

# Chlorpheniramine and escitalopram: Similar antidepressant and nitric oxide lowering roles in a mouse model of anxiety

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**Abstract.** There is a crosstalk between mood disorders and oxidative stress. Chlorpheniramine (CPA), a first generation antihistamine, is hypothesized to have an anxiolytic role at high doses; however, its antidepressant and antioxidant roles have not previously been investigated. The aim of the current study was to evaluate the antidepressant and anxiolytic effects of CPA treatment in association with nitric oxide (NO) and super oxide dismutase (SOD) activity in a mouse model of anxiety. BALB/c mice were divided into unstressed (naïve), control, and CPA- (0.5 mg/kg) and escitalopram- (ESC; 10 mg/kg) treated groups for 3 weeks. Subsequently, they were immobilized for 6 h and subjected to behavioural paradigms as follows: The open field test, the elevated plus maze (EPM) and the forced swim test to investigate motor function, anxiety and depression, respectively. The mice were sacrificed and serum was obtained to detect NO and SOD activity. Compared with the control group, the CPA-treated group demonstrated an antidepressant effect similar to that of the ESC-treated group. In addition, CPA prevented stress-induced NO without affecting SOD activity. CPA did not improve anxiety-like behaviour in the EPM, nor did it improve stress-induced locomotion and rearing, as demonstrated by the OFT. Thus, to the best of our knowledge, this is the first study to evaluate the antidepressant role of CPA in association with NO metabolism. However, further studies are required to elucidate the underlying mechanism.

## Introduction

Anxiety and depression are widespread neurological or psychological illnesses with high prevalence rates worldwide. According to epidemiological studies, approximately one-third of people will suffer from anxiety disorders (1). It is well accepted that neurotransmitter deficiency is associated with mood

disorders, specifically serotonin and other catecholamines. Therefore, antidepressants, such as the selective serotonin reuptake inhibitor (SSRI), escitalopram (ESC), represent a fundamental pharmacotherapeutic option.

Histamine is a neurotransmitter in the brain with numerous physiological functions; it has also been implicated in anxiety-like behaviour in animal models (2,3). Previous evidence indicates a potential role for the influence of histaminergic neurons in anxiety-associated behaviour via H1, H2 and H3 receptor activation. H3 receptor activation is hypothesized to be involved in diminishing the release of serotonin, dopamine and norepinephrine, which are all implicated in anxiety and mood disorders (3,4). In addition, H3 receptor knockout in mice demonstrated reduced anxiety (5).

The precise role of antihistamines in anxiety and depression remains controversial. First generation antihistamines have been widely used to alleviate anxiety and panic attacks (6). The anxiolytic and antidepressant effects of chlorpheniramine (CPA), a first generation antihistamine, are proposed to be associated with its serotonergic functions (7). Furthermore, the anxiolytic and antidepressant effect of CPA was evident in animal models (8,9). However, these results have been challenged by previous findings (10) in which CPA was demonstrated to exert anxiogenic effects in mice.

In addition to the histaminergic system, growing evidence from animal and human trials implicates oxidative stress in anxiety and stress disorders in the central nervous system and in peripheral tissues (11,12). Oxidative stress is defined as the overproduction of free radicals, such as reactive oxygen species and reactive nitrogen species accompanied by the inability of endogenous defensive antioxidant enzymes to detoxify these free radicals (11). Nitrates, a nitric oxide (NO) metabolite present in different tissues and the chief reactive nitrogen species constituent, is a marker of oxidative stress and is implicated in associated disorders (13); NO increases in acute anxiety (14,15). Furthermore, various animal studies have reported higher NOx in the hippocampus and brain cortex of a stressed animal (16). Furthermore, SSRIs were found to exert an antioxidant role peripherally (17). Super oxide dismutase (SOD) is an abundant antioxidant enzyme responsible for super oxide ( $O_2^-$ ) species detoxification to yield hydrogen peroxide ( $H_2O_2$ ). SOD activation occurs as a response to an oxidative stress status (18).

