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ORIGINAL ARTICLE

Synergistic effect of ascorbic acid and taurine in the treatment of a spinal cord injury-induced model in rats

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Abstract

Spinal cord injury (SCI) results in severe damage, which causes functional alterations together with loss of autonomic functions, sensations, and muscle functioning. This injury leads to apoptosis of neurons and oligodendrocytes, which further leads to dysfunction of the spinal cord due to axonal degeneration and demyelination. Taurine is non-proteogenic and an essential amino acid, which plays a major role in the growth and development of brain cells. Ascorbic acid, also known as vitamin C, is found in various foods and is known to prevent scurvy. In this study, we have investigated the therapeutic effect of ascorbic acid and taurine against SCI-induced rats. The rats were divided into the following groups: sham, control, 100 mg/kg of taurine, 100 mg/kg of ascorbic acid, and 100 mg/kg of taurine + 100 mg/kg of ascorbic acid. Treatment was continued daily for 45 consecutive days. The combined treatment of taurine and ascorbic acid decreased caspase-3, bax, pro-NGF, and p53 mRNA expression by more than 30% compared to individual treatments. The combined treatment of taurine and ascorbic acid reduced caspase-3 and p53 expression by 33.7% and 44%, respectively, compared to individual treatments. The combined treatment of taurine and ascorbic acid decreased mRNA expression of interleukin-6 (IL-6), cyclooxygenase-2, tumor necrosis factor-alpha (TNF-α), and inducible nitric oxide synthase (iNOS) compared to the individual treatments of taurine and ascorbic acid. The combined treatment of taurine and ascorbic acid also significantly recovered altered antioxidant markers, and induced lipid peroxidation to near normal levels. In summary, apoptotic, inflammatory and oxidative stress markers were significantly decreased in SCI-induced rats treated with taurine and ascorbic acid.

Keywords Taurine · Ascorbic acid · Spinal cord injury · Apoptosis · Inflammation

Introduction

Spinal cord injury (SCI) results in severe damage, which causes functional alterations along with loss of autonomic functions, sensations, and muscle functioning (Krishna et al. 2013). Previous studies have reported that SCI leads to apoptosis of neurons and oligodendrocytes, which further leads to dysfunction of the spinal cord due to axonal degeneration and demyelination (Minakov et al. 2018). Additional studies have reported that SCI induces apoptosis and a higher rate of inflammation and oxidative stress in neurons and oligodendrocytes (Bao and Liu 2002). The proinflammatory cytokines and reactive oxygen species (ROS) are released from microglia during inflammatory processes and neurodegeneration (Min et al. 2004). Hussein et al. (2018) reported that interleukins (ILs) are key cytokines that play important roles in immunological reactions. Thus, an effective therapeutic agent is needed against inflammation and oxidative stress.

Taurine is a non-proteogenic and essential amino acid, which plays a major role in the growth and development of brain cells (Schuller-Levis and Park 2003; Wang et al. 2016). Previous studies have reported the active role of taurine in osmoregulation, neurotransmission, calcium homeostasis, and prevention of seizures (Leon et al. 2009; Tsuboyama-Kasaoka et al. 2006; Olive 2002). An additional study has reported the antioxidant potential of taurine against oxidative stress (Zhang et al. 2004). Yanagita et al. (2008) reported the reduction of apolipoprotein B100 and lipid secretion in liver cancer cells, and Nakajima et al. (2010) reported the therapeutic potential of taurine against inflammatory responses in SCI. Ascorbic acid, also known as vitamin C, is found in various foods and is known to prevent scurvy (Yan et al.

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Table 1 List of primers used in real-time PCR reactions

S. no.	Gene name	Sense primer	Anti-sense primer
1	TNF-α	5′-CCCAGACCCTCACACTCAGAT-3′	5′-TTG TCC CTTGAA GAG AAC CTG-3′
2	IL-6	5′-AAGTTTCTCTCCGCAAGATAC TTCCAGCCA-3′	5′-AGG CAAATTTCCTGGTTATATCCA GTTT-3′
3	Cyclooxygenase-2	5′-CCA TGT CAA AAC CGT GGTGAATG-3′	5′-ATG GGAGTTGGGCAGTCATCAG-3′
4	iNOS	5′-CTCCATGACTCTCAGCACAGAG-3′	5′-GCACCGAAGATATCCTCATGAT-3′
5	p53	5′-TAACAGTTCCTGCATGGGCGGC-3′	5′-AGGACAGGCACAAACACGCACC-3′
6	Bax	5′-TGG AGCTGCAGAGGATGATTG-3′	5′-GAAGTTGCCGTCAGAAAACATG-3′
7	Caspase-3	5′-TTAATAAAGGTATCCATGGAGAACACT-3′	5′-TTAGTGATAAAAATAGAGTTCTTTTGTGAG-3’
8	Bcl-2	5′-CAC CCC TGG CAT CTT CTC CTT-3′	5′-AGC GTC TTC AGA GAC AGC CAG-3′
9	pro-NGF	5′-CTTCAGCATTCCCTTGACAC-3′	5′-TGAGCACACACACGCAGGC-3′
10	GAPDH	5′-TCCCTCAAGATTGTCAGCAA-3′	5′-AGATCCACAACGGATACATT-3′

2014). Yan et al. (2012) have reported the supplementation of high dose of vitamin C improves the function recovery of SCI. Wang et al. (2015) reported the therapeutic effects of ascorbic acid against SCI-induced rats, and Katoh et al. (1996) reported the protective effect of dietary ascorbic acid against spinal cord compression injury in a rat model. Thus, the present study evaluated the synergistic effects of ascorbic acid and taurine in SCI-induced male albino rats.

