-acetylcysteine and melatonin as remedies for tramadol associated renal toxicity.

Introduction

Tramadol (TD) is a synthetic, centrally acting analgesic, available in Europe since 1977 and in the United States since 1995 for the treatment of pain syndromes previously amenable only to the opiate analogues.[1] It has a wide range of applications mostly in the treatment of moderate to severe, acute or chronic pain.[2] The use of TD as an analgesia has been abused leading to psychological and physical addiction similar to that seen with other opiates and opioids causing acute overdose –related deaths.[3,4] TD is metabolized in the liver by cytochrome P 450 to active metabolites which are excreted through the kidneys; hence the kidney is one of the primary organs susceptible to TD toxicity.[5,6] Repeated TD administration could lead to the accumulation of toxic metabolites in the kidney; decrease clearance of TD, thus increasing its renal toxicity.[7,8] Renal toxicity characterized by glomerular hemorrhage, atrophied glomeruli with collapsed tufts, wide Bowman's space, and degenerated tubules were observed in the kidneys of TD-treated rats.[9] Studies have reported lipid peroxidation, mitochondria damage, inhibition of protein synthesis and decrease in antioxidants in the kidneys of TD-treated rats[10-12] Melatonin (MT), a hormone produced by the pineal gland, is a potent scavenger of reactive oxidative radicals.[13] Thus, MT can prevent free radical-induced cellular oxidative damage and can contribute to the physiological functions of the antioxidant defensive system.[14] Also, it can

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stimulate several antioxidant systems, thereby facilitating more antioxidant activities and stabilizing cell membranes. In addition, it modulates the gene expression of several protective enzymes and reduces apoptosis and lipid peroxidation.[15] MT and its metabolites have anti-inflammatory property and have been proven to be highly effective in a variety of disorders linked to inflammation and oxidative stress.[16,17] MT supplement can decrease oxidative stress in renal tissues and attenuate kidney scarring. In a model of cyclosporine-induced nephropathy, pretreatment with MT decreased tubular and glomerular damage.[18,19]

N-acetylcysteine (NAC) is an n-acetylated derivative of the naturally occurring amino acid cysteine.[20] It is a synthetic precursor of GSH, which can stimulate the intracellular synthesis of GSH and enhances glutathione *S*-transferase (GST) activity.[21] It is a small molecule that can be freely filtered, with easy access to intracellular compartments and is very effective in neutralizing free radicals intracellularly.[22,23] Studies using animals have confirmed renal protective effect of NAC in drug-induced nephrotoxicity.[24,25] Also, synergy in activity has been reported with concurrent administration of MT and NAC.[26] Despite the renal protective effects of NAC and MT, literature search showed lack of information on the effects of NAC and MT on TD-induced renal toxicity. Therefore, this study evaluated the effects of MT and NAC on TD-induced renal toxicity in albino rats.

Material and methods

Animals

Eighty five (85) adult male albino rats weighing 250-300 g were used for this study. Rats were housed under continuous observation in appropriate cages at room temperature with a 12-12 h light-dark cycle. The rats were housed five per cage, and fed with commercial standard diet and water *ad libitum*.

Drugs and chemicals

The tramadol used for this study was manufactured by Zahidi Enterprise Mumbai India while N-acetylcysteine and melatonin were obtained from Shijiazhuang AO Pharm Import and Export Co Ltd China. All other chemicals used for this study are of analytical grade. TD, (15, 30 and 45 mg/kg/day),[27] MT (10mg/kg/day) [28]and NAC (10mg/kg/day)[29] were used for this study. MT was dissolved in 1% ethanol and diluted with normal saline.[30]

Experimental design

Group A served as the control and was divided into two groups, A1 and A2 of 5 rats each. Rats in group A1 (placebo control) and group A2 (solvent control) were treated with normal saline and 1% ethanol for 7 days respectively.

Groups B-F contained 15 rats each which were further sub-divided into 3 groups of 5 rats each.

Group B was treated with 10mg/kg/day of MT, 10mg/kg/day of NAC and combined doses of MT and NAC intraperitoneally for 7 days.

Group C was treated with 15, 30 and 45 mg/kg/day of TD intraperitoneally for 7 days.

Group D was pretreated with 10 mg/kg/day of MT prior to treatment with 15, 30 and 45 mg/kg/day of TD intraperitoneally for 7 days respectively.

Group E was pretreated with 10 mg/kg/day of NAC prior to treatment with 15, 30 and 45 mg/kg/day of TD intraperitoneally for 7 days respectively.

Group F was pretreated with 10 mg/kg/day of MT and 10 mg/kg/day of NAC prior to treatment with 15, 30 and 45 mg/kg/day of TD intraperitoneally for 7 days respectively.

Collection of sample

Rats were sacrificed with diethyl ether; blood was collected via cardiac puncture in a non-heparinized sample container and allowed to clot. It was centrifuged at 1500 rpm for 15 minutes and serum extracted and evaluated for biochemical parameters. The kidney was surgically removed weighed and placed in iced beakers. The kidney was washed in ice cold KCl solution (1.15% w/v), then homogenized with 0.1M phosphate buffer (pH 7.2) and evaluated for kidney oxidative stress indices.

Evaluation of renal function and kidney oxidative stress indices

Creatinine was evaluated as described by Junge *et al*., 2004,[31] urea was measured as reported by Kaplan, 2007,[32] while uric acid was evaluated as described by Prabu *et al*., 2010.[33] Superoxide dismutase was assayed according to the method of Sun and Zigma 1978 [34] while catalase was evaluated as reported by Aebi, 1984.[35] Reduced glutathione was measured as described by Sedlak and Lindsay 1968[36] while malondialdehyde was evaluated according to Buege and Aust 1978.[37]

Histological examination

Kidneys of rats were excised and processed using appropriate laboratory technique. Processed kidneys were stained with Hand E and examined with the aid of a light microscope. Relevant stained sections were photographed.

