Cryptotanshinone Protects Against Acute Pulmonary Edema

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ABSTRACT

Objective: Cryptotanshinone (CTS) is a compound with anti-inflammatory, anti-bacterial, anti-oxidative and anti-aggregant functions. We aimed to determine the effects of CTS on α-naphtilthiourea (ANTU)-induced acute pulmonary edema.

Materials and Methods: In this study, 4 groups (control, sham, ANTU and ANTU+CTS) were established from a total of 40 rats. The ANTU+CTS group received intraperitoneal CTS for seven days, and both ANTU and ANTU+CTS received an ANTU administration for the induction of peripheral effusion. Four hours after the ANTU administration, the rats were subjected to a forced swim test and were decapitated. Swimming times of rats, amount of pleural effusion (PE), lung weight (LW)/body weight (BW), and PE/BW ratios were determined.

Results: At the end of the experiment, PE was not detected in the lungs of control and sham group rats. It was determined that PE, LW/BW and PE/BW were significantly decreased, while swimming time was increased after acute pulmonary edema in the CTS group (p<0.05).

Conclusion: CTS showed a protective effect against acute pulmonary edema, which indicates that it may be used as a new therapeutic agent against pulmonary toxicity.

Keywords: Cryptotanshinone; α-naphthylthiourea, pulmonary edema, pleural effusion, swimming duration

INTRODUCTION

Pleural diseases are extremely common today, affecting 3000 people out of every 1 million people annually. Pleural effusions (PE) constitute an important part of pleural diseases (1). It has been reported that approximately 90% of PE, which constitute an important part of pleural diseases, occur due to congestive heart failure, pneumonia, malignancy, and pulmonary embolism (2). Pulmonary edema occurs as a result of high pressure in the pulmonary microcirculation or an increase in the permeability of the alveolar-capillary barrier, or a combination of both (3). Pulmonary edema is an imbalance between the formation and reabsorption of lung tissue fluid, leading to massive tissue fluid reabsorption by pulmonary lymphatic and venous vessels insufficiency. Pulmonary edema can also be defined as abnormal fluid accumulation in the extravascular parts of the lung (4). Pulmonary edema is divided into two subgroups as cardiogenic and non-cardiogenic (5). Non-cardiogenic pulmonary edema occurs with increased alveolocapillary permeability as a direct or indirect result of a pathological process (multiple trauma, septic shock, influenza pneumonia, aspiration syndrome) (6). Cardiogenic pulmonary edema is characterized by increased secondary hydrostatic capillary pressure due to increased pulmonary venous pressure (7). Cardiogenic pulmonary edema leads to acute respiratory failure and has a high mortality rate (8).

Cryptotanshinone (CTS) is a lipophilic compound derived from the roots of the plant species *Salvia miltiorrhiza*, which is used in the treatment of cardiovascular diseases, hepatitis, diabetes, and chronic liver failure in China (9). This compound, which has the potential to prevent many

diseases, also has anti-inflammatory, antibacterial, anticancer, antioxidative and antiaggregant effects (10-13). The important therapeutic effects of CTS in the treatment of angiogenesis-related diseases by inhibiting endothelial cell proliferation have been emphasized (14). CTS shows antipermeability and antiangiogenic effects, as well as anti-inflammatory properties, in interaction with other cytokines and chemokines (15). It has been suggested that since CTS alleviates pulmonary fibrosis (16), it is a promising drug candidate for the treatment of fibrotic pulmonary diseases (17). In addition, inhalation of CTS is a safe and effective treatment strategy for chronic pulmonary fibrosis. (18). Although CTS has anti-inflammatory effects against acute lung injury, it also has therapeutic effects (19). Moreover, it can efficiently protect pulmonary function and decrease early pulmonary inflammation infiltration in radiation-induced lung injury, and it was found to be more effective than prednisone in alleviating pulmonary fibrosis (20).

For the identification of new therapeutic agents in the treatment of pulmonary edema, the α-naphthylthiourea (ANTU)-induced pulmonary edema model is the most widely used and most similar to the clinicopathological features of pulmonary edema in humans (21). ANTU, a chemical compound derived from thiourea, was first used as a rodenticide, and it causes an increase in pulmonary vascular permeability in the rat, leading to the development of pulmonary edema (22).

To date, several antioxidants have been used as an alternative treatment for the cure of various diseases, including pulmonary edema. The potential of numerous flavonoids isolated from natural sources has been demonstrated against various lung diseases. However, the protective effect of CTS, which is a strong antioxidant substance against acute pulmonary edema, has not been studied so far.

We aimed to determine the effects of CTS on the amount of PE, lung weight (LW)/body weight (BW), and PE/BW ratios and forced swim test time in the lungs of rats with acute pulmonary edema with ANTU.