Materials and methods

Rats

Male albino Wistar strain rats (180–210 g, 3–4 months old) were obtained from the Animal center of the Academy of Military medical sciences, China. All of the rats were kept in standard rat polypropylene cages (435 × 290 × 150 mm; six rats in each cage) and maintained under standard lighting condition (12 h light/dark) periods with a relative humidity

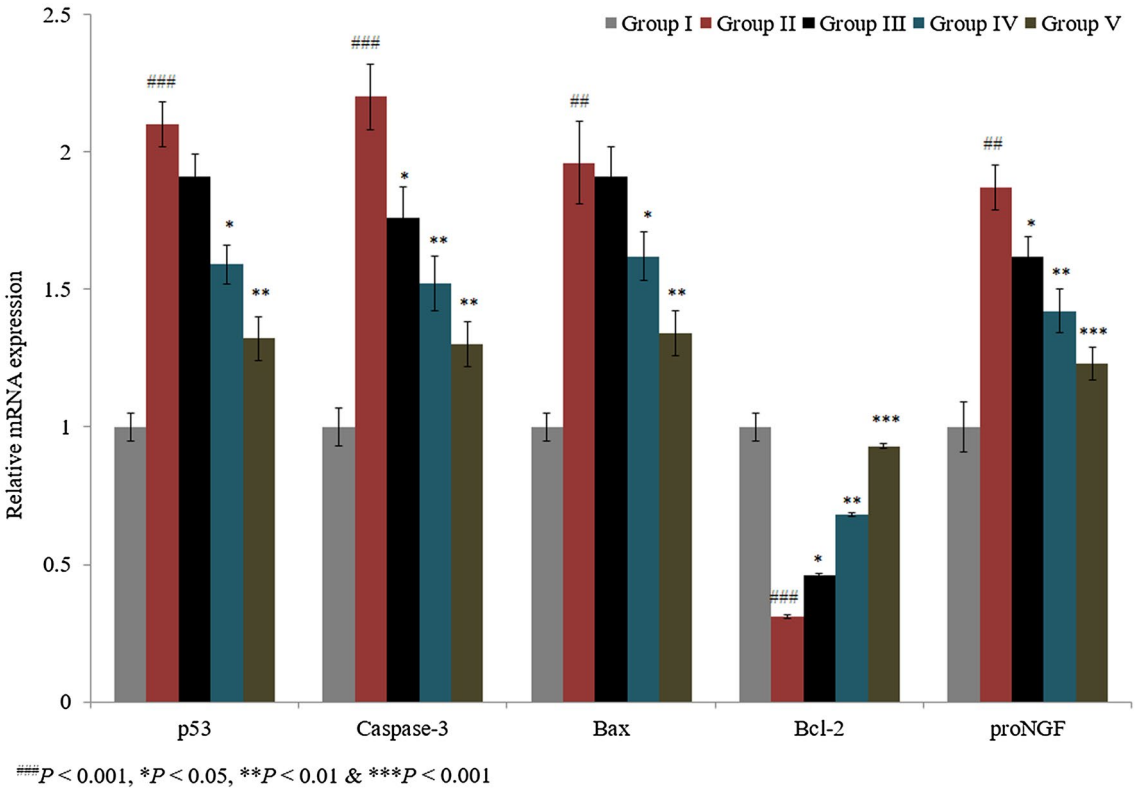

Fig. 1 Synergistic protective effect of taurine and ascorbic acid on p53, caspase-3, bax, bcl-2, and pro-NGF mRNA expression. The combined treatment of taurine and ascorbic acid recovered the altered mRNA expression levels. *$P < 0.05$, **$P < 0.01$, and ***$P < 0.001$ vs. control; ###$P < 0.001$ vs. sham control

of 60 ± 5% and temperature of 25 ± 0.5 °C with food and water provided ad libitum. Animal experiments were approved by the ethical committee (201308819) of Tianjin Hospital, Tianjin and China.

Experimental groups

SCI was induced in rats as previously reported (Krishna et al. 2013). Rats were divided into the following groups: group I, sham; group II, control (SCI); group III, 100 mg/kg of taurine; group IV, 100 mg/kg of ascorbic acid; and group V, 100 mg/kg of taurine + 100 mg/kg of ascorbic acid. Sham and control rats were given saline, and treated rats received 1 mL of taurine or ascorbic acid. Treatment was continued daily for 45 consecutive days.