Effects on renal function parameters

There were no significant ($p>0.05$) effects on serum levels of creatinine, urea and uric acid observed in rats administered with NAC and MT when compared to saline control (Table 1). In contrast, serum creatinine, urea and uric acid levels were significantly ($p<0.05$) increased in a doses-dependent manner in TD-treated rats. However, pretreatments with individual doses of NAC and MT prior to TD treatment decreased serum creatinine,

urea and uric acid levels. The serum levels of creatinine, urea and uric acid were further decreased in rats pretreated with combined doses of NAC and MT. The observed decreases with pretreatment using combined doses of NAC and MT differ significantly ($p<0.05$) when compared to pretreatments with their individual doses (Table 2-4)

Effects on oxidative stress indices and kidney histology

The kidney MDA, SOD, CAT and GSH levels were not significantly ($p>0.05$) altered in rats treated with NAC and MT when compared to control saline control (Table 1). On the contrary, kidney MDA levels were increased whereas SOD, CAT and GSH levels were decreased significantly ($p<0.05$) and in a dose-dependent manner in TD-treated rats. Nevertheless, kidney levels of SOD, CAT and GSH were increased whereas MDA levels were decreased significantly ($p<0.05$) in rats pretreated with individual doses of NAC and MT prior to treatment with TD. The highest increases in SOD, CAT and GSH levels with decreases in MDA levels were obtained in rats pretreated with combined doses of NAC and MT. The effects obtained with pretreatment using combined doses of NAC and MT differ significantly ($p<0.05$) when compared to their individual doses (Table 4-8). H and E stained sections of the kidneys of rats treated with 15mg/kg/day of TD showed tubular necrosis, collapsed glomueruli with inflammatory materials in the tubular lumen. On the other hand, kidneys of rats pretreated with 10mg/kg/day of MT and NAC showed tubular necrosis with signs of regeneration of tubular lining (Figure 1-5).

Table 1. Effects of melatonin and n-acetylcysteine on renal function and kidney oxidative indices of albino rats.

Dose (mg/kg)	Cr (mg/dL)	U (mg/dL)	UA (mg/dL)	GSH µg/mg protein	CAT u/mg protein	SOD u/mg protein	MDA nmole/mg protein
Control	1.05 ± 0.04	22.45 ± 1.29	1.13± 0.07	16.45 ± 0.13	30.54 ± 2.19	18.72 ± 1.05	0.53± 0.01
NAC	0.90 ± 0.05	20.73 ± 1.07	0.97 ± 0.02	17.40 ± 1.26	32.91 ± 2.06	20.05 ± 2.22	0.50 ± 0.02
MT	0.95 ± 0.01	21.57 ± 0.14	1.10 ± 0.06	16.91 ± 1.35	32.15 ± 3.17	19.36 ± 2.01	0.49 ± 0.06
NAC+MT	0.85 ± 0.06	19.06 ± 1.08	0.90 ± 0.07	18.72 ± 1.19	33.73 ± 2.02	21.50 ± 2.07	0.47 ± 0.07

TD= Tramadol. MT= Melatonin. NAC= N-acetylcysteine. Cr=creatinine, U=urea, UA=Uric acid n= 5.

Table 2. Effects of melatonin and n-acetylcysteine on tramadol-induced serum creatinine level in albino rats.

	Serum creatinine (mg/dL)			
Dose (mg/kg)	**TD**	**TD+ NAC**	**TD+MT**	**TD+NAC+MT**
Control (Saline)	1.05 ± 0.04	1.05 ± 0.04	1.05 ± 0.04	1.05± 0.04
15	1.96 ± 0.01	1.10 ± 0.02 [b]	1.12 ± 0.03 [b]	0.82± 0.01 [b]
30	2.71 ± 0.09	1.33 ± 0.07 [b]	1.39 ± 0.03 [b]	0. 89± 0.04 [c,d]
45	3.51 ± 0.08	2.00 ± 0.01 [b]	2.11 ± 0.05 [b]	1.10 ± 0.06 [c,d]

TD= Tramadol. MT= Melatonin. NAC= N-acetylcysteine. n= 5. Results are expressed as mean ± S.E.M.
[b]Significant ($p < 0.05$) difference when compared to TD- pretreated rats.
[c]Significant ($p < 0.05$) difference when compared to MT- pretreated rats.
[d]Significant ($p < 0.05$) difference when compared to NAC- pretreated rats

Table 3. Effects of pretreatments with melatonin and n-acetylcysteine on tramadol-induced serum urea level in albino rats.

	Serum urea (mg/dL)			
Dose (mg/kg)	**TD**	**TD+ NAC**	**TD+MT**	**TD+ NAC+MT**
Control (Saline)	22.45 ± 2.09	22.45 ± 2.09	22.45 ± 2.09	22.45 ± 2.09
15	47.26 ± 2.17	28.40 ± 1.02 [b]	30.31 ± 2.03 [b]	20.33 ± 2.02 [c,d]
30	68.10 ± 3.51	35.83 ± 1.74 [b]	39.35 ± 2.10 [b]	22.17 ± 1.16 [c,d]
45	80.22 ± 3.14	50.58 ± 2.33 [b]	52.38 ± 3.38 [b]	24.35 ± 2.15[c,d]

TD= Tramadol. MT= Melatonin. NAC= N-acetylcysteine. n= 5. Results are expressed as mean ± S.E.M.
[b]Significant ($p < 0.05$) difference when compared to TD- pretreated rats.
[c]Significant ($p < 0.05$) difference when compared to MT- pretreated rats.
[d]Significant ($p < 0.05$) difference when compared to NAC- pretreated rats

Table 4. Effects of melatonin and n-acetylcysteine on tramadol-induced serum uric acid level in albino rats.