MATERIALS AND METHODS

The study was performed with the approval of the Karabuk University Medical Faculty Experimental Animals Ethics Committee (Date: 04/10/2022, Decision number: 2022/09/15, Registration number: E-55212866-050.99-174960). A total of 40 male Wistar Albino rats with an average weight of 200-240 grams were included in the study. During the study, the rats were housed in a 12-hour light/12-hour dark cycle and a temperature of 21±2°C. The animals were fed *ad libitum* with standard rat pellet feed produced for experimental animals and were given tap water.

Experimental Groups and Experimental Design

Before the experimental stages, all the rats were weighted, and experimental groups were formed from those whose body weights were closest to each other. Experimental groups: They were randomly divided into 4 groups as control, sham, ANTU and ANTU+CTS (n=10, for each group).

Control: Rats of this group were given CTS solvent (saline containing 1% Tween 80) in a volume of CTS for 7 days from day 1 of the study.

Sham: The rats in this group were given a single intraperitoneal ANTU solvent (olive oil) (Sigma-Aldrich, USA) dose volume of ANTU on the 7th day of the study.

ANTU: The rats of this group were injected with a single dose of ANTU intraperitoneal at a dose of 10 mg/kg dissolved in olive oil (4 mg/mL) on the 7th day of the study.

ANTU+CTS: The rats of this group were administered CTS (10 mg/kg) (Sigma-Aldrich, USA) dissolved in saline containing 1% Tween 80 for 7 days from the 1st day of the study (23-25). Again, the rats in this group were injected with ANTU on the 7th day of the study.

Establishment of Experimental Lung Edema Model with ANTU

Severe pulmonary edema and lung damage were observed in rats injected with ANTU within 4 hours. As a result of this damage, PE formation, LW/BW and PE/BW ratios were found to be increased. Floatation exercises were performed on the rats 4 hours after the ANTU injection.

Forced Swim Test

The forced swim test is commonly used to evaluate fatigue behavior in animal experiments. The pulmonary edema and effusion we created experimentally lead to fatigue and respiratory failure because of the decreased oxygenation capacity and lung compliance of the lungs of rats. The forced swim test was utilized to evaluate the effect of CTS on induced PE.

For the rats to get used to the pool, they were allowed to swim free for 15 minutes the day before the experiment and were dried and returned to their cages. After seven days of CTS and solvent injections, on the 7th day of the experiment (4 hours after the ANTU injection), the rats were subjected to a forced swim test in a plastic pool (90 cm × 45 cm × 45 cm) filled with room temperature water. In this test, the depth of the water should have been sufficient to prevent the animals from touching the floor of the pool with their tails. The swimming period was taken as the duration spent by the rats swimming until they exhausted their strength and started struggling. The end point of the strenuous swimming time was accepted as the point where the nose of the rats remained below the water surface for 10 seconds. When obvious signs of fatigue appeared, the animals were removed from the pool, and returned to their cages (26).

Termination of Experiment

Following the swimming exercises, the rats were euthanized by taking blood from the abdominal aorta under anesthesia (intraperitoneal ketamine/xylazine (100 mg/kg / 5 mg/kg). The thorax was opened very carefully, and utmost care was taken not to make the surrounding tissues bleed and not to contaminate the effusion fluid with blood. The effusion fluid was collected from the pleural space with a syringe, and the lungs were carefully isolated and cleaned from the surrounding tissues. The removed lungs were weighed on a precision scale. The amount of PE, LW/BW, and PE/BW ratios were calculated.

Statistical Analyses

Statistical analyses were performed with IBM SPSS 25.0 program. The normal distribution was determined with the Shapiro Wilk test. When the data of the groups did not show normal distribution, the difference between the groups was evaluated with the Kruskal Wallis H test. When statistical significance was detected between groups, multiple/pair comparisons were analyzed with the Bonferroni correction-Mann Whitney U test. $p < 0.05$ was considered significant.

RESULTS

Effect of CTS on PE

It was detected that there was no PE in the lungs of the rats in the control and sham groups, when the study was terminated, and the thorax of rats were opened. Non-hemorrhagic PE with exudate characteristics was determined in the lungs of rats in the ANTU and ANTU+CTS groups. When the pleural effusions of groups were compared; it was determined that the amount of pleural fluid in the ANTU (4.1 ± 0.5 mL) group and ANTU+CTS