Collection of blood

Blood was collected at the end of treatment by cardiac puncture with the use of a syringe (5 mL) with a 23-gauge needle. The animals were then anesthetized using xylazine (10 mg/kg body weight) and ketamine hydrochloride (100 mg/kg body weight), and sacrificed by decapitation.

Tissue homogenate preparation

Rats were sacrificed by decapitation following anesthetization with ketamine hydrochloride (100 mg/kg body weight) and xylazine (10 mg/kg body weight). Spinal cord samples (12 mm) were obtained from the operated region of the rats. Spinal cord tissue was sliced into several pieces and homogenized in Tris–HCl buffer (50 mM, pH 7.4) for 10 min at 6000 rpm. The homogenate was then centrifuged, and the supernatant was preserved at 4 °C for experiments.

Apoptosis

p53, caspase-3, bax, bcl-2, and pro-NGF mRNA expression levels were determined as previously described (Bernal et al. 2005). Total RNA was isolated from the spinal cord tissue and transcribed into cDNA. Primers used for the

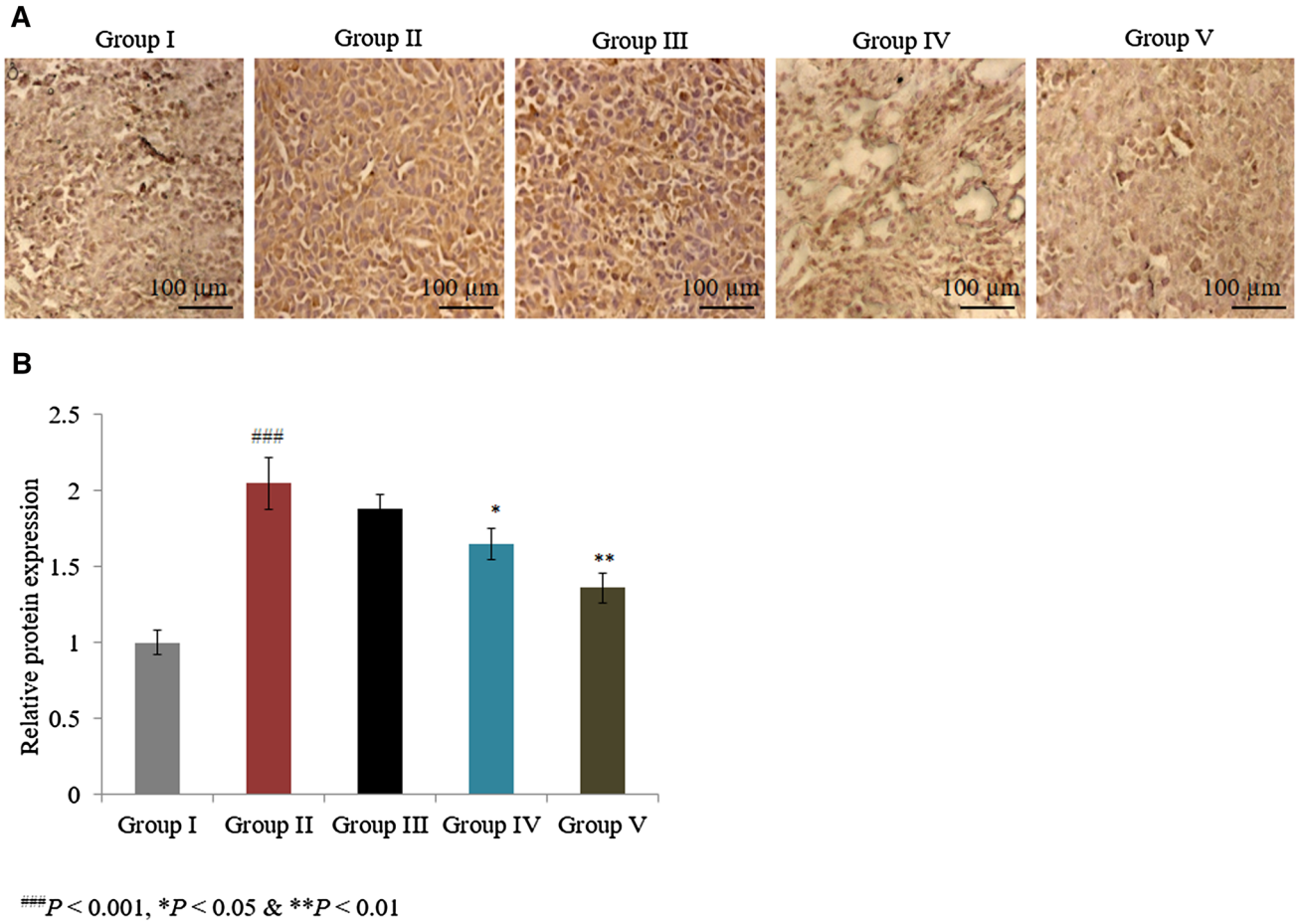

Fig. 2 Synergistic protective potential of taurine and ascorbic acid on the protein expression of caspase-3. The combined treatment of taurine and ascorbic acid recovered the altered protein expression. $^{*}P<0.05$, $^{**}P<0.01$, and $^{***}P<0.001$ vs. control; $^{\#\#\#}P<0.001$ vs. sham control. Scale bar 100 µm, $N=6$