Serum uric acid(mg/dL)				
Dose (mg/kg)	**TD**	**TD+ NAC**	**TD+MT**	**TD+ NAC+MT**
Control (Saline)	1.13 ± 0.07	1.13 ± 0.07	1.13 ± 0.07	1.13± 0.07
15	1.84 ± 0.03	1.00 ± 0.05 [b]	1.09 ± 0.01 [b]	0.76± 0.05 [c,d]
30	2.90 ± 0.02	1.39± 0.01 [b]	1.46 ± 0.05 [b]	0. 79± 0.09 [c,d]
45	3.73 ± 0.08	2.21± 0.01 [b]	2.35 ± 0.05 [b]	1.24± 0.05 [c,d]

TD= Tramadol. MT= Melatonin. NAC= N-acetylcysteine. n= 5. Results are expressed as mean ± S.E.M.
[b]Significant ($p < 0.05$) difference when compared to TD- pretreated rats.
[c]Significant ($p < 0.05$) difference when compared to MT- pretreated rats.
[d]Significant ($p < 0.05$) difference when compared to NAC- pretreated rats
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Table 5. Effects of melatonin and n-acetylcysteine on tramadol-induced kidney malondialdehyde level in albino rats.

kidney malondialdehyde (MDA) (U/mg protein)				
Dose (mg/kg)	**TD**	**TD+ NAC**	**TD+MT**	**TD+ NAC+MT**
Control (Saline)	0.53 ± 0.01	0.53 ± 0.01	0.53 ± 0.01	0.53 ± 0.01
15	1.00 ± 0.03	0.60 ± 0.08 [b]	0.69 ± 0.07 [b]	0.40 ± 0.06 [c,d]
30	2.11 ± 0.06	1.03 ± 0.07 [b]	1.20 ± 0.03 [b]	0.51 ± 0.03 [c,d]
45	3.10 ± 0.08	1.59 ± 0.07 [b]	1.60 ± 0.03 [b]	0.65 ± 0.04 [c,d]

TD= Tramadol. MT= Melatonin. NAC= N-acetylcysteine. n= 5. Results are expressed as mean ± S.E.M.
[b]Significant ($p < 0.05$) difference when compared to TD- pretreated rats.
[c]Significant ($p < 0.05$) difference when compared to MT- pretreated rats.
[d]Significant ($p < 0.05$) difference when compared to NAC- pretreated rats

Table 6. Effects of pretreatments with melatonin and n-acetylcysteine on tramadol-induced kidney superoxide dismutase level in albino rats.

Kidney superoxide dismutase (SOD) (U/mg protein)				
Dose (mg/kg)	**TD**	**TD+ NAC**	**TD+MT**	**TD+ NAC+MT**
Control (Saline)	18.72 ± 0.95	18.72 ± 0.95	18.72 ± 0.95	18.72 ± 0.95
15	10.31 ± 0.61	17.41 ± 0.16[b]	15.25 ± 0.31 [b]	18.53 ± 1.39 [c,d]
30	6.91 ± 0.73	13.35 ± 0.31 [b]	10.19 ± 0.23 [b]	16.76 ± 0.52 [c,d]
45	4.97 ± 0.26	8.43 ± 0.59 [b]	7.44 ± 0.08 [b]	16.00 ± 0.75 [c,d]

TD= Tramadol. MT= Melatonin. NAC= N-acetylcysteine. n= 5. Results are expressed as mean ± S.E.M.
[b]Significant ($p < 0.05$) difference when compared to TD- pretreated rats.
[c]Significant ($p < 0.05$) difference when compared to MT- pretreated rats.
[d]Significant ($p < 0.05$) difference when compared to NAC- pretreated rats
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Table 7. Effects of pretreatments with melatonin and n-acetylcysteine on tramadol-induced kidney catalase level in albino rats.

Kidney catalase (U/mg protein)				
Drug (mg/kg)	**TD**	**TD+ NAC**	**TD+MT**	**TD+ NAC+MT**
Control (Saline)	30.54 ± 2.19	30.54 ± 2.19	30.54 ± 2.19	30.54 ± 2.19
15	20.70 ± 1.01	29.25 ± 1.23[b]	27.01 ± 1.17 [b]	33.00 ± 2.27 [c,d]
30	12.24 ± 0.63	21.27 ± 1.39 [b]	18.06 ± 1.25 [b]	30.44 ± 2.02 [c,d]
45	7.88 ± 0.01	16.80 ± 0.82 [b]	13.83 ± 0.38 [b]	28.03 ± 1.31 [c,d]

TD= Tramadol. MT= Melatonin. NAC= N-acetylcysteine. n= 5. Results are expressed as mean ± S.E.M.
[b]Significant ($p < 0.05$) difference when compared to TD- pretreated rats.
[c]Significant ($p < 0.05$) difference when compared to MT- pretreated rats.
[d]Significant ($p < 0.05$) difference when compared to NAC- pretreated rats

Table 8. Effects of pretreatments with melatonin and n-acetylcysteine on tramadol-induced kidney glutathione level in albino rats.

Kidney glutathione (GSH) (µg/mg protein)				
Dose (mg/kg)	**TD**	**TD+ NAC**	**TD+MT**	**TD+ NAC+MT**
Control (Saline)	16.45 ± 1.03	16.45 ± 1.03	16.45 ± 1.03	16.45 ± 1.03
15	8.97 ± 0.01	13.63 ± 0.71[b]	13.17 ± 0.67 [b]	18.65 ± 1.59 [b]
30	4.46 ± 0.03	10.37 ± 0.15 [b]	8.73 ± 0.31 [b]	16.06 ± 1.07 [c,d]
45	2.90 ± 0.12	6.38 ± 0.02 [b]	5.65 ± 0.14 [b]	15.68 ± 0.92 [c,d]

TD= Tramadol. MT= Melatonin. NAC= N-acetylcysteine. n= 5. Results are expressed as mean ± S.E.M.
[b]Significant ($p < 0.05$) difference when compared to TD- pretreated rats.
[c]Significant ($p < 0.05$) difference when compared to MT- pretreated rats.
[d]Significant ($p < 0.05$) difference when compared to NAC- pretreated rats

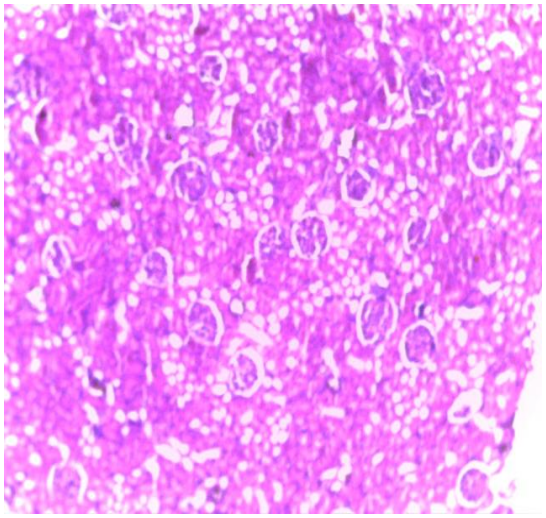

Figure 1. Micrograph of the kidney of control rat treated with normal saline for 7 days showing normal kidney architecture (Hand E X 200).