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Clinically, anxiolytics or antidepressants are used chronically, rather than as a single dose. Although CPA anxiolytic and antidepressant effects were investigated using a single dose (8,18,19), their effects, however, were not evaluated following repeated doses. Additionally, the role of CPA in oxidative stress modulation has yet to be addressed. Therefore, the aim of the present study was to evaluate the protective anxiolytic and antidepressant effects of three weeks of CPA administration in comparison to ESC, and to correlate CPA behavioural effects on serum NO<sub>x</sub> levels and SOD activity in mice anxiety.

## Materials and methods

**Study animals and design.** Forty BALB/c mice (Animal House Facility of the Applied Science University, Amman, Jordan) were used in the present study. The mice were aged 6–8 weeks and weighed ~25 g. The BALB/c line was selected due to its suitability in anxiety studies (20). Mice were maintained in separate cages, at a temperature of ~25°C, with 50–60% humidity and continuous air ventilation. The research was conducted according to the international ethical standards for the Care and Use of Laboratory Animals and the study was approved by the American University of Madaba research committee (Madaba, Jordan). The mice were divided into four groups (n=10 per group) as follows: Group 1, naïve (unstressed and untreated); group 2, control (treated with distilled water for 3 weeks subsequently stressed); group 3, CPA (treated for 3 weeks and subsequently stressed); group 4, ESC (treated for three weeks and subsequently stressed); and group 5 received a combination of 0.5 mg/kg CPA and 10 mg/kg ESC (treated for three weeks and subsequently stressed). A treatment period of 3 weeks was selected in order to investigate the antidepressant potential. Following completion of the 3-week treatment, groups 2, 3 and 4 were restrained in Falcon tubes for 6 h between 9:00 and 15:00, then all mice were subjected to behavioural tests. The mice were sacrificed, blood samples (~1.5 ml) were collected and the serum was obtained by centrifugation at 2,000 × g for 10 min at room temperature.

**Treatment strategies.** The CPA-treated group received a dose of 0.5 mg/kg intraperitoneally for 3 weeks. The ESC-treated group received a dose of 10 mg/kg orally for 3 weeks. CPA and ESC were generously donated by the Arab Pharmaceutical Manufacturing Co., Ltd. (Amman, Jordan) and the JOSWE Medical (Amman, Jordan), respectively. The control group received an equal volume of distilled water intraperitoneally for 3 weeks. The majority of similar studies employed acute dosing regimens (1 h prior to the behavioural tests); however, chronic dosing for 3 weeks with ESC has previously been performed (21). In addition, the effect of chronic dosing (at least 2 weeks) was evaluated in the present study to establish the possible antidepressant effect that may resemble clinical settings.

**Acute immobility stress.** In order to induce anxiety in mice, an acute immobility stress test was performed according to the method of Machawal and Kumar (22) with slight modifications. The current findings indicate that acute immobility stress increases serum levels of nerve growth factor (data not published), which is associated with anxiety (23).

In the present study, the mice were immobilised individually for 6 h in a 50-ml Falcon tube while proper ventilation was maintained. In the naïve group, the mice were maintained in an animal cage with soft bedding under the same experimental conditions. After performing the immobility stress test, the mice were subjected to behavioural tests.

### Behavioural tests

**Forced swim test (FST).** The FST is the most commonly used behavioural model for screening antidepressant-like activity in rodents (24). Mice were individually forced to swim for 5 min in an open glass chamber (25×15×25 cm<sup>3</sup>) containing fresh water to a height of 15 cm and maintained at 26±1°C. Floating time (FT) was defined as the time in which mice stop moving completely while in the water.

**Elevated plus maze (EPM).** The EPM test, a model for screening anxiolytics, was performed as described previously (25) with certain modifications. The apparatus was elevated 25 cm above the floor. The maze is composed of two closed arms (30×5×10 cm) and two open arms (30×5 cm). Mice were placed at the centre, facing the closed arm, and allowed to move freely for 10 min. The frequency of open arm entries (OAE) and the open arm time (OAT) spent were recorded by an experienced technician.

