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Epoetin alfa has a potent anxiolytic effect on naive female rats

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Abstract

Background Epoetin alfa is a derivative of the erythropoietin hormone. This study aims to investigate the epoetin alfa effect on anxiety-like behaviors.

Methods Adult female Wistar Albino rats were divided into Control ($n=8$), 1000 U Epoetien alfa, and 2000 U Epoetien alpha. Epoetin alfa was administered intraperitoneally once a week for 4 weeks. The animals were then subjected to open field test, elevated plus maze, light-dark box, and the behaviors were video recorded.

Results Epoetin alfa significantly reduced anxiety-like behaviors in both low- and high-dose groups in a dose-independent manner. This anxiolytic effect was seen in all three anxiety tests. Further, exploratory behaviors such as unsupported rearing and head-dipping behaviors increased with the application of Epoetin alfa. This protocol did not alter locomotor activity.

Conclusion The present study found beneficial effects of epoetin alfa on behaviors. Further studies on the effect of derivatives of erythropoietin hormone on anxiety-like behaviors are needed.

Keywords Anxiety-like behaviors, Epoetin alfa, Elevated plus maze, Light-dark box, Open field test

Introduction

Anxiety disorders include some unpleasant emotions, such as a predominance of exaggerated fear or worry [1, 2]. Anxiety disorders are the most common psychiatric disorders [3].

According to the Epidemiology of anxiety disorders study, Anxiety disorder prevalence is predicted at 4.05%, with nearly 301 million people. The number of people suffering from anxiety increased by more than 55% from 1990 to 2019 [4]. Further anxiety disorders put a severe burden on [4]. Anxiety disorder treatments are psychological therapy, pharmacotherapy, or a combination of both [3]. Preclinical studies test new treatment strategies alongside admitted treatment [5].

Erythropoietin (EPO) is a glycoprotein hormone [6]. EPO is secreted as a prehormone; the mature form consists of 165 amino acids. Molecular weight is – 34 Kda [6]. In adult humans, EPO is mainly produced by the kidneys and liver [7]. Non-renal tissues source approximately 10 % of circulating EPO [8]. Apart from the kidney, it has also been shown to be produced from the liver, heart, lung, brain, testis, uterus, and spleen [9]. EPO has two defined receptors. EPOR, the receptor of EPO, has two variations, defined as homodimeric and heterodimeric EPOR receptors. While it shows its erythropoietic effect by binding to its homodimeric subunit, it shows a protective effect in the cell by binding to its heterodimeric

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subunit. The homodimeric subunit consists of two EPOR subchains, whereas the heterodimeric subunit contains EPOR and beta-chain, and the heterodimeric receptor is also called the tissue repair receptor or cd111 receptor [10]. EPO's protective activity has been reported in different animal models, such as retinal ischemia, acute lung injury, renal fibrosis, and bone defect [11–14].

Peripherally administered EPO has been reported to cross the blood-brain barrier in animal studies, albeit to a limited extent [15–17]. Especially after crossing the blood-brain barrier, its positive effects on the central nervous system have been shown in different experimental models such as Parkinson's, Alzheimer's, and epilepsy [18–20].

Epoetin alfa has hematopoiesis activity and is therefore used to treat anemia. It binds to both heterodimeric and homodimeric receptors. It has been reported to cross the blood-brain barrier [21]. Therefore, its effects on the central nervous system have been studied in different experimental models, mainly neurological disorders [22, 23].

There are very few studies on the effects of EPO and its derivatives on anxiety, and studies have focused on cancer and chronic renal failure patients [24–26]. Miskoviak et al. reported that Epoetin alfa reduces neural and cognitive processing of fear in human functional magnetic resonance imaging study [27]. Another neuroimaging study by Msikoviak et al. points out that EPO would be estimated to decrease the exaggerated threat-relevant processing observed across depression and anxiety disorders [28].

The present study aimed to investigate the effect of chronic and different doses of Epoetin alfa on anxiety-like behaviors in adult naïve rats. This preliminary study evaluated different behavioral parameters in various unconditioned anxiety tests.

Methods

Animal

This study used adult female Wistar albino rats (n = 24) at 12 weeks. Rats were provided by Ankara University's Experimental Animals and Research Laboratory. Adaptation of rats to the environment 1 week Ankara University Faculty of Medicine Department of Physiology Animal Laboratory. Subjects were sheltered in a 12-light/dark cycle and had access to *ad libitum*. All experiments were performed in accordance with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health [29]. All experimental procedures were performed under the approval of the Ankara University Experimental Animals Ethics Committee (2022–15-144, committee meeting date: 07.09.2022). The experiment included three groups (n = 8 each): control (C), Epoetin alfa 1000 U/kg, and Epoetin alfa 2000 U/kg, respectively.

Drug treatment and experimental protocol

Epoetin alfa (EPOα), (DROPOETIN, Drogsan, Turkey), an erythropoietin derivative, was administered intraperitoneally (i.p.) at 1000 or 2000 U/kg for four consecutive weeks. A single dose of EPO alfa was administered every week. 1000 U and 2000 U/kg doses displayed beneficial effects on nervous systems from other clinical and preclinical studies [30–33]. The control group was administered physiological saline in group 1 ml/kg. After the anxiety tests, the subjects were sacrificed under anesthesia with 50 mg/kg sodium thiopental (i.p.) (See Table 1). Exsanguination from the heart was conducted under deep sodium thiopental anesthesia. At the end of the blood collection, the heart and other organs are separated from the body.

Behavioral tests

General procedures in previous studies established by the study team were followed [34–36]. Behavioral testing was performed in a unique behavior laboratory (Banu Ocakçıoğlu Laboratory in Ankara University Medicine Faculty Physiology Department). Rats were transported to the behavior laboratory for adaptation two hours before the start of the experiment. The tests (open field test [OFT], elevated plus maze [EP]) were conducted in the morning (09:00–14:00). The light-dark box test (LDB) was performed from 20:00 to 24:00 pm. Digital cameras recorded the behaviors of the animals. The test apparatus was cleaned with ethanol. At least two seconds were admitted as "freezing" [37]. The OFT was always applied before the EPM, and there was no break between the tests.