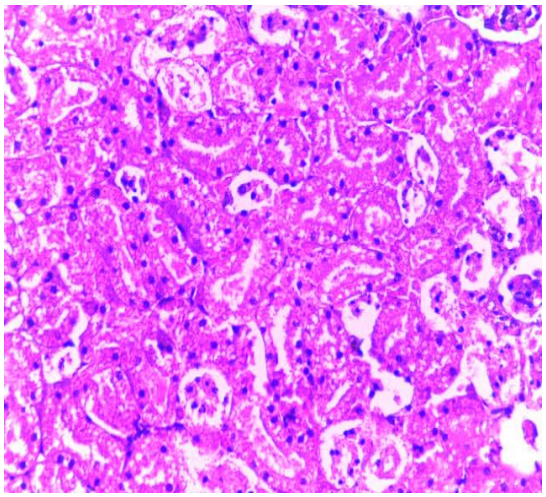

Figure 2. Micrograph of the kidney of rat treated with 15 mg/kg/day of TD for 7 days showing tubular necrosis, collapsed glumeruli with inflammatory materials in the tubular lumen (Hand E X200).

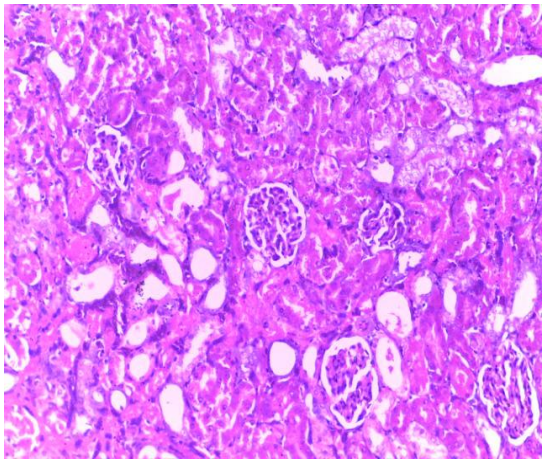

Figure 3. Micrograph of the kidney of rat treated with 15 mg/kg/day of TD and 10mg/kg/day of NAC for 7 days showing tubular necrosis with regeneration of tubular lining (Hand E X200).

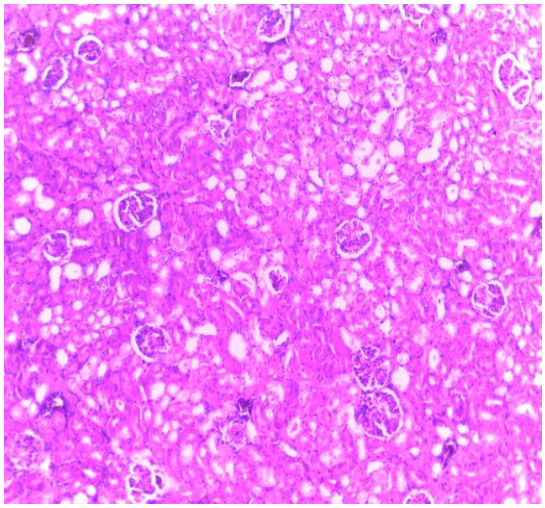

Figure 4. Micrograph of the kidney of rat treated with 15 mg/kg/day of TD and 10mg/kg/day of MT for 7 days showing tubular necrosis (Hand E X200).

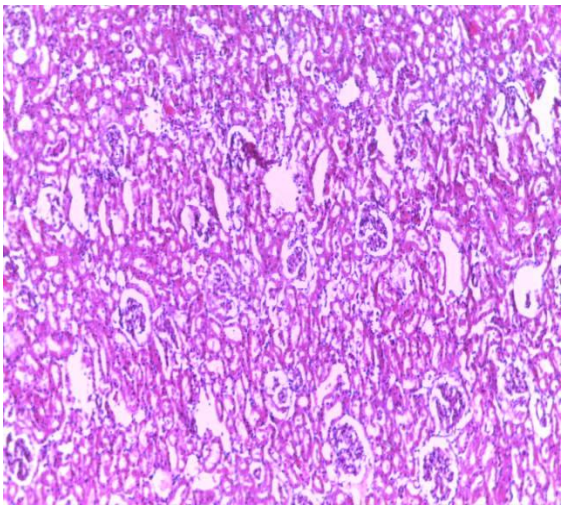

Figure 5. Micrograph of the kidney of rat treated with 15 mg/kg/day of TD and 10mg/kg/day of MT and 10mg/kg/day of NAC for 7 days showing tubular necrosis with regeneration of tubular lining (Hand E X 200).