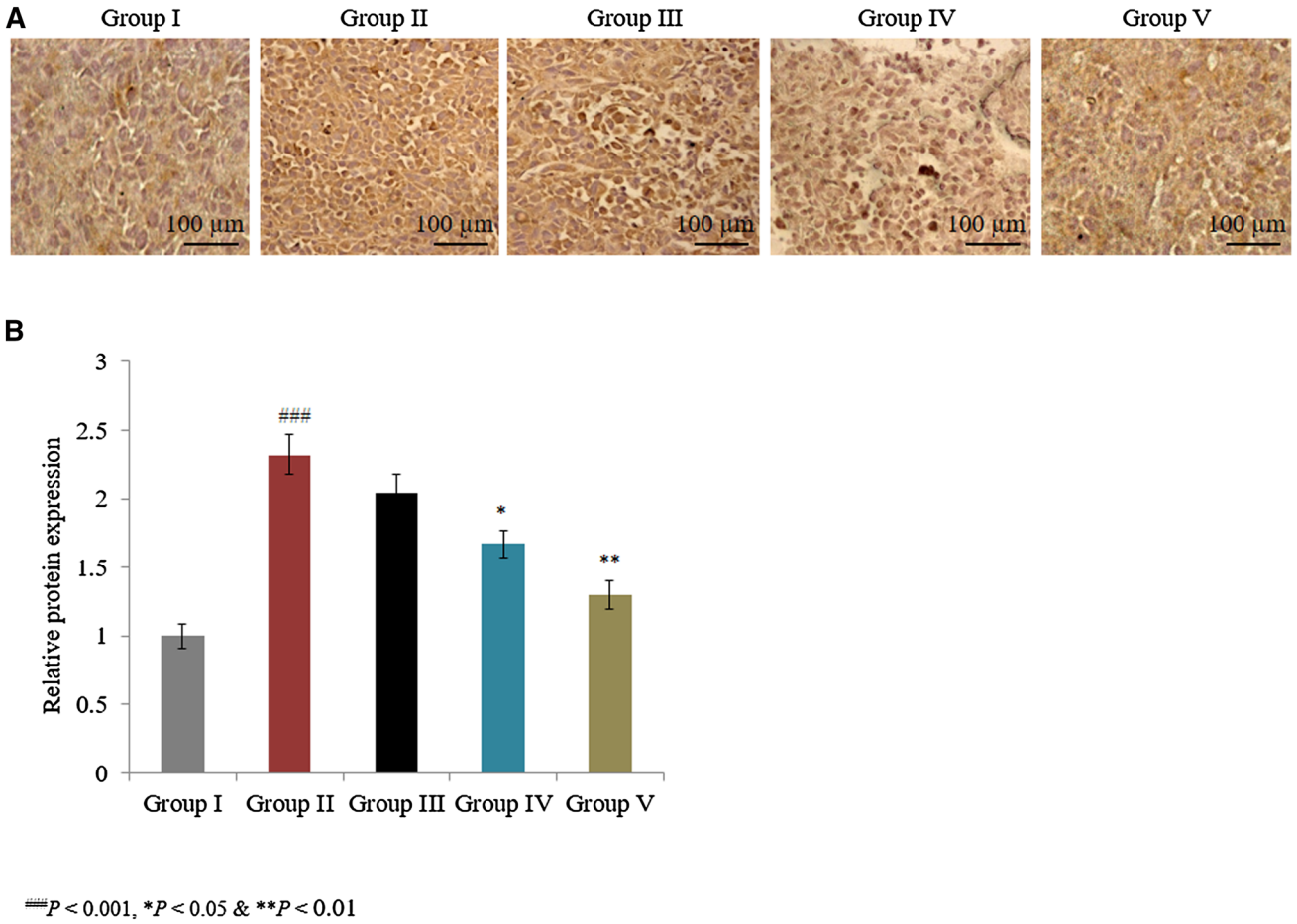

Fig. 3 Synergistic protective potential of taurine and ascorbic acid on the protein expression of p53. The combined treatment of taurine and ascorbic acid recovered the altered protein expression. $^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$ vs. control; $^{\#\#\#}P < 0.001$ vs. sham control. Scale bar 100 µm, $N = 6$

amplification of these genes using real-time PCR are listed in Table 1. Protein expression of caspase-3 (ab4051, Abcam) and p53 (ab131442, Abcam) in the spinal cord tissue was determined according to Ali et al. (2017).