Open field test (OFT)

The open-field test analyzed locomotor activity and anxiety-like behaviors (ALB) [34]. In this test, the time spent in the peripheral zone (tendency to stay close to the walls, also known as thigmotaxis) is the primary indicator of anxiety [34]. An increase in the time spent in the central zone and the number of entries into the central zone is considered a decrease in anxiety-like behaviors.

Furthermore, rats displayed rearing behaviors. If the animal rears without any contact with the walls of the behavioral apparatus, this is classified as "unsupported rearing" [36]. Unsupported rearing is more sensitive to stress and anxiety. 100 × 40 cm rectangular open area test experiment was performed. The experiments were performed once for 5 minutes. Subjects were always placed in the center region at the beginning of the experiment. In the center zone time, the number of entries into the center zone, total distance traveled (horizontal locomotor activity), supported rearing number, supported rearing latency, unsupported rearing number, unsupported

Table 1 Drug treatment and experimental protocol

	Experimental Schedule						
Experimental Groups	**0–7 Days**	**8th Day**	**15th Day**	**22th Day**	**29th Day**	**30th Day**	**31th Day**
Control (C) (n = 8)	Adaptation	1 ml/kg Saline (i.p.)	1 ml/kg Saline (i.p.)	1 ml/kg Saline (i.p.)	1 ml/kg Saline (i.p.)	X	Euthanization
Epoetin alfa (EPO*α*) (n = 8)		1000 U/kg EPO*α* (i.p.)	1000 U/kg EPO*α* (i.p.)	1000 U/kg EPO*α* (i.p.)	1000 U/kg EPO*α* (i.p.)	x	Euthanization
Epoetin alfa (EPO*α*) (n = 8)		2000 U/kg EPO*α* (i.p.)	(2000), U/kg EPO*α* (i.p.)	2000 U/kg EPO*α* (i.p.)	2000 U/kg EPO*α* (i.p.)	x	Euthanization
Injection Schedule	X	X	9 pm	9 pm	9 pm	x	
Behavioral Test Schedule	X	X	X	X	X	9–12 pm: OFT + EPM 8 pm: LDB	

OFT: open field test, EPM: elevated plus maze, LDB: light-dark box. X: No implementation was carried out, i.p.: Intraperitoneal injection

rearing latency, Unsupported rearing number in the center zone, total rearing behavior were analyzed.

Elevated plus maze

Elevated plus maze (EPM) is one of the rodents' most preferred tests for evaluating anxiety-like behaviors [36]. Depending on the decrease in anxiety, the time spent in the open arm, open-arm time (%), and the number of open-arm entries increase. Furthermore, the subjects' behavior of looking down the open arm (headdipping) for research purposes is analyzed and considered as anxiolytic-like behavior. We also analyzed stretch attend postüre (SAP). In SAP behavior, rats are petrified because of fear of a specific posture. This posture generally occurs in the open arm and is admitted as a clue to an increase in anxiety-like behaviors. 50 cm x 10 cm open arm, 50×50×10 cm closed arm, and an elevated plus maze made of white wood with a height of 70 cm from the floor was conducted. The test took 5 minutes. In the experiment, the time spent in the open arm, entry to the open arm number, frequency of head dipping, and stretch attend posture. The subjects' head-dipping behavior was also analyzed using the open arm. Head dipping behavior is known as exploratory behavior and is considered indicative of a reduction in anxiety-like behaviors. An anxiety index was also calculated in the presented study. According to the anxiety index, the closer it gets to 1, the more anxiety-like behaviors increase. Anxiety Index formula = 1 – [([Open arm time/Test duration] + [Open arms entries/Total number of entries])/2] [38].

Light dark box

The light-dark box (LDB) was performed to observe anxiety-like behaviors [35]. The separated from cage subject is exposed to new surroundings and high light, which causes anxiety-like behavior to increase. Anxiolytics generally increase the time spent in high-light illuminated areas, and anxiogenic applications decrease [39].

The box (40 cm width and 110 cm length) includes a light compartment (850 lx illumination intensity) and a dark compartment. A hole (7.5 × 7.5 cm^2) separates the light and dark zones. The rats were placed in the LDB for 5 min, beginning from the light compartment. The time spent in the light zones, the number of light-dark entrances, and the latency of crossing to the dark zone were evaluated.

Physiological measurements

Blood obtained from the tail vein of rats under deep anesthesia was used to measure hemoglobin and hematocrit. The measurements were made using a Mission Ultra Hemoglobin Meter (Acon, Lab, Inc., USA). A new strip was used for each measurement. After the stripe was dripped with blood, the data from the device was

recorded. Final Weights were also recorded while the animals were anesthetized.

Statistical methods

The first step, the Shapiro-Wilk test, was conducted as a normality test. If the results passed normality, a One-way analysis of variance (ANOVA) test was conducted. Tukey's test was performed as a post hoc test. $P < 0.05$ values were considered statistically significant. If there was no normal distribution, Kruskal Wallis was performed. Dunn was conducted as a post hoc test (Median values and interquartile range of parameters were also given). Behavioral and physiological findings were presented as mean (X) ± standard error of the mean (SEM) in bar graphs. Further, the relationship between hemoglobin values and anxiety index, central zone time, light zone time, and open arm time was also analyzed using the Pearson correlation test.

Result

Physiological result

We measured hematocrit, hemoglobin, and animal weight, which are given in Fig. 1. Hematocrit F(2,21 = 68,06) and hemoglobin F(2,21 = 57,87) were dramatically increased after EPOα treatment ($p < 0.0001$). The high-dose EPOα group differed significantly from the low-dose EPOα group regarding hemodynamic parameters (Hemoglobin: $0.005 < p$, hematocrit: $0.05 < p$). EPOα treatment did not affect animal weight F(2,21 = 0,79), ($p > 0.05$).

Open field result

We observed central zone time, central zone entrance number, freezing time, and total distance travelled in OFT, which are given in Fig. 2. According to central zone time F(2,21 = 31,82) and central zone entrance number parameters F(2,21 = 22,24), EPOα has potent an anxiolytic effect both 1000 U and 2000 U ($p < 0.0001$). After EPOα treatment, freezing time F(2,21 = 15,32) decreased positively ($p < 0.001$). EPOα administration did not affect locomotor activity, which is the total distance traveled F(2,21 = 0,13) versus control ($p > 0.05$). The Fecal boli number F(2,21 = 0,13) had no difference in all experiment groups ($p > 0.05$).