Discussion

Oxidative stress is defined as an imbalance between oxidation and antioxidants characterized by disturbance in the prooxidant–antioxidant balance in favor of the former, leading to tissue damage.[38] Studies have implicated oxidative stress as one of the possible mechanisms of drug-induced renal toxicity;[39] hence antioxidants could have potential as treatments for drug-induced renal toxicity. This study was aimed at investigating the effects of NAC and MT on TD-induced alterations in renal function parameters and kidney oxidative stress indices of albino rats. In the present study, the serum levels of creatinine, urea, uric acid and kidney MDA, SOD, CAT and GSH levels were not significantly altered in MT and NAC treated rats. These observations are in agreement with reports by some authors.[40,41] On the other hand, dose-dependent increases in serum

levels of creatinine, urea and uric acid were obtained in TD-treated rats. These observations are in agreement with previous studies which reported increases in these parameters, but not in a dose-dependent manner.[42,43] Also, dose-dependent increases in kidney MDA with decreases in SOD, CAT and GSH levels were observed in TD-treated rats. These observations are in conjunction with previous results.[44] Serum creatinine, urea and uric acid concentrations are used as measures for the estimation of renal function;[45] hence observed increases in serum creatinine, urea and uric acid levels in TD-treated rats are signs of kidney damage. The examination of the H and E stained sections of the kidneys of TD-treated rats showed varying degrees of histological damage which were dose-dependent. This finding is in resonance with previous report.[46] The observations in the present study could be attributed to TD-induced oxidative stress (through the production of the mediators of renal vasoconstriction) which might have decreased glomerular filtration rate leading to the accumulation of serum creatinine, urea and uric acid.[47] Oxidative stress occurs when the antioxidant defense system is overwhelmed by the excessive production of reactive oxygen species.[48] Excess intracellular levels of oxidative radicals can cause peroxidation of polyunsaturated fatty acids characterized by an increase in MDA level and decreases in antioxidant levels.[49] This makes the measurement of MDA and the altered levels of endogenous antioxidants as indices for free radical generation and oxidative stress.[50] In the present study, dose-dependent increases in kidney MDA levels observed in TD- intoxicated rats are indications of lipid peroxidation and oxidative stress. Kidney tissue contains antioxidants such as SOD, CAT, and GSH to protect itself from the hazardous effects of oxidative attack. These antioxidants could be depleted in the presence of an over whelming oxidative stress;[51] hence decreases in antioxidants (SOD, CAT and GSH) levels observed in the kidneys of TD-treated rats suggest oxidative stress.

However, in the current study increases in serum creatinine, urea, uric acid, and kidney MDA levels observed in TD-treated rats were decreased in rats pretreated with MT and NAC. Also, kidney architectural damage observed in TD-treated rats was ameliorated by pretreatments with MT and NAC. Furthermore, TD-induced decreases in kidney levels of SOD, CAT and GSH were increased in MT and NAC pretreated rats. Best effects on these parameters were observed in rats pretreated with combined doses of MT and NAC. These observations are pointers to the possible beneficial effects of MT and NAC on TD-induced renal toxicity. In conjunction with findings in the current study, authors have reported the ameliorative effect of NAC in human and animal models of drug associated renal toxicity.[52-55] Also, MT have been reported to mitigate various forms of xenobiotic-induced renal toxicity in animal models.[56-60] The observed decreases in serum levels of creatinine, urea and uric acid in MT and NAC pretreated rats could be attributed to their inhibitory effects on free radical-induced kidney damage and renal vasoconstriction. Studies have shown that treatment with NAC ameliorate decline in glomerular filtration rate and stabilized plasma creatinine by reducing vasoconstriction.[61] Also, Conesa*et al.* found that NAC reduced renal vasoconstriction and improved renal blood flow.[62] The observed increases in SOD, CAT and GSH levels in NAC and MT pretreated rats could be attributed to the stimulatory synthesis and regeneration of these endogenous antioxidants by NAC and MT. MT has been reported to stimulate the activities of endogenous antioxidants and increase gene expression that improves the total antioxidant defense capacity of organisms.[63] Studies showed that NAC can maintain –SH groups of enzymes and membrane proteins in reduced state.[64] It can prevent kidney GSH depletion, provides more substrate for reactive intermediates that promote detoxification mechanisms and restores other antioxidant enzymes such as SOD and CAT.[65] The observed decreases in MDA levels in MT and NAC pretreated rats could be attributed to the incorporation of these antioxidants into cell membranes, thereby inhibiting TD-induced lipid peroxidation.

Furthermore, NAC is a source of sulfhydryl groups to cells which served as acetylated precursor of reduced GSH. It scavenges reactive oxygen and nitrogen species, thereby reducing their activities.[66] Studies in humans and animals showed that it is safe and effective and can be used as remedy for many diseases associated with oxidative stress.[67] MT and its metabolites have potent antioxidant and anti-inflammatory properties and have been shown to be highly effective in a variety of disorders linked to inflammation and oxidative stress.[68,69] MT can neutralize RNS and ROS species, stimulate several antioxidant systems and stabilize cell membranes.[70] It can modulate the gene expression of several protective enzymes, reduce apoptosis and lipid peroxidation.[71]

Conclusion

The present study demonstrated the protective potential of NAC and MT on tramadol - induced renal toxicity in albino rats. Based on the observations in the present study, MT and NAC could be used for the treatments of tramadol associated renal toxicity.

Acknowledgement

The authors wish to appreciate the technical

assistance offered by Mr Eze Iheukwumere of the Faculty of Pharmacy, Madonna University, Elele, Rivers State

Conflict of interests

The authors claim that there is no conflict of interest.