Inflammation

The nitrite level was determined as a marker of nitric oxide (NO) content as previously described (Shaheen et al. 2016). Serum levels of tumor necrosis factor (TNF)-α and interleukin-6 (IL-6) were determined according to Afshari et al. (2005). IL-6, cyclooxygenase-2, inducible nitric oxide synthase (iNOS), and TNF-α mRNA expression levels were determined according to Bernal et al. (2005). Table 1 lists the primers used for real-time PCR.

Oxidative stress

Catalase, superoxide dismutase (SOD), and glutathione peroxidase (Gpx) activities were determined according to Kaddour et al. (2016). Reduced glutathione (GSH), ROS, and lipid peroxidation were also determined as previously described (Arutyunyan et al. 2016).

Statistical analysis

Experimental results are expressed as mean ± standard deviation. The results were compared using a one-way analysis of variance (ANOVA), followed by Tukey's post hoc test. The difference between control and treated samples was considered significant at $P < 0.05$.

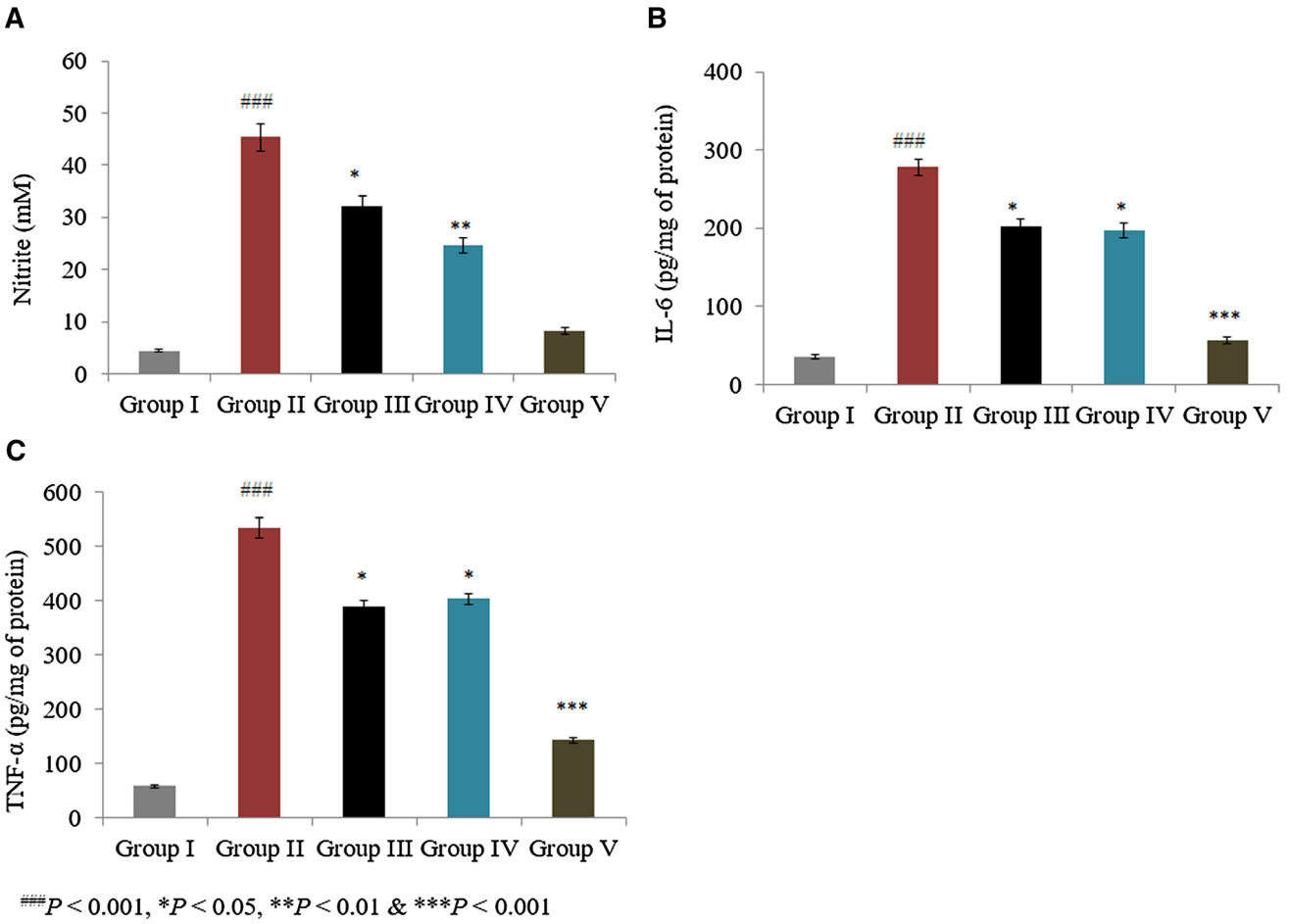

Fig. 4 Synergistic protective effect of taurine and ascorbic acid on the levels of nitric oxide (NO), interleukin (IL)-6, and tumor necrosis factor (TNF)-α. The combined treatment of taurine and ascorbic acid recovered the altered protein expression levels. Serum levels of NO (**a**), IL-6 (**b**), and TNF-α (**c**). $^{*}P<0.05$, $^{**}P<0.01$, and $^{***}P<0.001$ vs. control; $^{\#\#\#}P<0.001$ vs. sham control. Scale bar 100 µm, $N=6$