We also analyzed rearing behavior patterns in OFT, which are given in Fig. 3. The unsupported rearing number (total), F(2,21 = 9, $p < 0.005$) and unsupported rearing number (in the center zone) F(2,21 = 12,44, $p < 0.001$) increased remarkably compared to the control group. According to unsupported rearing, latency time decreased both 1000 U/kg EPOα and 2000 U/kg EPOα vs control group F(2,21 = 8,80, $p < 0.005$). Supported rearing number, supported rearing number latency, and total rearing number did not change among all groups ($p > 0.05$).

Elevated plus maze result

We also analyzed behavioral patterns in the elevated plus maze. We observed open arm time, open arm entrance number, head dipping behavior, and stretch-attend posture in EPM, which are given in Fig. 4. EPOα showed a

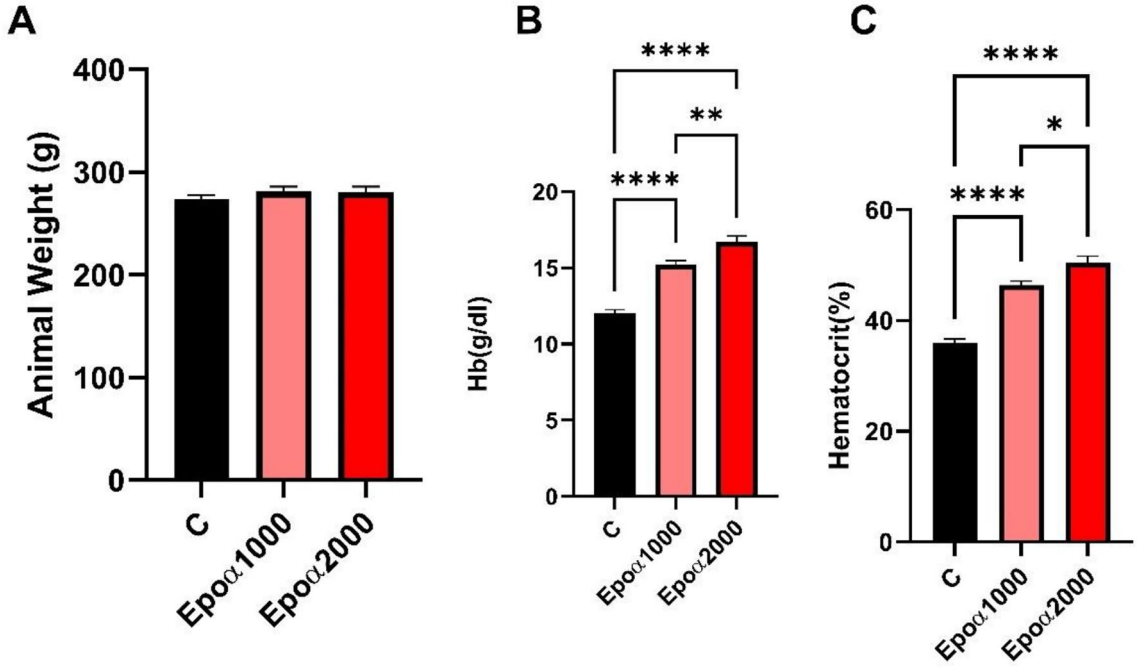

Fig. 1 Effect of Epoetin alfa on physiological parameters. Animal weight (**A**), Hemoglobin (**B**), and Hematocrit (**C**) were analyzed. Data are presented as mean ± SEM. ("one-way ANOVA", *$p < 0.05$, **$p < 0.005$, ****$p < 0.0001$)

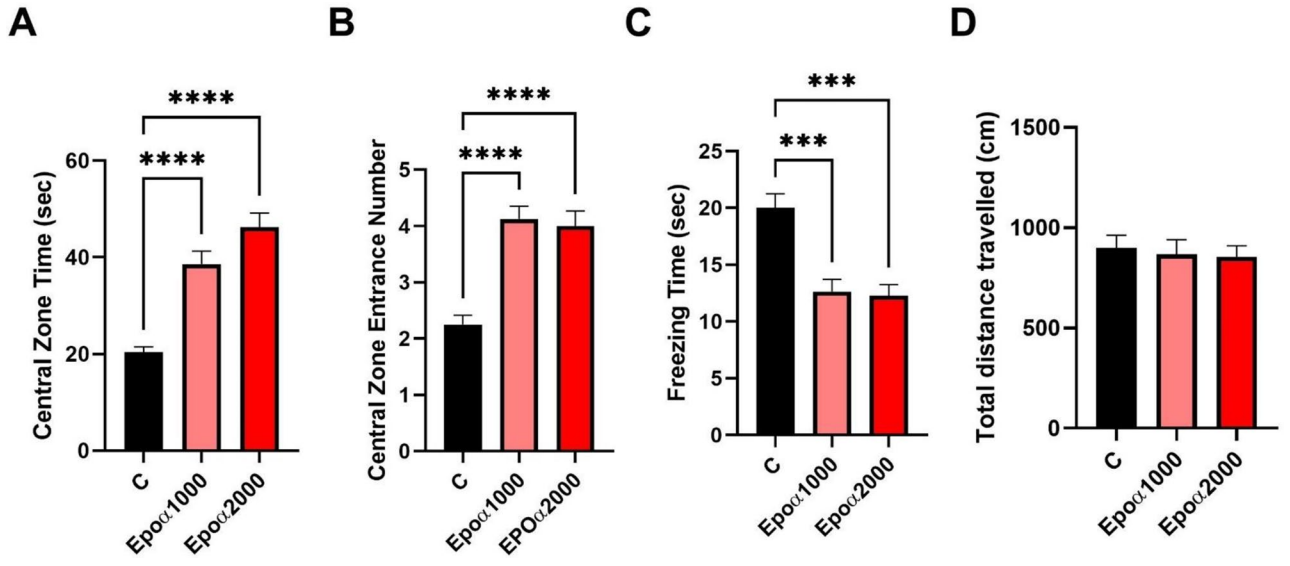

Fig. 2 Behaviors in the open-field test. Central zone time (**A**), central zone entries number(**B**), freezing time (**C**), and total distance (**D**) traveled. Data are presented as mean ± SEM. ("one-way ANOVA", ****p < 0.001, ****p < 0.0001 vs control*)