(1.8 ± 0.4 mL) groups were statistically significantly higher than in the control and sham groups ($p < 0.05$). In addition, when the ANTU group and ANTU+CTS groups were compared, it was found that the pleural fluid of the ANTU+CTS group was statistically reduced compared to the ANTU group ($p < 0.05$) (Figure 1). It can be said that CTS provides protection against the formation of PE.
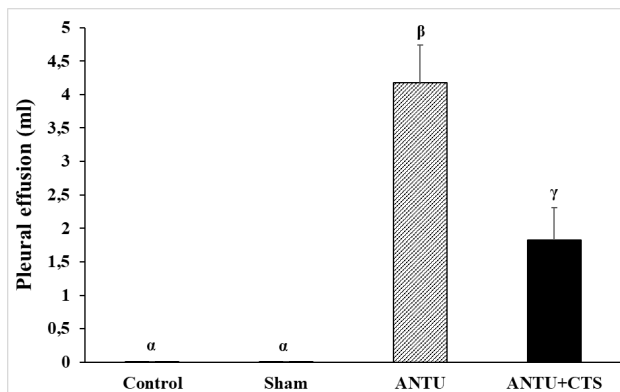
Effect of CTS on PE/BW $\times 10^4$

When the PE/BW ratios of the groups were compared; the PE/BW ratio of the ANTU (144.9 ± 3.8) and ANTU+CTS (83.6 ± 1.8) groups was statistically significantly higher than the control and sham groups ($p < 0.05$). However, when ANTU and ANTU+CTS groups were compared in terms of PE/BW ratio, it was determined that CTS statistically significantly reduced the PE/BW weight ratio ($p < 0.05$) (Figure 2).

Effect of CTS on LW/BW $\times 10^4$

By the end of the experiment, when the LW/BW ratios of the groups were compared; the LW/BW ratio of the ANTU (93.6 ± 9.4) and ANTU+CTS (79.7 ± 3.8) groups was statistically significantly higher than the control (47.7 ± 2.6) and sham (46.3 ± 2.4) groups ($p < 0.05$), while it was statistically lower in the ANTU+CTS group than ANTU ($p < 0.05$) (Figure 3).

Effect of CTS on Swimming Time

On the seventh day of the experiment, four hours after ANTU was given to ANTU and ANTU+CTS groups, all experimental groups were given a swimming test. Regarding the swimming times of the groups; it was statistically significantly lower in ANTU (18.8 ± 6.9) and ANTU+CTS (30.2 ± 5.8) than in the control (50.2 ± 5.1) and sham (48.4 ± 4.9) groups ($p < 0.05$). Moreover, the swimming time of the ANTU+CTS group was statistically
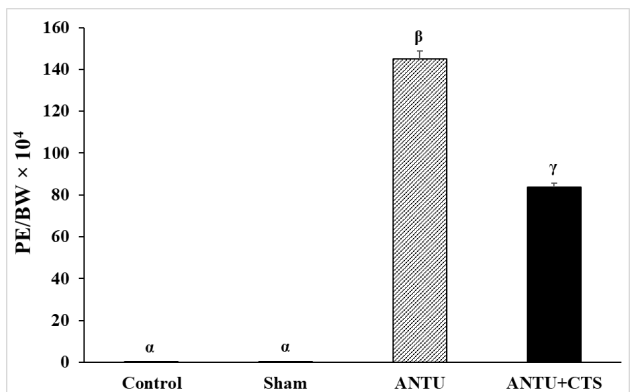

Figure 1. Effect of CTS on PE.
α:There was no significance between the control and sham groups.
β:The ANTU group is significantly higher than the control and sham groups.
γ: The ANTU+CTS group was significantly reduced compared to the ANTU group.

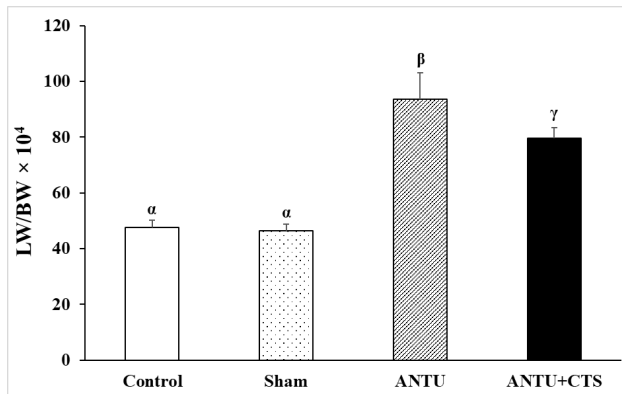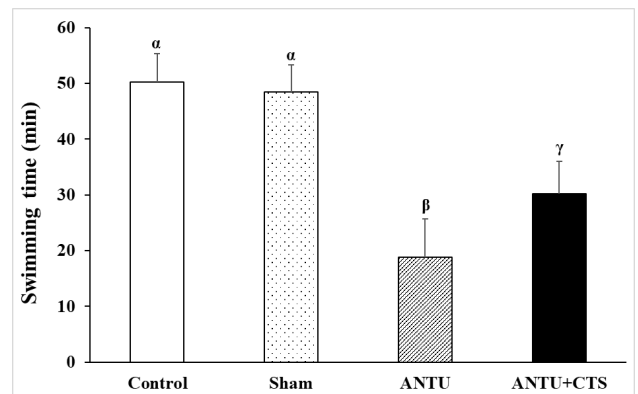**Figure 2.** Effect of CTS on PE/BW $\times 10^4$.
α:There was no significance between the control and sham groups.
β:The ANTU group is significantly higher than the control and sham groups.
γ: The ANTU+CTS group was significantly reduced compared to the ANTU group.

