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Research Article

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

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A B S T R A C T

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.

Dose (mg/kg)	Serum uric acid(mg/dL)			
	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.

Dose (mg/kg)	kidney malondialdehyde (MDA) (U/mg protein)			
	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.

Dose (mg/kg)	Kidney superoxide dismutase (SOD) (U/mg protein)			
	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.

Drug (mg/kg)	Kidney catalase (U/mg protein)			
	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.

| Dose (mg/kg)	Kidney glutathione (GSH) (µg/mg protein)			
	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.
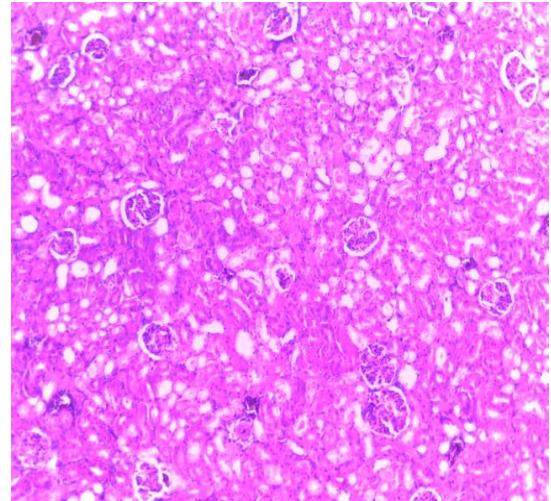[b]Significant ($p < 0.05$) difference when compared to TD- pretreated rats.
[c]Significant ($p < 0.05$) difference when compared to MT- pretreated rats.
[d]Significant ($p < 0.05$) difference when compared to NAC- pretreated rats
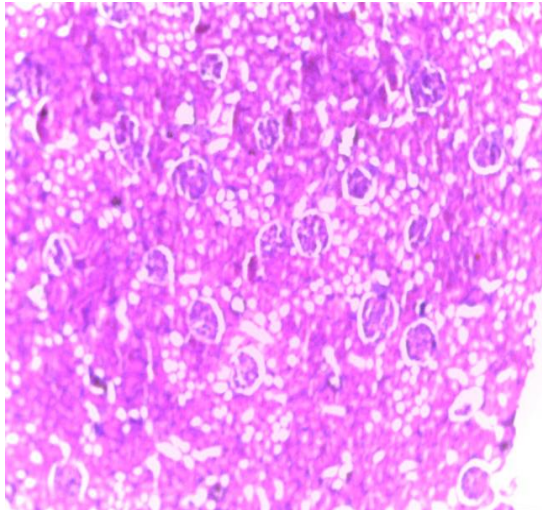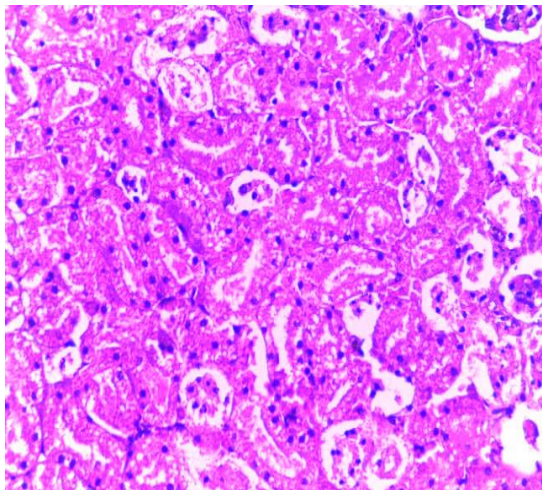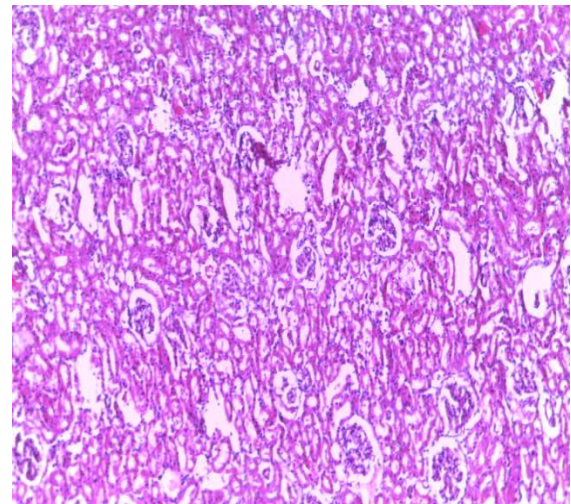
Figure 1. Micrograph of the kidney of control rat treated with normal saline for 7 days showing normal kidney architecture (Hand E X 200).

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).
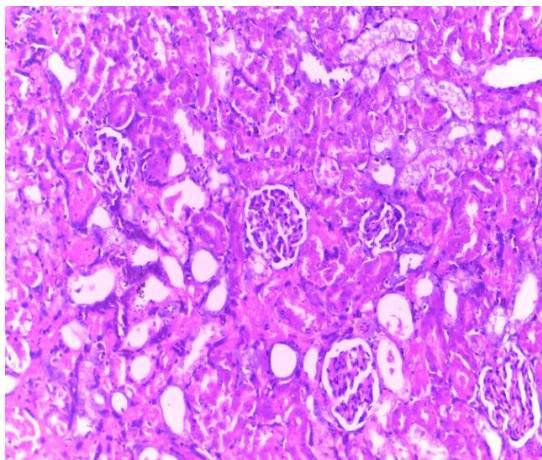

Figure 4. Micrograph of the kidney of rat treated with 15 mg/kg/day of TD and 10mg/kg/day of MT for 7 days showing tubular necrosis (Hand E X200).

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

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

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