powerful anxiolytic effect in both low and high-dose experiment groups. The time spent in the open arm $F(2,21 = 5,86$, $p < 0.005)$ and the number of open arm entries $F(2,21 = 29,61)$ were significantly increased in subjects receiving EPOα ($p < 0.0001$). Head dipping behavior $F(2,21 = 5,62)$, an exploratory behavior associated with decreased anxiety, increased in both two EPOα administered groups ($p < 0.05$). We also examined stress-related stretch-attend posture $F(2,21 = 9,72)$, which was significantly reduced in the EPOα vs control ($P < 0.005$). The fecal boli $F(2,21 = 0,26)$ number displayed no difference among all groups ($p > 0.05$). Anxiety index $F(2,21 = 13,39)$ had a lower score in EPOα treatment groups (1000 U vs. C: $p < 0.005$, 2000 U vs. C: $p < 0.001$). We also analyzed open arm time % was significantly increased both 1000 EPOα (median: 17, interquartile range: 13.33, $p < 0.05$) and 2000 EPOα (median: 19.33, interquartile range: 22.91, $p < 0.005$) compared to the control group (median: 9.66, interquartile range: 3.34, "Kruskal Wallis").

Light dark box result

We also examined the effects of EPOα in the light-dark box in Fig. 5. A remarkable increase was observed in subjects after EPO administration in light zone time $F(2,21 = 8,11$, $p < 0.005)$ and light-dark zone entrance number $F(2,21 = 8,43$, $p < 0.05)$. All groups found Light-dark zone latency time insignificant ($p > 0.05$).

Fecal boli results

Fecal boli numbers were also observed in three anxiety tests. The number of defecations was not significantly different among the groups in all three tests (See Fig. 6) ($p > 0.05$).

Correlation result

In naive rats receiving EPOα treatment, a statistically significant correlation was not found between behavior patterns (including anxiety index, central zone time, light zone time, and open arm time) and hemoglobin values (See Figs. 7 and 8).

Discussion

The present study showed that chronic administration of Epoetien alfa decreased anxiety-like behaviors in female rats. Consistent with the data we present, in our previous study with darbepoetin alfa (another EPO derivation) in male rats, brain-derived neurotrophic factor (BDNF) and serotonin levels increased, and anxiety-like behaviors decreased [36]. Depending on the decrease in anxiety, the time spent in the center zone, which is the risky zone in the open field test, and the number of entries into this risky zone increased without dose dependence. Another open-field results study by Aghaei et al. reported that the time spent in the center zone increased slightly. However, a non-significant anxiolytic effect was observed in adult male rats [40]. Fathi et al. reported a 500 U/kg EPO anxiolytic effect in OFT but not EPM. In this study, EPO 500 U/kg was administered for 10 days, and only the time elapsed in the central region and the time elapsed in the open arm were analyzed [41].

Another finding that supports the reduction of anxiety-like behaviors is the increase of unsupported rearing behavior, which is an exploratory behavior. Similar effects were demonstrated in the acute EPO treatment [42]. Passive defensive behaviors such as freezing also occur in anxiety tests [34]. Freezing time also decreased positively

