BMC Complementary Medicine and Therapies

RESEARCH

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Xiao Yao San mitigates corticosterone stimulation-induced hippocampal neuronal damage by inhibiting GR phosphorylation and nuclear translocation via FKBP4 involvement

Xuedi Kang[1†], Ting Wang[2†], Haiping Wan[1], Wenjun Fu[2] and Songjun Lin[1*]

Abstract

Background Corticosterone stimulation has profound physiological and neurological effects on individuals, necessitating effective interventions to mitigate its impact. Current therapeutic approaches for corticosterone stimulation injury have limitations, including addiction and tolerance issues. In contrast, historical formulations such as Xiao Yao San, a traditional Chinese medicine formula, have shown promise in addressing changes in corticosterone stimulation-related neuroplasticity. This study aimed to explore the potential of Xiao Yao San in modulating the glucocorticoid receptor (GR) signaling pathway and its downstream effects on hippocampal neuroplasticity under corticosterone stimulation conditions.

Methods Primary hippocampal neurons were cultured and exposed to corticosterone to establish a corticosterone stimulation model. Cellular viability, apoptosis, and protein expression were assessed via CCK-8 assays, flow cytometry, and immunoblotting, respectively. Interactions between FK506 binding protein 51 (FKBP51), GR, and p-GR were analyzed via coimmunoprecipitation and GST pull-down assays. The influence of FKBP4 on the competitive binding of GR was explored via similar techniques. The functional consequences of gene knockdown and overexpression were evaluated through cellular assays.

Results Xiao Yao San attenuated corticosterone-induced reductions in cell viability and apoptosis, counteracting the detrimental effects of corticosterone stimulation. It downregulated FKBP51 expression and suppressed GR phosphorylation and nuclear translocation. Additionally, it hindered the interaction between FKBP51 and GR/p-GR. FKBP4 overexpression rescued hippocampal neuron viability and protected against the GR phosphorylation and nuclear translocation induced by corticosterone.

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Conclusion Xiao Yao San exhibited promising effects in ameliorating changes in corticosterone stimulation-induced neuroplasticity through the modulation of the GR signaling pathway. By inhibiting FKBP51-mediated GR phosphorylation and nuclear translocation, Xiao Yao San has potential as an alternative therapeutic strategy for corticosterone stimulation-related conditions. Further clinical investigations and mechanistic studies are warranted to validate its therapeutic efficacy and elucidate its mechanisms of action.

Keywords Xiao Yao San, Corticosterone stimulation, Glucocorticoid receptor, FK506-binding protein 51, Brain-derived neurotrophic factor

Background

Stress refers to neuroendocrine responses primarily characterized by sympathetic excitation and hypothalamic–pituitary–adrenal (HPA) axis hyperactivity upon exposure to diverse stressors, resulting in a series of neuroendocrine reactions along with associated physiological and metabolic alterations [1, 2]. Moderate stress plays a crucial role in organism survival; however, prolonged stress reactions stemming from continuous exposure to various stressors or ineffective adaptation lead to subsequent pathological changes, namely, chronic stress damage [3, 4]. The central role of the hypothalamic–pituitary–adrenal (HPA) axis in the stress response, which is marked by excessive activation and dysfunction, contributes to the pathophysiology of chronic stress [4, 5]. The efficacy of the HPA axis relies on a cascade of events initiated from the hypothalamus, culminating in glucocorticoid (GC) molecules that regulate various functions via receptor binding to target organs, including the hippocampus, where neuronal plasticity is vulnerable to injury [6].

Corticosterone stimulation-induced impairments in brain neuronal plasticity are widely acknowledged in academia; however, the underlying mechanisms remain incompletely understood [7, 8]. Our research team has dedicated years to exploring the role of traditional Chinese medicine in combating damage caused by corticosterone stimulation, revealing that the FKBP-GR-BDNF signaling pathway is a critical regulator of hippocampal neuronal plasticity under corticosterone stimulation conditions.