**Open field test (OFT).** An OFT was performed to assess the locomotion activity and sedation of the mice (26). Briefly, the mice were placed in a central square and allowed to move freely for 5 min. The field was located in a test room and lit by indirect lighting. The procedure was performed in an empty room to minimise noise and distractions. The open field maze was cleaned between each mouse, using 70% ethyl alcohol. The locomotion activity (represented by the number of lines crossed) and the sedation (represented by the rearing frequency) were recorded by an experienced technician.

**Biochemical tests.** Subsequent to performing the behavioural tests, the mice were sacrificed, blood (~1.5 ml) was collected and serum was obtained using standard protocols. The accumulation of nitrate, an indicator of the production of NO, was determined using a colorimetric assay with a Griess reagent (27). Serum nitrate was assayed using a Nitric Oxide Assay kit (cat. no. ab65328; Abcam, Cambridge, MA, USA) according to the manufacturer's instructions. The nitrate concentration was obtained according to the standard curve generated after measuring absorbance at a wavelength of 540 nm using a Multiskan™ FC Microplate Photometer (Thermo Fisher Scientific, Waltham, MA, USA). All samples and standards were processed in duplicate.

**SOD activity was assayed using an SOD Assay kit (Sigma-Aldrich; Merck KGaA, Darmstadt, Germany; cat. no. 19160) as previously described (28).** The kit utilises Dojindo's highly water-soluble tetrazolium salt, WST-1 [2-(4-Iodophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2H-tetrazolium, monosodium salt], which produces a water-soluble formazan dye upon reduction with a superoxide anion. The rate of the reduction with O<sub>2</sub> is linearly associated with the xanthine oxidase activity, and is inhibited by SOD. The SOD activity, as

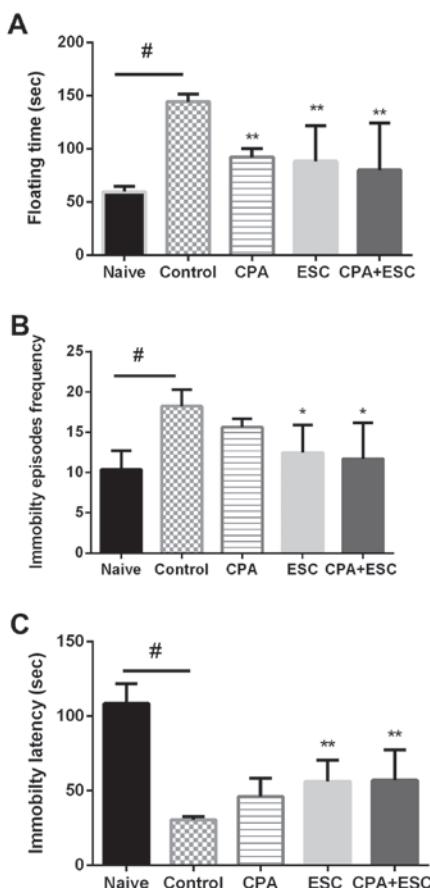


Figure 1. (A) Effect of 3 weeks of treatment with CPA (0.5 mg/kg), ESC (10 mg/kg) and a combination of the two on FT following immobilization stress. Values are expressed as the mean  $\pm$  SEM (ANOVA followed by Tukey's test). F(4, 45)=10.83; \*P<0.05 and \*\*P<0.00001. (B) Effect of treatment with CPA (0.5 mg/kg), ESC (10 mg/kg) and a combination of the two for 3 weeks on OAE. ANOVA followed by Tukey's test. F(4, 39)=3.725; #P<0.05. CPA, chlorpheniramine; ESC, escitalopram; FT, floating time; OAE, open arm exists; SEM, standard error of the mean.

an inhibition activity, is quantified by measuring the decrease in the colour development at a wavelength of 440 nm.

**Statistical analysis.** All data obtained from the behavioural tests, and the NO and SOD activity were analyzed with a one-way ANOVA and a subsequent Tukey's post hoc test. P<0.05 was considered to indicate a statistically significant difference and quantitative data are presented as the mean  $\pm$  standard error of the mean.