Results

The effect of taurine and ascorbic acid on apoptotic markers

We showed the synergistic effect of ascorbic acid and taurine in a spinal cord injury-induced rat model. Caspase-3, p53, bax, bcl-2, and pro-NGF mRNA expression levels were quantified using RT-PCR. p53, caspase-3, bax, and pro-NGF mRNA expression levels were substantially increased by 110%, 120%, 96%, and 87%, respectively, in control rats, whereas bcl-2 mRNA expression was decreased by 69% in control rats (Fig. 1; $P<0.05$). The individual treatments of taurine and ascorbic acid reduced mRNA levels of p53 by 9% and 24.3% in groups III and IV, respectively. However, the combined treatment of taurine and ascorbic acid decreased p53 mRNA by 37.1% in group V (Fig. 1; $P<0.05$).

In a similar manner, the combined treatment of taurine and ascorbic acid decreased caspase-3, bax, and pro-NGF mRNA expression by more than 30% in group V (Fig. 1; $P<0.05$). The individual treatments of taurine and ascorbic acid increased the mRNA expression of bcl-2 by 48% and 119.4% in groups III and IV, respectively, and the combined treatment of taurine and ascorbic acid significantly increased bcl-2 mRNA expression by 200% in group V (Fig. 1; $P<0.05$). Immunohistochemical analysis revealed that caspase-3 and p53 protein expression was significantly increased by 105% and 132%, respectively, in control rats (Figs. 2, 3; $P<0.05$). The individual treatments of taurine and ascorbic acid reduced caspase-3 protein expression by 8.3% and 19.5% in groups III and IV, respectively, whereas p53 expression was reduced by 12.1% and 28%, respectively

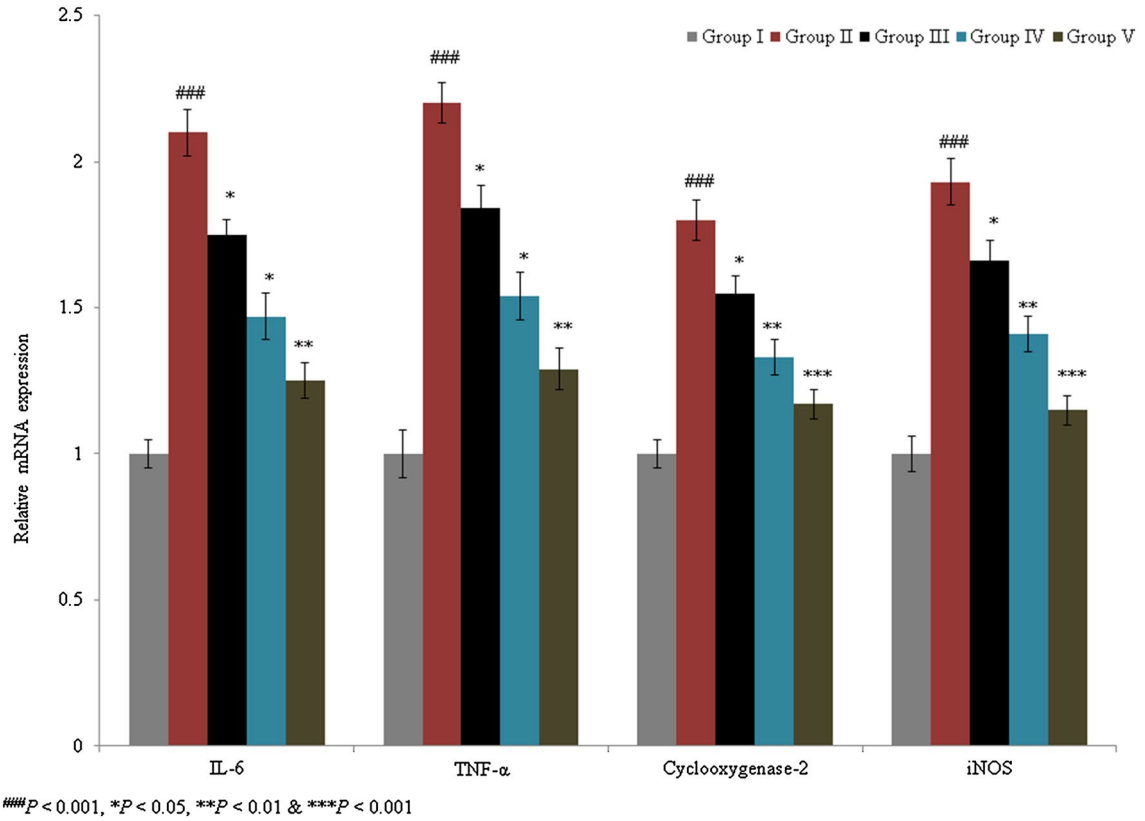

Fig. 5 Synergistic protective effects of taurine and ascorbic acid on interleukin (IL-6), cyclooxygenase-2, tumor necrosis factor (TNF-α), and inducible nitric oxide synthase (iNOS) mRNA expression. The combined treatment of taurine and ascorbic acid recovered the altered mRNA expression levels. $^{*}P<0.05$, $^{**}P<0.01$, and $^{***}P<0.001$ vs. control; $^{\#\#\#}P<0.001$ vs. sham control