"Xiao Yao San," documented in the "Tai Ping Hui Min He Ji Fang," comprises a combination of eight herbs, including Bupleuri Radix, Paeoniae Radix Alba, Atractylodis Macrocephalae Rhizoma, Poria, Angelicae Sinensis Radix, Glycyrrhizae Radix and Rhizoma, Zingiberis Rhizoma Recens, and Menthae Haplocalycis Herba [9, 10]. While reports indicate that Xiao Yao San can harmonize the liver, ameliorate mood, and respond to stress, the pharmacological actions of Chinese herbal medicine are inherently intricate; [11, 12] therefore, further clinical research and scientific validation are warranted to determine whether Xiao Yao San has therapeutic potential in addressing corticosterone stimulation-related disorders and clarifying its specific site of action.

Corticosterone binds to glucocorticoid receptors (GRs) via FKBP51 as a cochaperone, participating in the regulation of the glucocorticoid signaling pathway [13]. This transduction process plays pivotal roles in stress response modulation, immune regulation, and numerous physiological processes [14]. An in-depth investigation of these mechanisms contributes to an enhanced understanding of cell signaling and associated disease progression [15, 16]. Preliminary studies by our team confirmed that corticosterone stimulation induces elevated hippocampal FKBP5 expression concomitant with GR downregulation. The administration of antistress injury drugs results in decreased FKBP5 expression, increased GR expression, and elevated BDNF expression. This mechanism possibly involves increased FKBP5 expression, which represses GR expression, leading to BDNF downregulation and consequent alterations in hippocampal neuroplasticity.

In summary, the phosphorylation and nuclear translocation of GR and the subsequent regulation of the expression of target genes are focal points in the context of corticosterone stimulation. Taking into account domestic and international research, we posit that the FKBP-GR-BDNF signaling pathway during corticosterone stimulation significantly governs neuronal plasticity. Given the limited depth of related investigations, further refinement is essential to unravel these mechanisms comprehensively.

Methods

Cell culture

Mouse hippocampal neurons were cultured according to the modified protocol outlined in Sect. [17]. The pregnant mice were purchased from Sun Yat-sen University Laboratory Animal Center, and then the mouse were narcotized with intraperitoneal injection of 50 mg/kg of pentobarbital sodium (P3761, Sigma) and subsequently were euthanized by carbon dioxide inhalation. Then, the primary separation of hippocampal neurons from E18 mouse embryos was performed as previously reported [18], which in compliance with the approval of the Fourth Clinical Medical College of Guangzhou University of Chinese Medicine's institutional animal care and use committee. In brief,

hippocampal neurons were cultured at low density on coverslips coated with poly-D-lysine or at high density in six-well plates coated with poly-D-lysine. The culture medium consisted of neural basal medium (Gibco) supplemented with 2% B27 supplement (Gibco). The cells were maintained at 37 °C under a 5% CO_2 atmosphere. Corticosterone (CORT, catalog number C104537) was obtained from Aladdin (Shanghai, China). To establish an in vitro stress model, cells were treated with CORT (250 μmol/L) for 24 h to induce stress, and DMSO was used as the vehicle control. This model was created to simulate the effects of corticosterone stimulation on cells in vitro.

Preparation and composition analysis of Xiao Yao San

Xiaoyaosan, a traditional Chinese herbal formula, is composed of eight herbs in the following proportions: Paeonia lactiflora (root), Bupleurum chinense (root), Atractylodes macrocephala (rhizome), Angelica sinensis (root), Rhizoma Zingiberis (rhizome of Zingiber officinale Rosc.), Poria cocos (dried fungus of Pori cocos (Schw.) Wolf.), Radix Glycyrrhizae (roots of Glycyrrhiza uralensis Fisch.), and Herba of Mentha haplocalyx Briq [19, 20], with ratios of 5:5:5:5:5:5:4:1 (Table 1). Xiaoyaosan (batch number J2447) was obtained from JiuZhiTang Co., Ltd. (Changsha, China) and was prepared as described previously (Yuan et al., 2020). The original herbal materials (2.10 g in total) yielded 1 gram of finely powdered Xiaoyaosan. To analyze the botanical constituents of Xiaoyaosan, UPLC-MS (Dionex Utimate 3000 UHPLC Plus Focused coupled with an LTQ/Orbitrap MS system, Thermo Scientific, USA) was employed [21, 22]. This analytical approach efficiently identifies and quantifies compounds in Xiaoyaosan, aiding in understanding its composition and potential therapeutic components. XYS-medicated serum was prepared as previously described [23].