## Results

**FST.** The results of the FST are presented in Fig. 1. The results of the FT revealed that the CPA- and the ESC-treated groups exhibited a similar and significant decrease when compared with the control group. The result of combination therapy did not vary when compared with that of each therapy alone (P>0.05).

In addition, the control group demonstrated increased FT when compared with the naïve group (P<0.00001) and the CPA group demonstrated higher FT than the naïve

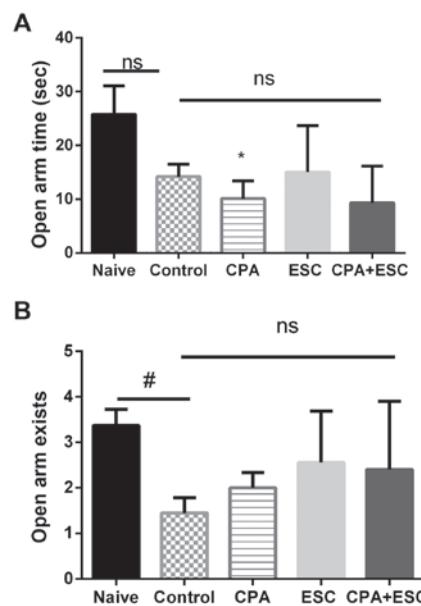


Figure 2. (A) Effect of 3 weeks of treatment with CPA (0.5 mg/kg), ESC (10 mg/kg) and a combination of the two on OAT following immobilization stress. Values are expressed as the mean  $\pm$  SEM (ANOVA followed by Tukey's test). F(4, 38)=3.648; \*P<0.05. (B) Effect of treatment with CPA (0.5 mg/kg), ESC (10 mg/kg) and a combination of the two for 3 weeks on OAE. ANOVA followed by Tukey's test. F(4, 39)=3.725; #P<0.05. CPA, chlorpheniramine; ESC, escitalopram; FT, floating time; OAE, open arm exists; SEM, standard error of the mean; ns, non-significant.

group (Fig. 1A; P<0.05). Regarding the immobility episodes frequency (Fig. 1B), the control group demonstrated a higher frequency of immobility episodes when compared with the naïve group (P<0.05). The ESC-treated group demonstrated a significant decrease in FT when compared with the control group (P<0.05), the CPA-treated group exhibited lower, but non-significant immobility episodes when compared with the control, with no difference between the CPA- and ESC-treated groups (P>0.05). The result of combination therapy did not vary when compared with that of each therapy alone (P>0.05).

The results of the immobility latency (Fig. 1C) revealed significantly lower immobility latency in the control group when compared with the naïve group (P<0.05). The ESC-treated group and the combination therapy group significantly improved the immobility latency (P<0.05). The CPA-treated group demonstrated higher, but non-significant immobility latency when compared with the control group. The result of combination therapy did not vary when compared with that of each therapy alone (P>0.05).

**EPM.** The OAT and OAE were significantly higher in the naïve group when compared with the control group (P<0.05). The administration of CPA (0.5 mg/kg), ESC (10 mg/kg) and a combination of the two for 3 weeks did not alter the OAT and OAE measurements when compared with the control group (P>0.05; Fig. 2A and B).

**OFT.** The results demonstrated a significant decrease in the locomotion activity in the control group when compared with the naïve group (P<0.0001). The administration of CPA (0.5 mg/kg), ESC (10 mg/kg) and a combination of the two for 3 weeks did not increase the ambulation frequency (P>0.05). Similar results were