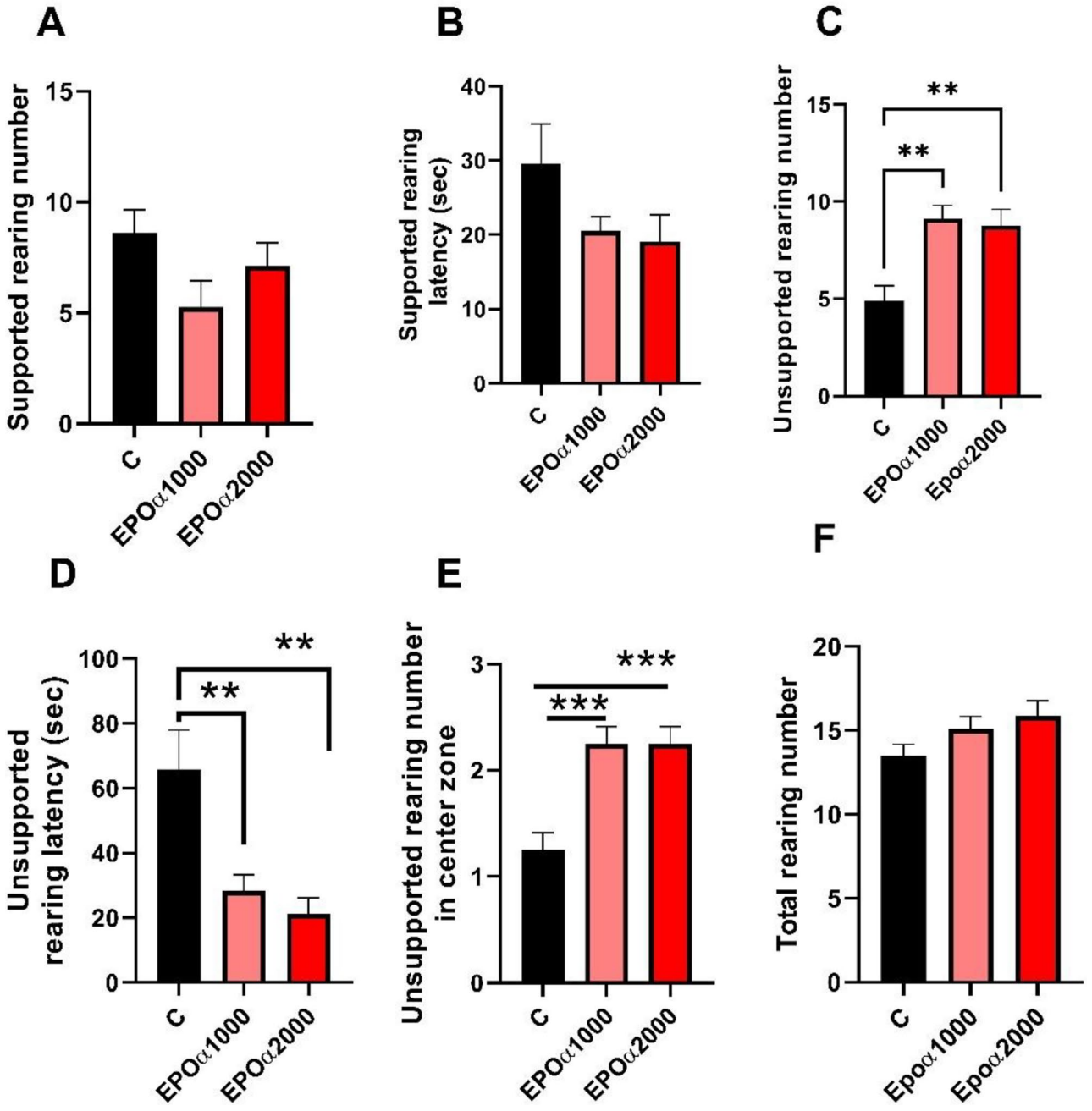

Fig. 3 Behaviors in the open-field test. Supported rearing number (**A**), Supported rearing latency (**B**), Unsupported rearing number (**C**), Unsupported rearing latency **(D)**, Unsupported rearing number in the center zone **(E)**, Total rearing behavior (**F**) Data are presented as mean ± SEM. ("one-way ANOVA", $^{**}p < 0.005$, $^{***}p < 0.001$, vs control)

with the treatment. Fecal boli count, a marker of the autonomic system, was not significant among all groups.

The open-field test also examines locomotor activity and anxiety-like behaviors. There was no significant increase in horizontal and vertical direction after chronic EPOα treatment. In other rodent studies, locomotor activity did not change after EPO administration [40, 43–46].

In the literature, studies on EPO and its derivatives mainly focus on the locomotion effect of EPO in subjects with neurological damage. Neurological damage models such as focal hypoxia, cerebral edema, and neuroinflammation deteriorated locomotor activity. In these studies, locomotor activity improved after EPO treatment [47, 48]. Apart from these studies, acute EPO treatment results showed that locomotor activity increased after a

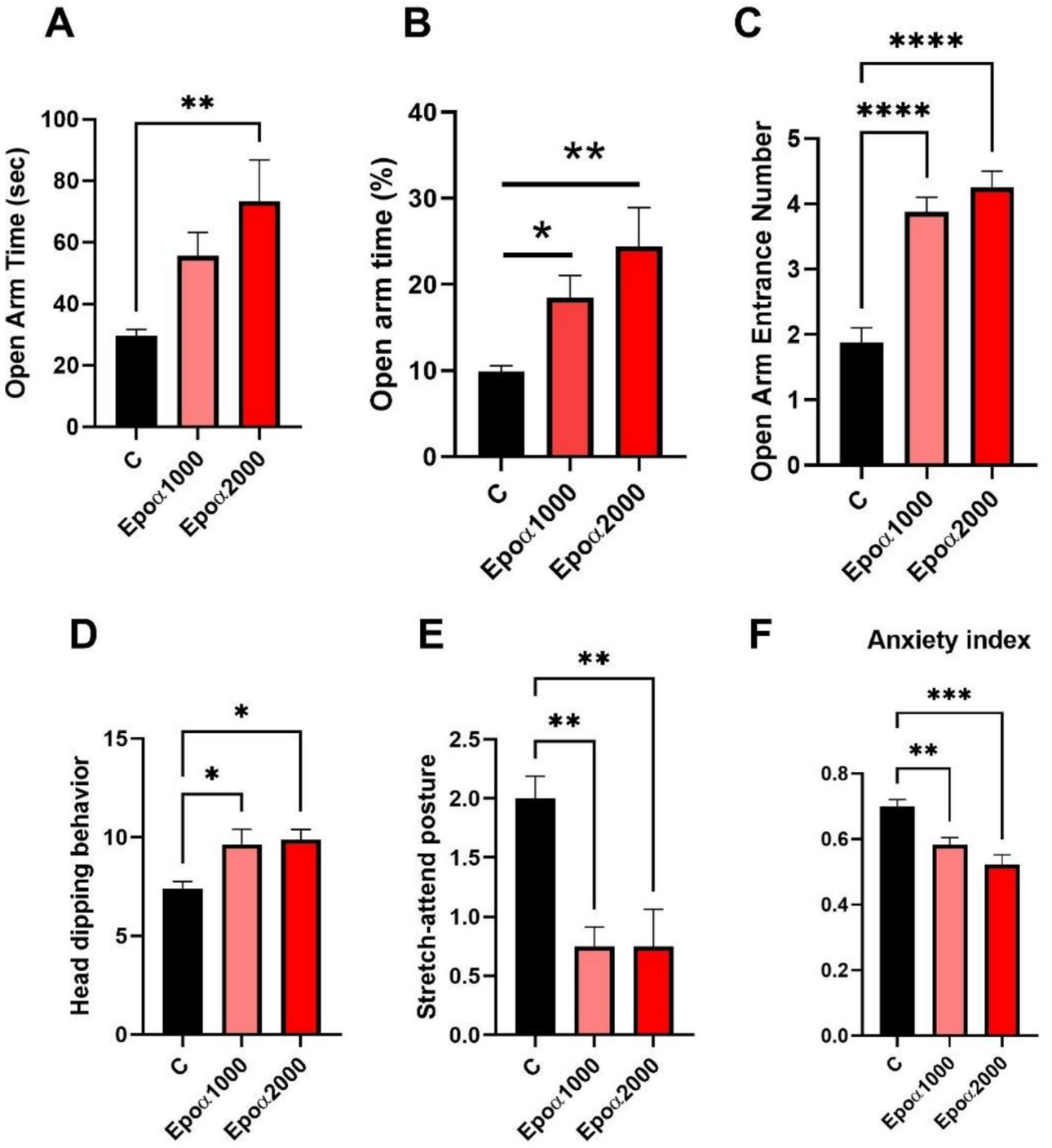

Fig. 4 Behaviors in the elevated plus maze Open arm time (**A**), Open arm time % (**B**), Open arm entrance number (**C**), Head dipping behavior (**D**), Stretch-attend posture (**E**), Anxiety index (**F**) Data are presented as mean ± SEM. ("one-way ANOVA", *$p < 0.05$, **$p < 0.005$, ***$p < 0.001$, ****$p < 0.0001$ vs control), (Open arm time %, "Kruskal Wallis," *$p < 0.05$, **$p < 0.005$, vs. control)

short period (24 h) and was the same as the control after 96 h [42].