Table 1 Components of Xiaoyaosan

Name	Part	Content
Paeonia lactiflora	Root	5
Bupleurum chinense	Root	5
Atractylodes macrocephala	Rhizome	5
Rhizoma Zingiberis	Rhizome of Zingiber officinale Rosc.	5
Poria cocos	dried fungus of Pori cocos (Schw.) Wolf.	5
Radix Glycyrrhizae	Root of Glycyrrhiza uralensis Fisch.	4
Herba of Mentha haplocalyx Briq	Total	1

Preparation of Xiao Yao san-treated serum

Sixteen Sprague–Dawley rats were randomly divided into a blank control group and a Xiao Yao San administration group according to body weight. In accordance with the equivalent clinical doses used for humans and rats, the clinical equivalent dose of Xiao Yao San was 0.945 g/kg, which was calculated according to 10 times the clinical equivalent dose. When the drug-containing serum was prepared, the rats were given a 9.45 g/kg intragastric dose, and the blank group was given an equal volume of normal saline. Seven days after continuous intragastric administration, the animals were fasted without water 12 h before the last administration, and 2 h after administration, blood was taken from the abdominal aorta. The blood was centrifuged at 4 °C and 3500 r/min for 10 min, and the supernatant was collected and inactivated at 56 °C for 30 min. After filtration through a 0.22 μm filter membrane, the serum was divided into centrifuge tubes at -80 °C and stored for later use. The animal experiments were approved by the ethics committee of The Fourth Clinical Medical College of Guangzhou University of Chinese Medicine.

FITC/PI staining

Membrane-associated protein (FITC) staining and propidium iodide (PI) staining were conducted via the Annexin V-FITC Apoptosis Detection Kit (#BD 556547, BD Bioscience, Franklin Lakes, NJ, USA). Analysis was performed following the manufacturer's instructions. After treatment, the cells were suspended in binding buffer. Annexin V-FITC and PI were added to the samples, which were subsequently incubated at room temperature for 15 min. Cell apoptosis was detected via flow cytometry (Quanta SC; Beckman Coulter), and data were collected via CytExpert 2.3 software provided by Beckman Coulter.

Immunoprecipitation

Primary antibodies were immobilized via protein G magnetic beads (Bio-Rad). The fixed magnetic beads were incubated with cell lysates (300 μg) at 4 °C overnight. The beads were then pulled down via a magnet and collected. The antibody-bound proteins were eluted via incubation in elution buffer (Thermo Fisher). Protein analysis was performed via Western blotting, with anti-mouse IgG antibodies used as negative controls.

Assessment of cell viability

Cell viability was assessed via a Cell Counting Kit-8 (CCK-8; Dojindo Molecular Technologies, Kumamoto, Japan). Briefly, cells were seeded in a 96-well plate at a density of 100 μL per well and incubated for 12 h. The cells were subsequently treated with either 0.5% DMSO or CORT (250 μmol/L) for 24 h. Following the treatment,

the culture medium was aspirated, and 10 μL of CCK-8 solution along with 90 μL of culture medium were added to each well, followed by incubation at 37 °C for 2 h. Finally, the absorbance at 450 nm was measured via an enzyme-linked immunosorbent assay (ELISA) reader (ELX 800 UV, BIO-TEK, USA) to quantify cell viability. This study provides insights into the impact of CORT treatment on cell survival under stress conditions.

Protein extraction and Western blot analysis

Total protein extraction was performed via a protein extraction kit (KenGen Biotechnology Co., Ltd., Nanjing, China) with a 1% protease inhibitor cocktail (Biosharp, BL630B). The cells were lysed to isolate total protein, and the protein concentrations were determined via the bicinchoninic acid (BCA) method (KenGen Biotechnology Co., Ltd., Nanjing, China) following the manufacturer's instructions. Equal amounts of protein (30 μg) were loaded onto polyvinylidene fluoride (PVDF) membranes (Bio-Rad, California, USA) and separated via sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE). Following separation, the PVDF membranes were blocked with 5% (w/v) bovine serum albumin (BSA) in Tris-buffered saline with Tween 20 (TBST) for 1 h. The membranes were then incubated overnight at 4 °C with primary antibodies against FKBP4, FKBP51, GAPDH, GR, p-GR, histone H3, and BDNF (all the antibodies used in this study were purchased from Abcam). After three washes with TBST, the membranes were incubated with horseradish peroxidase-conjugated secondary antibodies for 1.5 h at room temperature. Bands were visualized via an enhanced chemiluminescence (ECL) detection kit (Thermo Fisher Scientific, Inc.). The protein bands were scanned via a bioimaging analyzer (Bio-Rad, USA), and quantitative analysis of the band intensities was performed via Bio-Rad Quantity One software v.4.6.3 (Bio-Rad Laboratories, Hercules, CA, USA). This method allowed the determination of protein expression levels and changes in response to different treatments.