References

1. Moore KA, Cina SJ, Jones R, Selby DM, Levine B, Smith ML. Tissue distribution of tramadol and metabolites in an overdose fatality. Am J Forensic Med Pathol. 1999;20(1):98-100. doi:10.1097/00000433-199903000-00023
2. Nossaman VE, Ramadhyani U, Kadowitz PJ, Nossaman BD. Advances in perioperative pain management: Use of medications with dual analgesic mechanisms, tramadol & tapentadol. Anesthesiol Clin. 2010;28(4):647-66. doi:10.1016/j.anclin.2010.08.009
3. Senay EC, Adams EH, Geller A, Inciardi JA, Muñoz A, Schnoll SH, et al. Physical dependence on Ultram (tramadol hydrochloride): both opioid-like and atypical withdrawal symptoms occur. Drug Alcohol Depend. 2003;69(3):233-41. doi:10.1016/s0376-8716(02)00321-6
4. Lee HJ, Cha KE, Hwang SG, Kim JK, Kim GJ. In vitro screening system for hepatotoxicity: comparison of bone-marrow-derived mesenchymal stem cells and Placenta-derived stem cells. J Cell Biochem. 2011;112(1):49-58. doi:10.1002/jcb.22728
5. Dickman A. Tramadol: a review of this atypical opioid. Eur J Palliat Care. 2007;14(5):181-5.
6. Singhal PC, Sharma P, Sanwal V, Prasad A, Kapasi A, Ranjan R. et al. Morphine modulates proliferation of kidney fibroblasts. Kidney Int. 1998;53(2):350-7. doi:10.1046/j.1523-1755.1998.00758.x
7. Shadnia S, Soltaninejad K, Heydari K, Sasanian G, Abdollahi M. Tramadol intoxication: a review of 114 cases. Hum Exp Toxicol. 2008;27(3):201-5.
8. Awadalla E, Salah-Eldin A. Histopathological and Molecular Studies on Tramadol Mediated Hepato-Renal Toxicity in Rats. IOSR J Pharm Biol Sci. 2015;10(6):90-102.
9. Youssef SH, Zidan AHM. Histopathological and Biochemical Effects of Acute and Chronic Tramadol Drug Toxicity on Liver, Kidney and Testicular Function in Adult Male Albino Rats. Journal of Medical Toxicology and Clinical Forensic Medicine. 2016;2(4):(27)1-8. doi:10.15406/frcij.2016.02.00060
10. Droge W. Free radicals in the physiological control of cell function. Physiol Rev. 2002;82(1);47–95. doi:10.1152/physrev.00018.200
11. Masini A, Gallesi D, Giovannini F, Trenti T, Ceccarelli D. Membrane potential of hepatic mitochondria after acute cocaine administration in rats - the role of mitochondrial reduced glutathione. Hepatology. 1997;25(2): 385-90. doi:10.1053/jhep.1997.v25.pm0009021951
12. Abdel-Zaher AO, Abdel-Rahman MS, Elwasei FM. Protective effect of Nigella sativa oil against tramadol-induced tolerance and depend-ence in mice: role of nitric oxide and oxidative stress. Neurotoxicology. 2011;32(6):725-33. doi:10.1016/j.neuro.2011.08.001
13. Reiter RJ, Tan DX, Osuna C, Gitto E. Actions of melatonin in the reduction of oxidative stress. J Biomed Sci. 2000;7(6):444-58. doi:10.1007/bf02253360
14. Kumar KV, Naidu MU, Shifow AA, Prayag A, Ratnakar KS. Melatonin: An antioxidant protects against cyclosporineinduced nephrotoxicity. Transplantation. 1999;67(7):1065-8.
15. Reiter RJ, Guerrero JM, Garcia JJ, Acuna-Castroviejo D. Reactive oxygen intermediates, molecular damage, and aging: relation to melatonin. Ann N Y Acad Sci. 1998;854(1):410-24. doi:10.1111/j.1749-6632.1998.tb09920.x
16. Carrillo-Vico A, Guerrero JM, Lardone PJ, Reiter RJ. A review of the multiple actions of melatonin on the immune system. Endocrine. 2005;27(2):189-200. doi:10.1385/endo:27:2:189
17. Mayo JC, Sainz RM, Tan DX, Hardeland R, Leon J, Rodriguez C, et al. Anti-inflammatory actions of melatonin and its metabolites, N1-acetyl-N2-formyl-5-methoxykynuramine (AFMK) and N1-acetyl-5-methoxykynuramine(AMK), in macrophages. J Neuroimmunol. 2005;165(1-2):139-49. doi:10.1016/j.jneuroim.2005.05.002
18. Kumar KV, Naidu MU, Shiwow AA, Prayag A, Ratnakar KS. Melatonin: an antioxidantprotects against cyclosporine-induced nephropathy. Transplantation. 1999;67(7):1065-8. doi:10.1097/00007890-199904150-00022
19. Stacchiotti A, Lavazza A, Rezzani R, Bianchi R. Cyclosporine A-induced kidney alterations are limited by melatonin in rats: An electron microscope study. Ultrastruct Pathol. 2002;26(2):81-7. doi:10.1080/01913120252959254
20. Gass JT, Olive MF. Glutamatergic substrates of drug addiction and alcoholism. Biochem Pharmacol. 2008;75(1):218-65. doi:10.1016/j.bcp.2007.06.039
21. Tylicki L, Rutkowski B, Horl WH. Antioxidants: a possible role in kidney protection. Kidney Blood Press Res. 2003;26(5-6):303-14. doi:10.1159/000073936
22. Aruoma OI, Halliwell B, Hoey BM, Butler J. The antioxidant action of N-acetylcysteine: Its reaction with hydrogen peroxide, hydroxyl radical, superoxide, and hypochlorous acid. Free