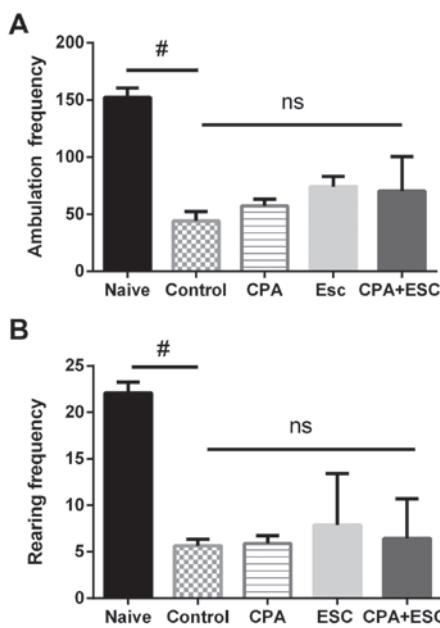


Figure 3. (A) Effect of treatment with CPA (0.5 mg/kg), ESC (10 mg/kg) and a combination of the two for 3 weeks on the ambulation frequency. Values are expressed as the mean  $\pm$  SEM (ANOVA followed by Tukey's test). F(4,38)=33.21;  $P=0.0001$ . No difference in ambulation frequency was observed between the treated groups and the control despite a trend of increase. (B) Effect of treatment with CPA (0.5 mg/kg), ESC (10 mg/kg) and a combination of the two for 3 weeks on ambulation frequency. Values are expressed as the mean  $\pm$  SEM (ANOVA followed by Tukey's test). F(4, 41)=33.5;  $P=0.0001$ . CPA, chlorpheniramine; ESC, escitalopram; SEM, standard error of the mean; ns, non-significant.

observed for the rearing frequency. Acute restraint significantly decreased rearing in the control group when compared with the naïve group ( $P<0.0001$ ). The administration of CPA (0.5 mg/kg), ESC (10 mg/kg) and a combination of the two for 3 weeks did not increase the rearing frequency ( $P=0.98$ ; Fig. 3A and B).

#### Biochemical tests

**Serum NO.** The immobility stress resulted in a significant increase in serum NOx in the control group when compared with the naïve group ( $P=0.0002$ ). The CPA- and the ESC-treated groups demonstrated lower NOx when compared with the control group ( $P<0.05$ ). However, the combination therapy did not result in any reduction ( $P>0.05$ ; Fig. 4A).

**SOD activity.** SOD serum activity did not vary among the study groups ( $P>0.05$ ; Fig. 4B).

#### Discussion

The current study revealed an antidepressant, but not anxiolytic role of CPA following 3 weeks of administration in mice. Additionally, CPA prevented stress-induced NOx increase, although it did not alter SOD activity with respect to the control.

In the current study, CPA treatment demonstrated antidepressant action similar to that of ESC treatment. A possible explanation could be attributed to its possible serotonergic activity, as CPA shares structural properties with SSRIs (29). Another explanation is that CPA exerts an antidepressant role by enhancing neurotransmitters additional to serotonin, such

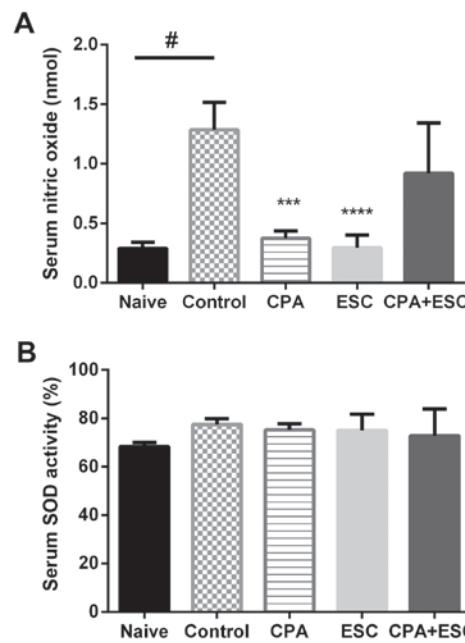


Figure 4. (A) Effect of treatments with CPA (0.5 mg/kg), ESC (10 mg/kg) and a combination of the two for 3 weeks on serum NO following immobilization stress. Values are expressed as the mean  $\pm$  SEM (ANOVA followed by Tukey's test). F(4, 35)=11.98;  $\#P<0.0001$ , \*\*\* $P<0.001$  and \*\*\*\* $P<0.00001$ . (B) Effect of treatments with CPA (0.5 mg/kg), ESC (10 mg/kg) and a combination of the two for 3 weeks on serum SOD activity after immobilization stress. Values are expressed as the mean  $\pm$  SEM (ANOVA followed by Tukey's test). F(4, 35)=1.662;  $P=0.183$ . CPA, chlorpheniramine; ESC, escitalopram; SEM, standard error of the mean.