Statistical analysis

Statistical analysis and graphical representation were performed via GraphPad Prism 8 software (GraphPad Software, La Jolla, CA, USA). The results are presented as the means ± standard deviations (SDs). Statistical significance was determined via one-way analysis of variance (ANOVA), followed by Tukey's post hoc test for multiple comparisons. A significance level of $P < 0.05$ was considered statistically significant. This approach allowed the identification of significant differences between experimental groups and the assessment of the impact of treatments on various parameters.

Results

Inhibition of FKBP51 expression and GR phosphorylation by Xiao Yao San

To investigate the impact of Xiao Yao San on corticosterone stimulation, primary hippocampal neurons were cultured, and a corticosterone stimulation model was established via corticosterone treatment. FKBP51, a cochaperone protein that binds to FK506, has a negative regulatory effect on the function of the GR and HPA axes. Fkbp51 plays an important regulatory role in various mental and metabolic disorders caused by stress. Corticosterone, by binding to glucocorticoid receptors (GRs) via FKBP51, participates in the modulation of glucocorticoid signaling pathways, which play crucial roles in the stress response, immune regulation, and various physiological processes. The CCK-8 data indicated that corticosterone treatment significantly suppressed cell viability, which was alleviated by Xiao Yao San treatment (Fig. 1A). Flow cytometric analysis demonstrated that corticosterone treatment notably induced cell apoptosis, whereas Xiao Yao San treatment mitigated this effect (Fig. 1B and C). Immunoblotting revealed that corticosterone treatment upregulated nuclear and cytoplasmic FKBP51 expression and enhanced GR nuclear translocation and phosphorylation (pGR), suggesting the potential involvement of FKBP51-mediated GR phosphorylation and the nuclear translocation pathway in corticosterone stimulation-induced cellular signal transduction. Xiao Yao San treatment significantly inhibited this process, suggesting a potential mechanism for treating corticosterone stimulation (Fig. 1D-F).

Suppression of FKBP51-GR binding by Xiao Yao San

Building upon the aforementioned results, we further delved into the underlying mechanisms involved. Our data indicated that corticosterone treatment significantly increased FKBP51-GR binding, whereas Xiao Yao San treatment notably inhibited this process (Fig. 2A and C). Concurrently, the data revealed a similar trend in the binding of FKBP51 to phosphorylated GR (pGR) (Fig. 2B and C). Moreover, the FKBP51-GST pull-down experiments demonstrated that corticosterone treatment increased the levels of pGR and GR in the eluate, which decreased upon Xiao Yao San treatment (Fig. 2D and E). Collectively, our data suggested that corticosterone treatment promoted the binding of FKBP51 to GR and pGR, a process that was significantly suppressed by Xiao Yao San treatment.

Enhancement of competitive binding of FKBP4 to GR by Xiao Yao San

FKBP4 (FK506 binding protein 4), a member of the FKBP family, is associated with the glucocorticoid receptor (GR). FKBP4 interacts with GR, influencing the