Figure 3. Effect of CTS on LW/BW.
α:There was no significance between the control and sham groups.
β:The ANTU group is significantly higher than the control and sham groups.
γ: The ANTU+CTS group was significantly reduced compared to the ANTU group.

Figure 4. Effect of CTS on swimming time.
α:There was no significance between the control and sham groups.
β:The ANTU group is significantly lower than the control and sham groups.
γ: The ANTU+CTS group was significantly increased compared to the ANTU group.

higher ($p<0.05$) than ANTU (Figure 4). It can be said that CTS reduces the formation of PE and increases swimming time.

DISCUSSION

ANTU gives rise to acute pulmonary vascular injury in rodents, with the most prominent manifestations including increased lung weight due to PE and pulmonary edema due to damage to endothelial cells and pneumocytes in the lung. Since the effects of ANTU are highly specific to the lungs, it is widely used in generating animal models to investigate the pathology of acute pulmonary edema (27). CTS, a key ingredient derived from *Salvia miltiorrhiza*, has many pharmacological properties such as anti-inflammatory, anti-oxidative, anti-angiogenic, and anti-proliferative properties. CTS is used in the treatment of many diseases including acute lung injury (18). Additionally, orally administered CTS is an alternative treatment agent for pulmonary fibrosis (17). However, its poor oral absorption and sensitivity to light greatly limit its clinical application (28).

Regarding its anti-cancer properties, it has been stated that CTS induces apoptosis in lung cancer cells and inhibits tumor formation in the lung both *in vitro* and in *vivo* (29). Furthermore, CTS prevents invasion/metastasis in non-small cell lung cancer (NSCLC) via inhibiting the expression of carcinogenic microRNAs (30, 31). It has also been stated that CTS potentiates the effects of chemotherapeutic drugs on NSCLC cells through transketolase inhibition both *in vitro* and *in vivo* (32). When this information is compiled, it can be concluded that CTS has suppressive effects on lung cancer, can be used as a treatment agent in *in vivo* and *in vitro* studies.

It has been stated that CTS reduces allergic airway inflammation (33), and might be a curative agent for the treatment of asthma (34). Moreover, CTS has been shown to have an anti-inflammatory effect by down-regulating pro-inflammatory

inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2) in the mouse macrophage cell line (35). In addition, CTS also performed anti-inflammatory effects specifically by inhibiting COX-2 activity in lipopolysaccharide (LPS)-stimulated pro-monocyte cell line and paw edema model (13). Tang et al. have shown that CTS reduces LPS-induced acute lung injury through its anti-inflammatory effects by inhibiting toll like receptor 4 (TLR4)-mediated nuclear factor kappa B (NF-kB) signaling pathways (36). Moreover, CTS maintain to pulmonary fibrosis by suppressing the cascade of Smad and STAT3 (37). CTS treatment has been shown to reduce radiation-induced lung injury and pulmonary fibrosis in rats by suppressing inflammatory and fibrotic factors (38). Similarly, its inhalation has also been shown to alleviate pulmonary fibrosis; therefore, CTS may be an alternative treatment method in the treatment of pulmonary fibrosis (39).

To date, the effects of CTS have not been examined on pulmonary edema. However, considering the studies on CTS, its protective role in pulmonary diseases and edema is due to its anti-inflammatory effects through inhibition of the inflammatory pathway. In this study, it was determined that the use of CTS improves swimming scores by reducing pleural fluid in the ANTU-induced pulmonary edema model. CTS may have shown a protective effect against pulmonary edema by inhibiting the anti-inflammatory pathway.

CONCLUSION

Our findings have demonstrated that CTS shows a protective effect against ANTU-induced acute pulmonary edema. CTS decreased PE, LW/BW, and PE/BW following pulmonary edema, while increasing swimming time. CTS seems like it may be considered as an alternative treatment approach in the treatment of pulmonary edema following more detailed

studies. The lack of molecular analyses is one of the limiting factors of our study, however, it provides important preliminary information for studies that will attempt to understand the effects of CTS on pulmonary edema with molecular analysis.

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