In the elevated plus maze test, the time spent in the open arm, which is the risk zone, and the number of entries increased positively in a dose-independent manner, consistent with the open field test. Head dipping, the exploratory behavior in the elevated plus maze test, increased with decreased anxiety. Stress-induced tense posture decreased after EPOα treatment. In the present study, the anxiety index decreased in the treatment groups. Osborn et al. administered recombinant mouse erythropoietin for 2 weeks [49]. Although a moderate

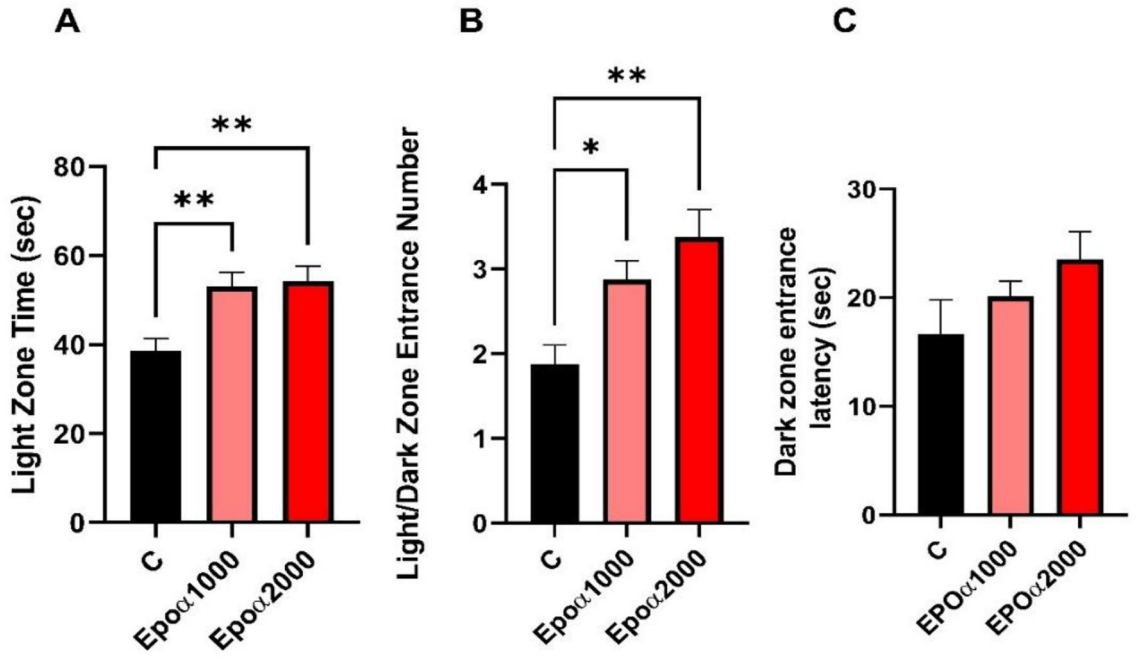

Fig. 5 Behaviors in light-dark box. Light zone time (**A**), Light/dark zone entrance number (**B**), Dark zone entrance latency (**C**), Data are presented as mean ± SEM. ("one-way ANOVA", **p < 0.05, **p < 0.005,* vs control)

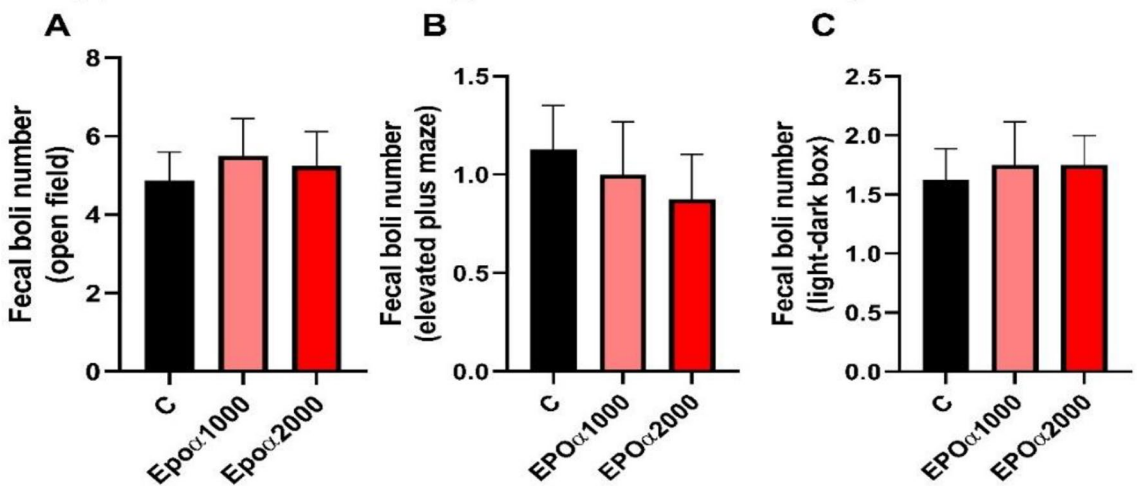

Fig. 6 Fecal boli number. Open field (**A**), Elevated plus maze (**B**), Light-dark box (**C**), Data are presented as mean ± SEM. ("one-way ANOVA")

increase in the number of open-arm entries was observed as a result of this application, no anxiolytic effect was observed, and the time spent in the open arm, which is an important parameter related to anxiety, was not analyzed in this study [49]. In Other rodent studies, recombinant EPO showed an anxiolytic effect in the sleep apnea model [50]. Beta-hydroxybutyrate, a hematopoietic agent, also decreased anxiety-like behavior in rats exposed to chronic stress [51].

EPOα chronic treatment also exhibited an anxiolytic effect in the light-dark box. Time spent in the light zone and exploration of light-dark compartments increased dose-independently. Carbamylated erythropoietin attenuated similarly anxiety-like behaviors in the light-dark box [52]. Different types of EPO have been shown to reduce depression-like behaviors [49]. It has also been reported to improve spatial learning [50]. Positive effects on mental disorders have also been reported in human imaging studies [27, 53].