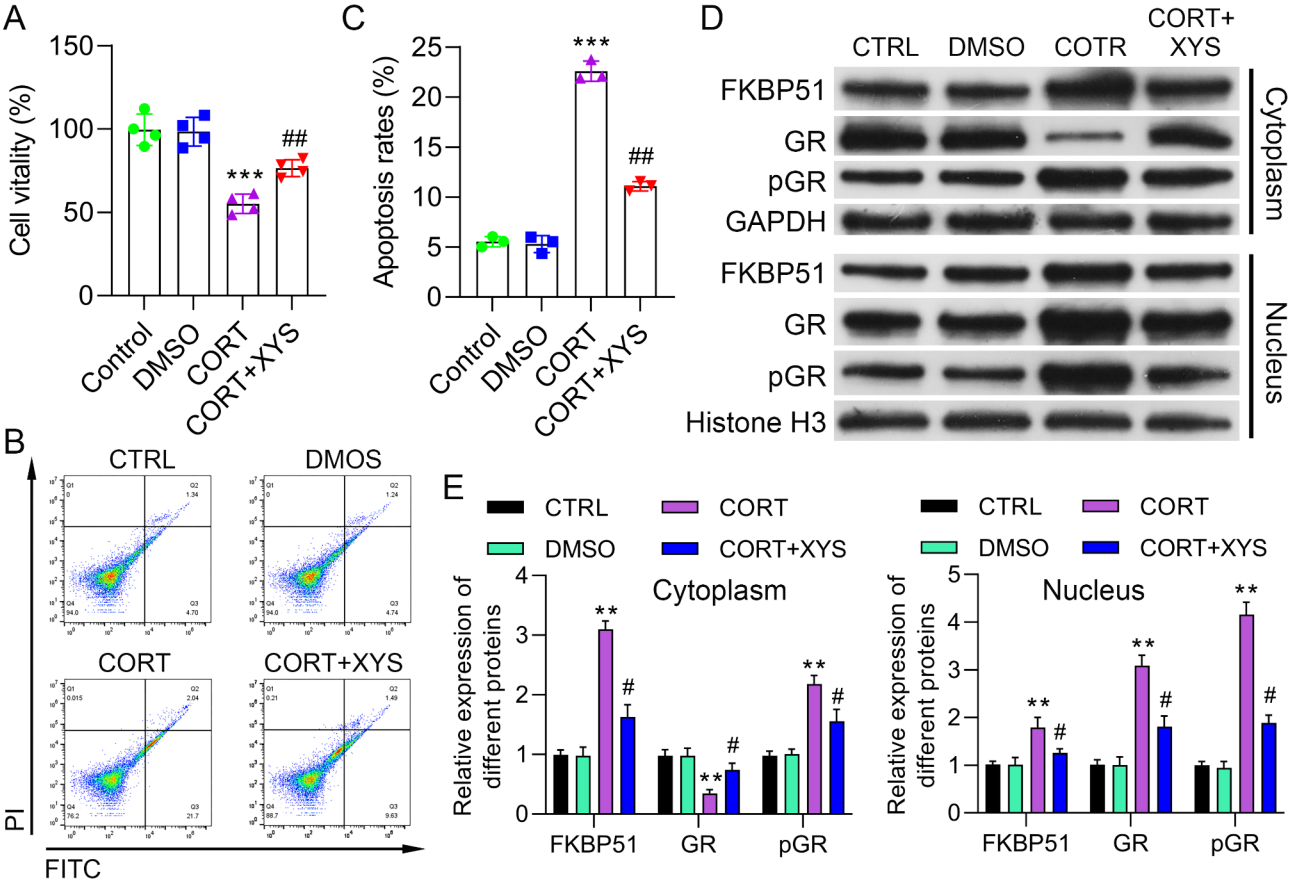

Fig. 1 Xiao Yao San Suppresses FKBP51 Expression and Inhibits GR Phosphorylation. (**A**) Primary hippocampal neurons treated with corticosterone (CORT) or dimethyl sulfoxide (DMSO, control), with or without Xiao Yao San. Cell viability was assessed by a CCK-8 assay. (**B**, **C**) Flow cytometry analysis of apoptosis in different treatment groups. (**D**, **E**, **F**) Protein expression of FKBP51, GR, and p-GR in isolated cell nuclei and the cytoplasm, as detected by immunoblotting. GAPDH was used as a cytoplasmic internal reference, and histone H3 was used as a nuclear internal reference. The samples were derived from the same experiment, and the gels/blots were processed in parallel

regulation of glucocorticoid signaling pathways. The binding of FKBP4 to GR competitively interacts with other cochaperones, impacting the formation of GR complexes and potentially inhibiting GR phosphorylation. We investigated the interaction between FKBP4 and GR. Coimmunoprecipitation (co-IP) data revealed that corticosterone treatment significantly suppressed FKBP4-GR binding, whereas Xiao Yao San treatment notably inhibited this process (Fig. 3A and C). Similar trends were observed for the binding of FKBP51 to pGR (Fig. 3B and C). Additionally, the FKBP4-GST pull-down experiment indicated that corticosterone treatment decreased the levels of pGR and GR in the eluate, which increased following Xiao Yao San treatment (Fig. 3D and E). Overall, our data demonstrated that Xiao Yao San significantly enhanced the binding of FKBP4 to GR and p-GR.