as norepinephrine (30). A previous study (8) demonstrated a CPA antidepressant effect via an FST in rodents following repeated acute doses of 7.5 and 15 mg/kg that were administered in the 24 h prior to the FST. In the current study, a lower dose of CPA (0.5 mg/kg) was used for 3 weeks, which demonstrated antidepressant properties. A reduced dose of CPA was selected in order to minimise the cholinergic and sedative side effects (31). Notably, the combination treatment of CPA with ESC was not synergistic. Typically, synergism is achieved by combining drugs that act on different receptors. Although further studies are required to explain this finding, one hypothesis is that CPA may be competing for the same serotonin transporter as ESC, based on its postulated serotonergic activity.

An unexpected finding was that CPA failed to exert anxiolytic effects in the EPM and the OFT. These findings are consistent with Serafim *et al.* (10) who demonstrated the anxiogenic effect of CPA in mice at various doses. It was proposed that the H1 receptor antagonists suppress acetylcholine release from the ventral striatum; furthermore, acetylcholine is associated with anxiety-like behaviours in rodents (10).

Conversely, certain studies reported the anxiolytic role of CPA in animals (25,32). Indeed, it has been suggested that CPA anxiolysis may be attributed to modulation of the serotonergic and cholinergic systems (7). In fact, CPA inhibits the serotonin (5-hydroxytryptamine) transporter (6) and, therefore, inhibits serotonin reuptake.

Our findings may be due to methodological differences; for example, prior to performing the anxiety behavioural tests in the present study, the mice were restrained. Previous findings

indicate that acute restraint induces anxiety (22). Therefore, it is hypothesized that the low dose of CPA employed in the present study was insufficient to alleviate anxiety-like behaviours. This explanation is supported by the fact that previous studies did not use immobility stress. Furthermore, other studies employed higher CPA doses (8). Therefore, it may be inferred that a low dose of CPA is only useful in mild anxiety.

Acute anxiety provokes an increase in NOx in various tissues, including the hippocampus, in blood and in saliva (13,14). To the best of our knowledge, this is the first study demonstrating the antioxidant effects of CPA. A novel finding in the current study was the ability of CPA treatment to normalize the stress-induced increase in serum NOx. Similarly, ESC treatment reduced serum NOx. Although the exact mechanism is yet to be established, this may be due to similarities in the mechanisms between CPA and ESC.

Although SOD activity was increased following acute stress induction, neither CPA nor ESC diminished its activity. The exact cause of the rise in SOD activity due to anxiety is yet to be clarified. It may be a compensatory mechanism to overcome oxidative stress. Recently, SOD has been gaining attention in mood disorders. For example, SOD mRNA levels were upregulated following acute stress exposure (31) while in another study, mild chronic stressful events decreased SOD activity in the hippocampus and cortex of mice (33). This may indicate a potential role for the antioxidant enzyme in acute stress. The authors suggest that investigating the transcripts of antioxidant enzymes could be more beneficial than analyzing enzymatic activity. It was reported that long term use of antidepressants upregulated the mRNA expression levels of SOD and other antioxidant enzymes (34).

The present study presents novel ideas, which may lay the foundation for future investigations; however, there were certain limitations. Only a single concentration of CPA was evaluated for its antidepressant and antioxidant effects. Future studies should administer different doses that could determine potential dose-dependent effects and potential synergism between CPA and ESC. Furthermore, future studies may focus on the potential role of CPA or ESC in modulating the levels or the activity of the inducible NO synthase [the enzyme responsible for NOx synthesis under stressful conditions (13)], which may demonstrate the cross talk between mood disorders and oxidative stress in the serum, as well as in the brain cortex, allowing definitive conclusions to be derived.

In conclusion, this is the first study, to the best of our knowledge, describing the antidepressant and the potential antioxidant role of CPA in a mouse model of anxiety. This preliminary finding provides novel hypotheses for future studies regarding depression. Thus, further investigations are required to clarify the underlying mechanisms and the possible implementation in clinical practice.

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