(Figs. 2, 3). However, the combined treatment of taurine and ascorbic acid significantly reduced caspase-3 and p53 expression by 33.7% and 44%, respectively, in group V (Figs. 2, 3; $P<0.05$).

The effect of taurine and ascorbic acid on inflammatory markers

Serum nitrite level was determined as a marker of NO levels. Serum NO content was 3.7 mM in sham rats, which was significantly increased by more than 900% in control rats. The individual treatments of taurine and ascorbic acid reduced NO levels by 28.9% and 45.5%, respectively, in groups III and IV. However, the combined treatment of taurine and ascorbic acid decreased the NO level by 81.9% in group V (Fig. 4a; $P<0.05$). In a similar manner, the combined treatment of taurine and ascorbic acid significantly reduced IL-6 and TNF-α expression by more than 30% in group V (Fig. 4b, c; $P<0.05$). IL-6, cyclooxygenase-2, TNF-α, and iNOS mRNA levels were substantially increased by 110%, 80%, 120%, and 93%, respectively, in control rats. The combined treatment of taurine and ascorbic acid decreased IL-6, cyclooxygenase-2, TNF-α, and iNOS mRNA levels when compared with the individual treatments of taurine and ascorbic acid (Fig. 5; $P<0.05$).

The effect of taurine and ascorbic acid on oxidative markers

The ROS level was substantially increased by 444.2% in control rats. The individual treatments of taurine and ascorbic acid reduced ROS levels by 30.4% and 33.1%, respectively, in groups III and IV. However, the combined treatment of taurine and ascorbic acid significantly reduced ROS levels by 76.9% in group V (Fig. 6; $P<0.05$). SOD, catalase, and Gpx activities, as well as GSH content, were significantly decreased in control rats. However, the combined treatment of taurine and ascorbic acid significantly recovered these altered oxidative markers to normal levels (Table 1; $P<0.05$). Lipid peroxidation was significantly reduced to near normal levels following combined treatment of taurine and ascorbic acid.