FKBP51 knockdown alleviates corticosterone-induced hippocampal neuronal cell stress

We further verified the role of FKBP51 in corticosterone-induced cellular stress. We knocked down FKBP51 in hippocampal neurons and subjected them to corticosterone treatment (Fig. 4A). The CCK-8 data indicated that FKBP51 knockdown rescued the decrease in cell viability induced by corticosterone treatment (Fig. 4B). Flow cytometric analysis revealed that FKBP51 knockdown attenuated the increase in apoptosis caused by corticosterone treatment (Fig. 4C and D). Immunoblotting of separated cytoplasmic and nuclear proteins revealed that FKBP51 knockdown significantly inhibited the GR phosphorylation and nuclear translocation induced by corticosterone treatment (Fig. 4E-H). Collectively, our data suggested that FKBP51 knockdown significantly mitigated corticosterone-induced hippocampal neuronal stress.

FKBP4 knockdown abolishes Xiao Yao San's protective effect on hippocampal neuronal cell stress

The FKBP4 protein is an immunophilin with peptidylprolyl cis-trans isomerase (PPIase) and cochaperone activities. It is an essential component of the heterocomplexes formed with steroid receptors. This interaction suggests a potential role in facilitating the

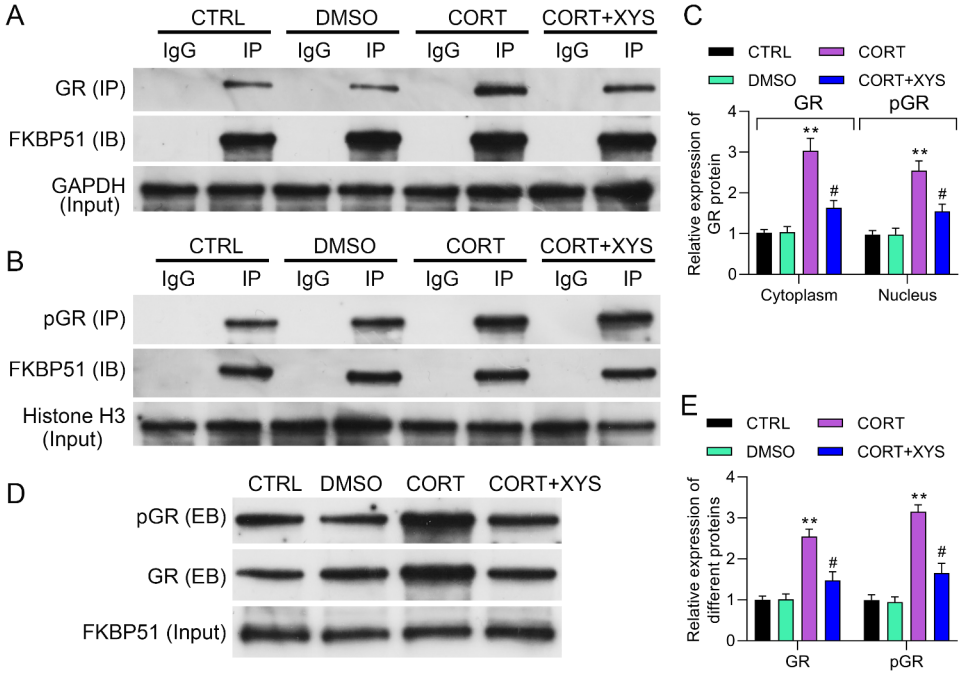

Fig. 2 Xiao Yao San Inhibits FKBP51 Interaction with GR. (**A**) Immunoprecipitation (co-IP) analysis of the interaction of FKBP51 with GR. (**B**) Co-IP analysis of the interaction of FKBP51 with p-GR. (**C**) Statistical analysis of GR and p-GR expression. (**D**, **E**) FKBP51-GST pull-down assay and Western blot analysis of GR and p-GR in the eluted samples. The samples were derived from the same experiment, and the gels/blots were processed in parallel