Radic Biol Med. 1989;6(6):593-7. doi:10.1016/0891-5849(89)90066-x
23. Harrison PM, Keays R, Bray GP, Alexander GJ, Williams R. Improved outcome of paracetamol-induced fulminant hepatic failure by late administration of acetylcysteine. Lancet. 1990;335(8705):1572-3. doi:10.1016/0140-6736(90)91388-q
24. Tariq M, Morais C, Sobki S, Al Sulaiman M, AlKhader A. *N*-acetylcysteine attenuates cyclosporin-induced nephrotoxicity in rats. Nephrol Dial Transplant. 1999;14(4):923-9. doi:10.1093/ndt/14.4.923
25. Patel Manali B, Deshpande S, Shah G. Evaluation of vitamin E and N-acetyl cysteine in gentamicin-induced nephrotoxicity in rats. Ren Fail. 2011;33(3):341-7. doi:10.3109/0886022x.2011.560987
26. Sener G, Tosun O, Özer şehirli A, Kaçmaz A, Arbak S, Ersoy Y, et al. Melatonin and N-acetylcysteine have beneficial effects during hepatic ischemia and reperfusion. Life Sci. 2003;72(24):2707-18. doi:10.1016/s0024-3205(03)00187-5
27. Hafez ME, Issa YS, Abdel Rahman MS. Parenchymatous Toxicity of Tramadol: Histopathological and Biochemical Study. J Alcohol Drug Depend. 2015;3(5):1-6. doi:10.4172/2329-6488.1000225
28. Koksal M, Kurcer Z, Erdogan D, Iraz M, Tas M, Eren MA, et al. Effect of melatonin and n-acetylcysteine on hepatic injury in rat induced by methanol intoxication: a comparative study. Eur Rev Med Pharmacol Sci. 2012;16(4):437-44.
29. Efrati S, Averbukh M, Berman S, Feldman L, Dishy V, Katchko L, et al. N-acetylcysteine ameliorates amphotericin-induced nephropathy in rats. Nephrol Dial Transplant. 2005;20(1):65-70.
30. Kaplan M, Atakan IH, Aydoğdu N, Aktoz T, Puyan, FO, Şeren G, et al. The effect of melatonin on cadmium-induced renal injury in chronically exposed rats. Turk J Urol. 2009;35(2):139-47.
31. Junge W, Wilke B, Halabi A, Klein G. Determination of referenceintervals for serum creatinine, creatinine excretion and creatinineclearance with an enzymatic and a modified Jaffé method. Clin Chim Acta. 2004;344(1-2):137-48. doi:10.1016/j.cccn.2004.02.007
32. Kaplan A. The Determination of Urea, Ammonia and Urease. In: Glick D editor. Methods of Biochemical Analysis. California: John Wiley and Sons; 2007. Vol. 17.
33. Milton Prabu S, Shagirtha K, Renugadevi J. Quercetin in combination with vitamins (C and E) improves oxidative stress and renal injury in cadmium intoxicated rats. Eur Rev Med Pharmacol Sci. 2010;14(11):903-14.
34. Sun M, Zigman S. An improved spectrophotometric assay for superoxide dismutase based on epinephrine autoxidation. Anal Biochem. 1978;90(1):81-9. doi:10.1016/0003-2697(78)90010-6
35. Aebi H. Catalase in vitro. In: Colowick SP, Kaplane NO, editors. Method in Enzymology. New York: Academic Press; 1984.
36. Sedlak J, Lindsay RH. Estimation of total, protein bound, and nonprotein sulfhydryl groups in tissue with Ellman'sreagent. Anal Biochem. 1968;25:192-205. doi:10.1016/0003-2697(68)90092-4
37. Buege JA, Aust SD. Microsomal lipid peroxidation. Meth Enzymol 1978;52:302-10. doi:10.1016/s0076-6879(78)52032-6
38. Sies H. Oxidative stress: from basic research to clinical application. Am J Med. 1991;91(3C):31-8.
39. Santos NAG, Catão CS, Martins NM, Curti C, Bianchi MLP , Santos AC. Cisplatin-induced nephrotoxicity is associated with oxidative stress, redox state unbalance, impairment of energetic metabolism and apoptosis in rat kidney mitochondria. Arch Toxicol. 2007;81(7):495-504. doi:10.1007/s00204-006-0173-2
40. Kamalakkannan N, Rukkumani R, Aruna K, Suresh P, Viswanathan P, Padmanabhan V. Protective Effect of N-Acetyl Cysteine in Carbon Tetrachloride-Induced Hepatotoxicity in Rats. Iranian J Pharmacol Ther. 2005;4(2):118-23.
41. Adikwu E, Braimbaifa N, Obianime AW. Melatonin and Alpha Lipoic Acid: Possible Mitigants for Lopinavir/Ritonavir- Induced Renal Toxicity in Male Albino Rats. Physiol Pharmacol. 2015;19(4):232-40.
42. Atici S, Cinel I, Cinel L, Doruk N, Eskandari G, Oral U. Liver and kidney toxicity in chronic use of opioids: an experimental long term treatment model. J Biosci. 2005;30(2):245-52. doi:10.1007/bf02703705
43. Ali OK, Ahmed A, Mawlood A. Effects of Tramadol on Histopathological and Biochemical Parameters in Male Rabbits. American Journal of Biology and Life Sciences. 2015;3(3):85-90.
44. Elkhateeb A, El Khishin I, Megahed O, Mazen F. Effect of *Nigella sativa Linn* oil on tramadol-induced hepato- and nephrotoxicity in adult male albino rats. Toxicol Rep. 2015;2:512-9. doi:10.1016/j.toxrep.2015.03.002
45. Yasmeen T, Qureshi GS, Perveen S. Adverse effects of Diclofenac sodium on renal parenchyma of adult albino rats. J Pak Med Assoc. 2007;57 (7):349-51.
46. Traynor J, Mactier R, Geddes CC, Fox JG. How to Measure Renal Function in Clinical Practice.