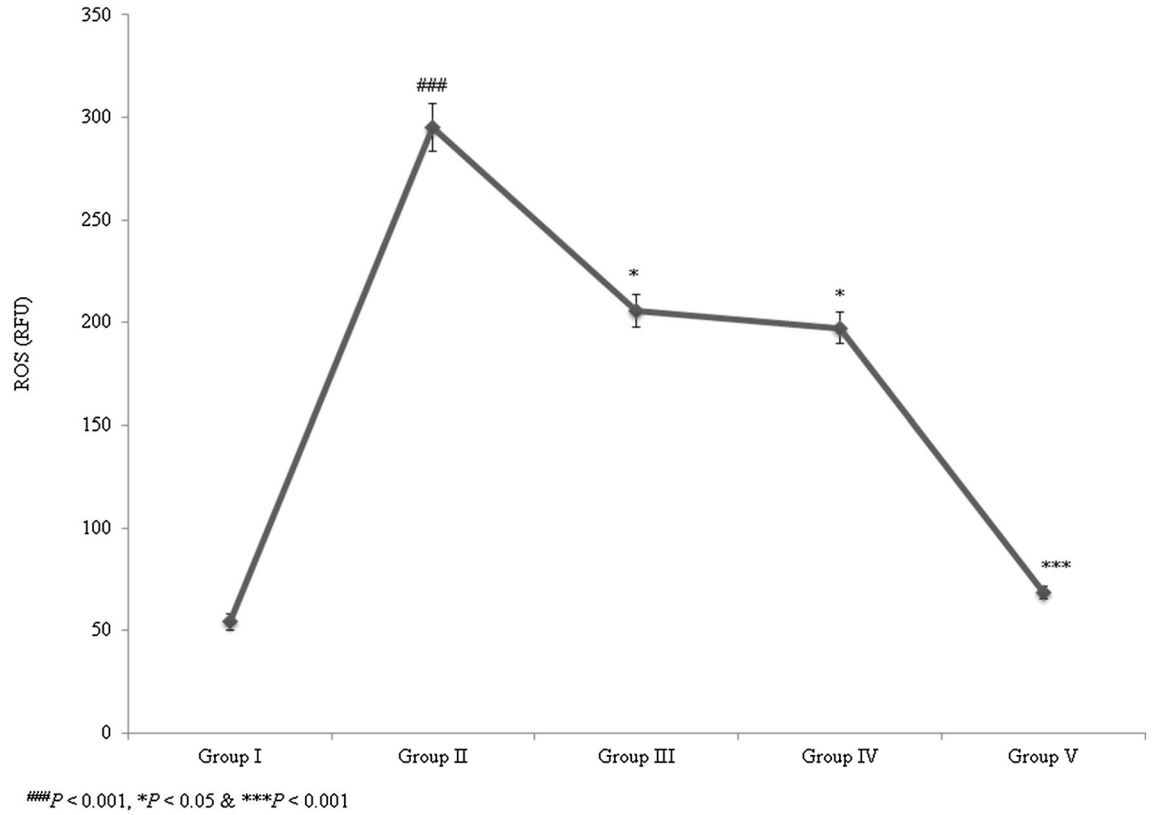

Fig. 6 Synergistic protective effect of taurine and ascorbic acid on reactive oxygen species (ROS) levels. The combined treatment of taurine and ascorbic acid reduced ROS levels. $^{*}P<0.05$, $^{**}P<0.01$, and $^{***}P<0.001$ vs. control; $^{\#\#\#}P<0.001$ vs. sham control

Table 2 Therapeutic effect of taurine and ascorbic acid on MDA, GSH, SOD, catalase and Gpx in spinal cord injury-induced male albino rats

Oxidative markers	Group I (Sham)	Group II (control)	Group III (100 mg/kg of taurine)	Group IV (100 mg/kg of ascorbic acid)	Group V (100 mg/kg of taurine + 100 mg/kg of ascorbic acid)
GSH (mg/g)	81.4 ± 3.15	30.2 ± 1.2$^{\#\#\#}$	45.2 ± 2.2*	48.4 ± 2.5*	75.9 ± 3.8***
SOD (U/mg)	8.2 ± 0.2	3.3 ± 0.1$^{\#\#\#}$	4.9 ± 0.2*	5.2 ± 0.2*	7.8 ± 0.3***
Catalase (U/g)	14.2 ± 1	4.1 ± 0.1$^{\#\#\#}$	7.8 ± 0.2*	8.6 ± 0.3*	12.7 ± 0.7***
Gpx (mg/protein)	0.98 ± 0.001	0.31 ± 0.005$^{\#\#}$	0.52 ± 0.003*	0.67 ± 0.005*	0.84 ± 0.01***
MDA (nmol/g)	20.5 ± 1.1	77 ± 3$^{\#\#\#}$	51.6 ± 2*	49.5 ± 2.5*	28.4 ± 1.2***

$^{\#\#}P<0.01$, $^{\#\#\#}P<0.001$, $^{*}P<0.05$, $^{***}P<0.001$

Discussion

In the present study, we showed the synergistic effect of taurine and ascorbic acid in the SCI-induced rat model. Studies have reported that SCI leads to apoptosis of neurons and oligodendrocytes, which further leads to dysfunction of the spinal cord due to axonal degeneration and demyelination (Minakov et al. 2018). Studies have reported that SCI induces apoptosis and a higher rate of inflammation and oxidative stress in neurons and oligodendrocytes (Bao and Liu 2002). The proinflammatory cytokines and reactive oxygen species (ROS) are released from microglia during inflammatory processes and neurodegeneration. Hussein et al. (2018) have reported that ILs are key cytokines that play vital roles in immunological reactions. In the present study, expression levels of caspase-3, bax, bcl-2, p53, and pro-NGF were significantly reduced in SCI-induced rats treated with taurine and ascorbic acid, which confirmed the protective role of taurine and ascorbic acid against SCI (Table 2).

Studies have reported that treatment with sufficient amount of antioxidants was effective against various neurodegenerative diseases (Liu et al. 2002). Studies have also

reported the functional role of taurine in osmoregulation, neurotransmission, calcium homeostasis, and the prevention of seizures (Leon et al. 2009; Tsuboyama-Kasaoka et al. 2006; Olive 2002), and other studies have reported the antioxidant potential of taurine against oxidative stress (Zhang et al. 2004). Yanagita et al. (2008) reported the reduction of apolipoprotein B100 and lipid secretion in liver cancer cells, and Nakajima et al. (2010) reported the therapeutic potential of taurine against inflammatory responses in SCI. Yan et al. (2012) have reported the supplementation of a high dose of vitamin C improves the function recovery of SCI. Wang et al. (2015) further reported the therapeutic effects of ascorbic acid against SCI-induced rats, and Katoh et al. (1996) reported the protective potential of dietary ascorbic acid on spinal cord compression injury in a rat model. Another study reported the downregulation of p53 and caspase-3 with a concomitant elevation in the ratio of bax/bcl-2o (Yu et al. 2016). Our results are consistent with these findings; decreased levels of oxidative, apoptotic, and inflammatory markers were observed in rats with a SCI.

Conclusion

Apoptotic, inflammatory, and oxidative stress markers were significantly decreased in spinal cord injury-induced rats treated with taurine and ascorbic acid.

Compliance with ethical standards

Conflict of interest Authors declare that they have no conflict of interest.

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