EPO may exert its possible anxiolytic effects by regulating neurotrophic factors and neurotransmitters. Brain-derived neurotrophic factor (BDNF) is responsible for neuronal survival, the secretion of several neurotransmitters, neurogenesis, and synaptic plasticity [36, 54]. BDNF

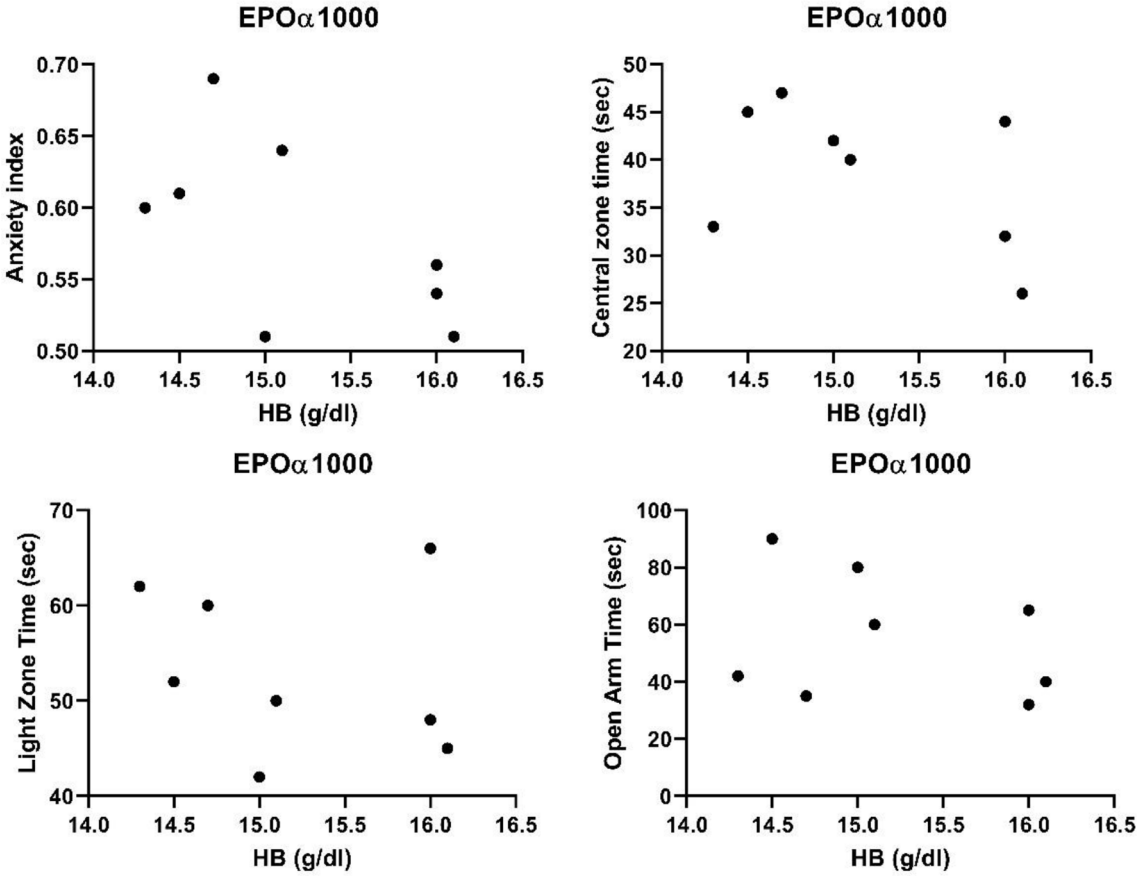

Fig. 7 EPOα1000 correlation results. The correlation between anxiety index and HB (r = - 0.66), ($p > 0.05$), the correlation between central zone time and HB (r = - 0.44), ($p > 0.05$), the correlation between light zone time and HB (r = – 0.21), ($p > 0.05$), the correlation between open arm time and HB (r = – 0.29), ($p > 0.05$), ("Pearson correlation test")

plays a significant role in the emergence of psychiatric disorders such as depression and anxiety [55]. Anxiety Clinical studies and rodent studies investigating anxiety-like behaviors have shown a relationship with BDNF levels. Different stressors have been reported to cause a decrease in BDNF expression [56]. Indeed, the association of EPO with BDNF, a neurotrophic factor, has been reported in many studies [57]—an important EPO study on anxiety concerns patients diagnosed with generalized anxiety disorder. EPO serum levels were lower value in patients diagnosed with generalized anxiety disorder. A negative correlation was found between decreased EPO serum levels and increased anxiety scores [58]. EPO and its derivatives have been shown to increase BDNF levels in different animal model studies [36, 57, 59–61]. Epoetin alfa may have shown anxiolytic effects with similar effects.

There is also an important relationship between EPO and the hypothalamic-pituitary-adrenal (HPA) axis [62, 63]. As a product of the HPA axis, glucocorticoid hormones are secreted that regulate fight-or-flight responses during stress. HPA dysfunction and dysregulation are seen in anxiety disorders. The EPO derivative darbepoetin alfa has been shown to reduce cortisol levels in both healthy rats and a neuroinflammation group after 4 weeks of administration [36]. Epoetin alfa may have reduced anxiety-like behaviors by affecting the HPA axis with similar effects.

There is also an important relationship between EPO and serotonin. It has been shown that EPO production decreases with decreased serotonin production and is secreted from nearby neurons [64] depressive-like behavior in ovariectomized rats by activating the EPO signaling pathway [65]. In our previous study, chronic darbepoetin alfa administration increased the level of serotonin in the striatum and prefrontal cortex, two critical brain regions related to anxiety, and anxiolytic effects were observed [36].