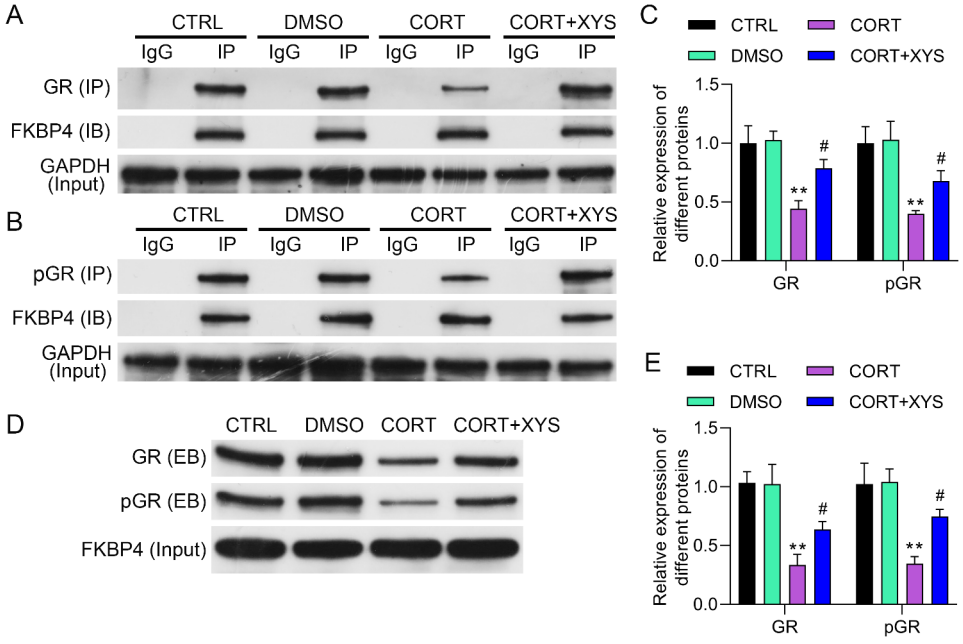

Fig. 3 Xiao Yao San Enhances Competitive Binding of FKBP4 with GR. (**A**) Co-IP analysis of the interaction of FKBP4 with GR. (**B**) Co-IP analysis of the interaction of FKBP4 with p-GR. (**C**) Statistical analysis of GR and p-GR expression. (**D**, **E**) FKBP4-GST pull-down assay and Western blot analysis of GR and p-GR in the eluted samples. The samples were derived from the same experiment, and the gels/blots were processed in parallel

intracellular trafficking of hetero-oligomeric forms of steroid hormone receptors between the cytoplasmic and nuclear compartments. Therefore, we further verified the role of FKBP4 in corticosterone-induced cellular stress. We knocked down FKBP4 in hippocampal neurons and treated them with Xiao Yao San (Fig. 5A). The CCK-8 data indicated that FKBP4 knockdown abolished the protective effect of Xiao Yao San on cell viability (Fig. 5B). Flow cytometric analysis revealed that FKBP4 knockdown abolished Xiao Yao San's