Br Med J. 2006;333:733-7. doi:10.1136/bmj.38975.390370.7C
47. Garcia-Cohen EC, Marin J, Diez-Picazo LD, Baena AB, Salaices M, Rodriguez-Martinez MA. Oxidative stress induced by tert-butylhydroperoxide causes vasoconstriction in the aorta from hypertensive and aged rats: role of cyclooxygenase- 2 isoform. J Pharmacol Exp Ther. 2000;293(1):75-81.
48. Nencini C, Giorgi G, Micheli L. Protective effect of silymarin on oxidative stress in rat brain. Phytomedicine. 2007;14(2-3):129-35. doi:10.1016/j.phymed.2006.02.005
49. Rahman K. Studies on free radicals, antioxidants, and co-factors. Clin Interv Aging. 2007;2(2):219-36.
50. El-Khatib AS, Moustafa AM, Abdel-Aziz H, Al-Shabanah OA, El-Khassef HA . Effect of aminoguanidineand desferrioxamine on some biochemical changes associated with streptozotocin induced hyperglycemiain rats. Pharmacol Res. 2001;43(3):233-40. doi:10.1006/phrs.2000.0772
51. Sies H. Oxidative Stress: Oxidants and Antioxidants. London: Academic Press; 1991.
52. Li Z, Nickkholgh A, Yi X, Bruns H, Gross ML, Hoffmann K, et al. Melatonin protects kidney grafts from ischemia/reperfusion injury through inhibition of NF-kB and apoptosis after experimental kidney transplantation. J Pineal Res. 2009;46(4):365-72. doi:10.1111/j.1600-079x.2009.00672.x
53. Luo J, Tsuji T, Yasuda H, Sun Y, Fujigaki Y, HishidaA .The molecular mechanisms of the attenuation of cisplatin-induced acute renal failure by N-acetylcysteine in rats. Nephrol Dial Transplant. 2008;23(7):2198-205. doi:10.1093/ndt/gfn090
54. Kim JH, Lee SS, Jung MH, Yeo HD, Kim HJ, Yang JI, et al. N-acetylcysteine attenuates glycerol-induced acute kidney injury by regulating MAPKs and Bcl-2 family proteins. Nephrol Dial Transplant. 2010;25(5):1435-43. doi:0.1093/ndt/gfp659
55. Heloisa M, Shimizu M, Coimbra TM, De Araujo M, Menezes LF, Seguro AC . N-acetylcysteine attenuates the progression of chronic renal failure. Kidney Int. 2005;68(5):2208-17. doi:10.1111/j.1523-1755.2005.00677.x
56. Lee IC, Kim SH, Lee SM, Baek HS, Moon C, Kim SH, et al. Melatonin attenuates gentamicin-induced nephrotoxicity and oxidative stress in rats. Arch Toxicol. 2012;86(10):1527-36. doi:10.1007/s00204-012-0849-8
57. Nasri H, Mubarak M. Contrast induced nephropathy has to be differentiated from kidney injury due to atheroembolic disease. J Renal Inj Prev. 2013;2(3):107-8. doi:10.12861/jrip.2013.34
58. Kilic U, Kilic E, Tuzcu Z, Tuzcu M, Ozercan IH, Yilmaz O, et al. Melatonin suppresses cisplatin-induced nephrotoxicity via activation of Nrf-2/HO-1 pathway. Nutr Metab. 2013;10(1):1-7. doi:10.1186/1743-7075-10-7
59. Ozguner F, Oktem F, Armagan A, Yilmaz R, Koyu A, Demirel R, et al. Comparative analysis of the protective effects of melatonin and caffeic acid phenethyl ester (CAPE) on mobile phone-induced renal impairment in rat. Mol Cell Biochem. 2005;276(1-2):31-7. doi:10.1007/s11010-005-2734-8
60. Zararsiz I, Sarsilmaz M, Tas U, Kus I, Meydan S, Ozan E. Protective effect of melatonin against formaldehyde-induced kidney damage in rats. Toxicol Ind Health. 2007;23(10):573-9. doi:10.1177/0748233708089022
61. Nitescu N, Ricksten SE, Marcussen N, Haraldsson B, Nilsson U, Basu S, et al. N-acetylcysteine attenuates kidney injury in rats subjected to renal ischaemia-reperfusion. Nephrol Dial Transplant. 2006;21(5):1240-7. doi:10.1093/ndt/gfk032
62. Conesa EL, Valero F, Nadal JC, Fenoy FJ, López B, Arregui B, et al. N-acetyl-L-cysteine im proves renal medullary hypoperfusion in acute renal failure. Am J Physiol Regul Integr Comp Physiol. 2001;281(3):730-7.
63. Antolin I, Rodriguez C, Sainz RM, Mayo JC, Uria H, Kotler ML, et al. Neurohormone melatonin prevents cell damage:effect on gene expression for antioxidant enzymes. FASEB J. 1996;10(8):882-90.
64. Wagner PD, Mathien-Castello O, Bebout DE, Gray AT, Natterson PD, Glennow C. Protection against pulmonary O2 toxicity by N-acetyl cysteine. Eur Respir J. 1989;2(2):116-26.
65. Zhao C, Shichi H. Prevention of acetaminophen-induced cataract by a combination of diallyl disulfide and N-acetyl cysteine. J Ocul Pharmacol Ther. 1998;14(4):345-55. doi:10.1089/jop.1998.14.345
66. Zafarullah M, Li WQ, Sylvester J, Ahmad M. Molecular mechanisms of N-acetylcysteine actions. Cell Mol Life Sci. 2003;60(1):6-20. doi:10.1007/s000180300001
67. Cotgreave IA. N-acetylcysteine: pharmacological considerations and experimental and clinical applications. Adv Pharmacol. 1997;38:205-27. doi:10.1016/s1054-3589(08)60985-0
68. Sezgin G, Oztürk G, Güney S, Sinanoğlu O, Tunçdemir M. Protective effect of melatonin and 1, 25-dihydroxyvitamin D3 on renal ischemia-reperfusion injury in rats. Ren Fail. 2013;35(3):374-9. doi:10.3109/0886022x.2012.760409
69. Mayo JC, Sainz RM, Tan DX, Hardeland R, Leon J, Rodriguez C. et al. Anti-inflammatory

actions of melatonin and its metabolites, N1-acetyl-N2-formyl-5-methoxykynuramine (AFMK) and N1-acetyl-5-methoxykynuramine (AMK), in macrophages. J Neuroimmunol. 2005;165(1-2):139-49. doi:10.1016/j.jneuroim.2005.05.002

70. Rodriguez C, Mayo JC, Sainz RM, Antolin I, Herrera F, Martin V, et al. Regulation of antioxidant enzymes: a significant role for melatonin. J Pineal Res. 2004;36(1):1–9. doi:10.1046/j.1600-079x.2003.00092.x
71. Reiter RJ, Guerrero JM, Garcia JJ, Acuna-Castroviejo D. Reactive oxygen intermediates, molecular damage, and aging. Relation to melatonin. Ann N Y Acad Sci. 1998;854(1):410-24. doi:10.1111/j.1749-6632.1998.tb09920.x