Serum, cerebrospinal fluid, and postmortem studies have shown that cytokines play a role in inflammation in psychiatric disorders [66–69]. Inflammation contributes to pathophysiology through increased proinflammatory cytokines and damaging neurotrophic factors and neurotransmitters [36]. According to rodent studies,

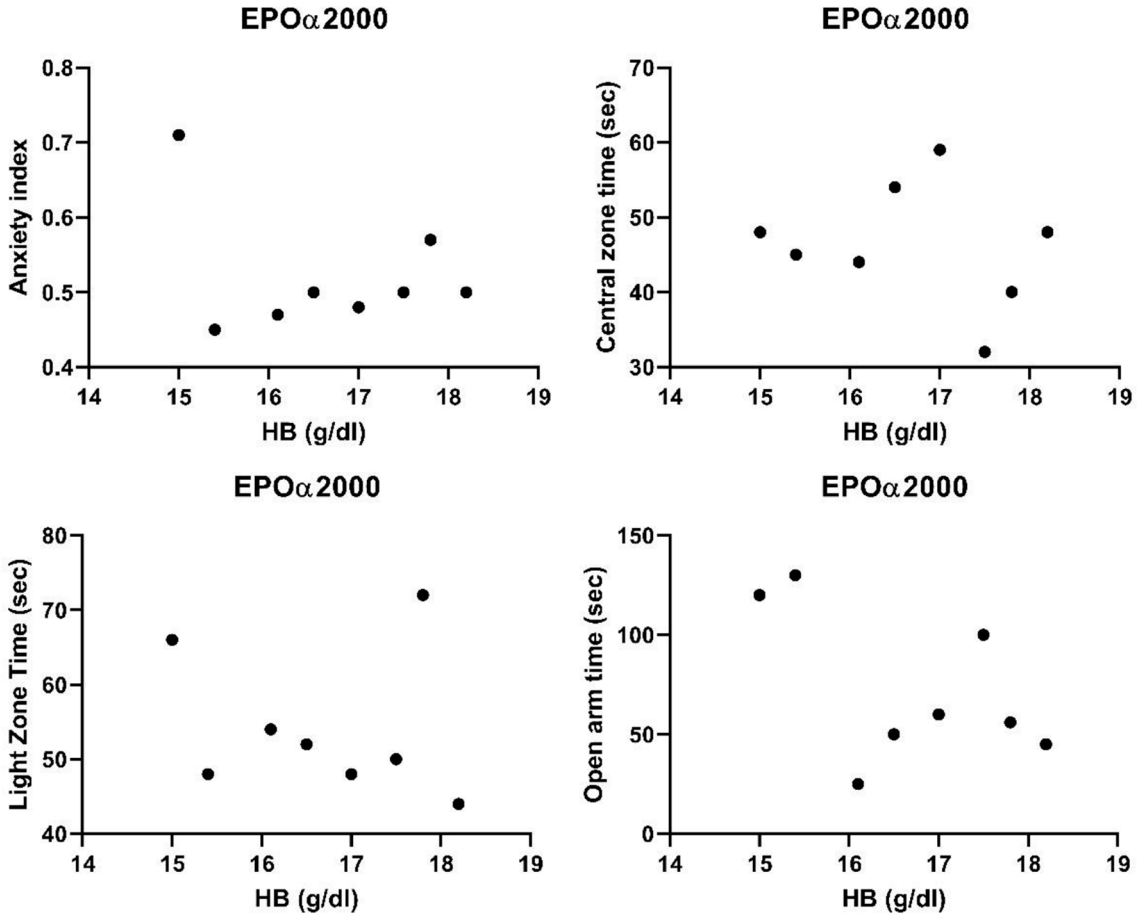

Fig. 8 EPO*α*2000 correlation results. The correlation between anxiety index and central zone time (r = − 0.30), ($p > 0.05$), the correlation between central zone time and HB (r = - 0.20), ($p > 0.05$), the correlation between light zone time and HB (r = − 0.15), ($p > 0.05$), the correlation between open arm time and HB (r = − 0.53), ($p > 0.05$), ("Pearson correlation test")

neuroinflammation causes anxiety-like behaviors [36, 70, 71]. Anti-inflammatory effects of different EPO derivatives have been reported [36]. Epoetin alfa may be responsible for anxiolytic effects with similar effects.

EPO*α* significantly increased hemoglobin, suggesting EPO*α* treatment may have increased oxygenation. Therefore, EPO*α* may also have caused similar anxiolytic effects seen with hyperbaric oxygen therapy [36]. However, in the present study, we could not measure oxygenation. Hematocrit increased with increasing hemoglobin. With increased hematocrit, viscosity may increase, and capillaries may develop a resistance to perfusion. We did not observe any correlation between hemoglobin levels and the anxiety behavioral parameters we examined. EPO therapy can mimic hyperbaric oxygen therapy when it leads to much higher hemoglobin values.

There were some limitations in the present study. Some basic physiological measurements were not conducted. Changes in blood pressure can be studied due to increased hematocrit in EPO studies [72]. In tail blood pressure and heart rate measurements, the swelling of the tail handle increases anxiety and stress [73, 74]. In those with increased anxiety, the hemodynamic parameters are affected [75]. The presented study did not measure blood pressure to avoid affecting behavior. Other studies methodologically looking at the effect of EPO derivatives on behavior have often not measured blood pressure [49, 52]. Separate animals should be used for measurements such as behaviors and molecular aspects [76, 77]. The proposed use of additional animals to study different parameters raises ethical and financial concerns. Instead of using additional animals, physiological parameters can be measured using more advanced technologies. Due to the absence of advanced technological applications such as telemetry, the study of some physiological measurements has been limited. Further, EPO administration increased hemoglobin levels. Increased hemoglobin levels may cause oxidation. Oxidation occurs due to increased hemoglobin and autooxygenation mechanisms [78, 79]. We could not examine oxidative stress for financial reasons; however, we aim to examine it in future studies. The elucidation of these positive effects,

especially in the central nervous system, will be useful in studying pharmacological parameters such as half-life to better adjust and administer the epoetin alfa dose.

Conclusion

The present study found beneficial effects of epoetin alfa on anxiety-like behaviors. Epoetin alfa has potent anxiolytic effects without dose dependency. Further studies on the effect of derivatives of erythropoietin hormone on anxiety-like behaviors are needed.

Abbreviations

ALB	Anxiety-like behaviors
BDNF	Brain-derived neurotrophic factor
EPO	Erythropoietin
EPO*α*	Epoetin alpha
EPM	Elevated plus maze
LDB	Light dark box
OFT	Open field test
HPA	Hypothalamic-pituitary-adrenal

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Author contributions

H.Ç. and E.G. designed the project. H.Ç. performed the animal experiments. H.Ç. and S.K. analyzed the data. E.G., H.Ç., and S.K. interrupted data. E.G., H.Ç., and S.K. wrote the manuscript. E.G., H.Ç., and S.K. read and approved the final manuscript.

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Data availability

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethical approval

All experiments were carried out as approved by the Ankara University Experimental Animals Ethics Committee, with the approval reference number 2022-15-144 (committee meeting date 07.09.2022).

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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