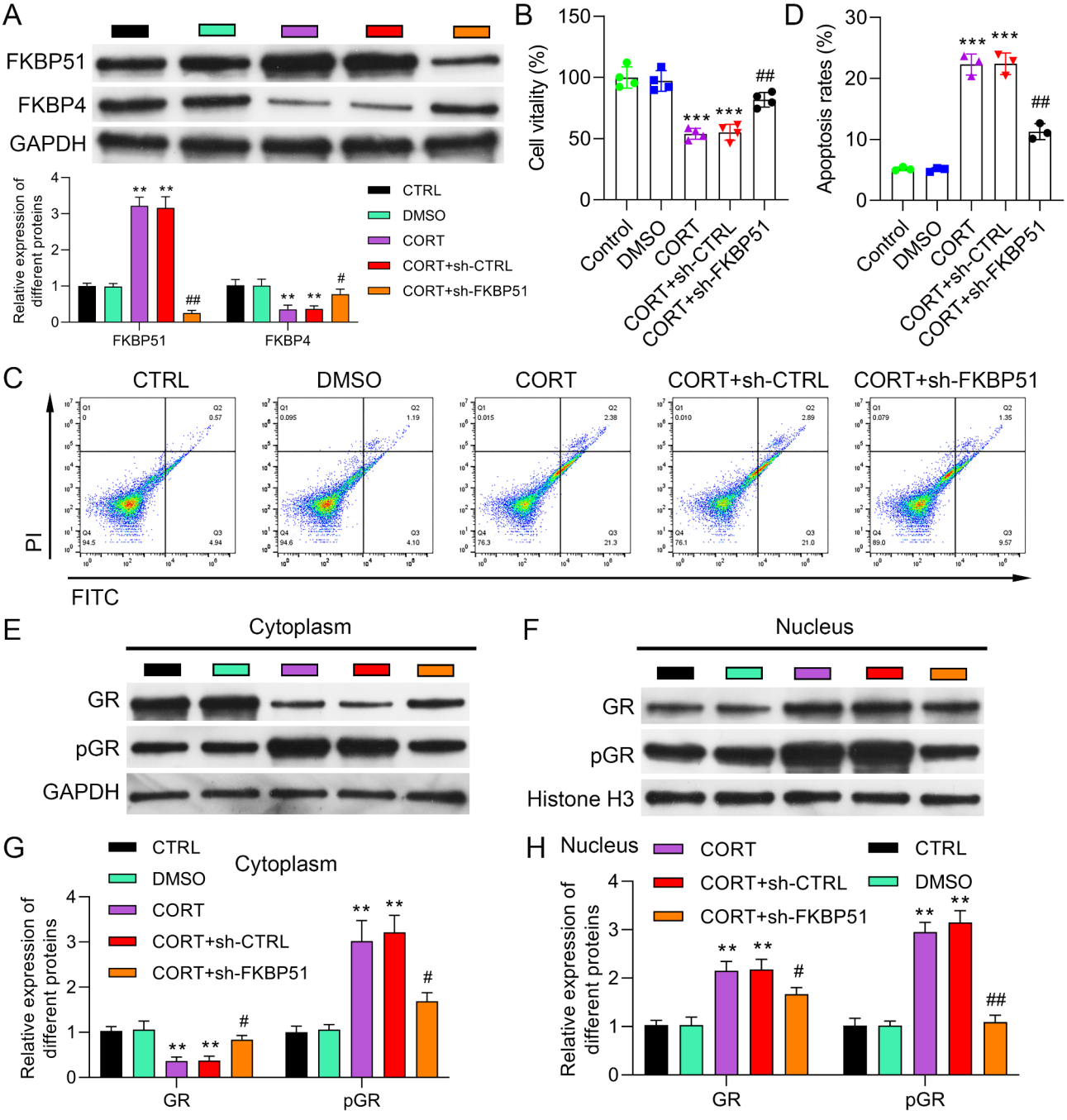

Fig. 4 Knocking down FKBP51 alleviated cellular stress. (**A**) FKBP51 knockdown in corticosterone-treated neurons and immunoblotting for FKBP51 and FKBP4 expression. (**B**) CCK-8 assay assessing cell viability in different groups. (**C**, **D**) Flow cytometry analysis of apoptosis in different treatment groups. (**E**, **G**) GR and p-GR expression in the cytoplasm of different groups. (**F**, **H**) GR and p-GR expression in the nuclei of different groups. The samples were derived from the same experiment, and the gels/blots were processed in parallel

inhibition of apoptosis (Fig. 5C and D). Immunoblotting of separated cytoplasmic and nuclear proteins revealed that FKBP4 knockdown reversed the Xiao Yao San-mediated inhibition of GR phosphorylation and nuclear translocation (Fig. 5E-H). In summary, our data suggested that FKBP4 knockdown abolished the protective effect of Xiao Yao San on hippocampal neuronal stress.

GR overexpression abolished the protective effect of Xiao Yao San on BDNF expression

We further investigated how GR/p-GR influences neuronal cells. We knocked down GR in cells (Fig. 6A-C)

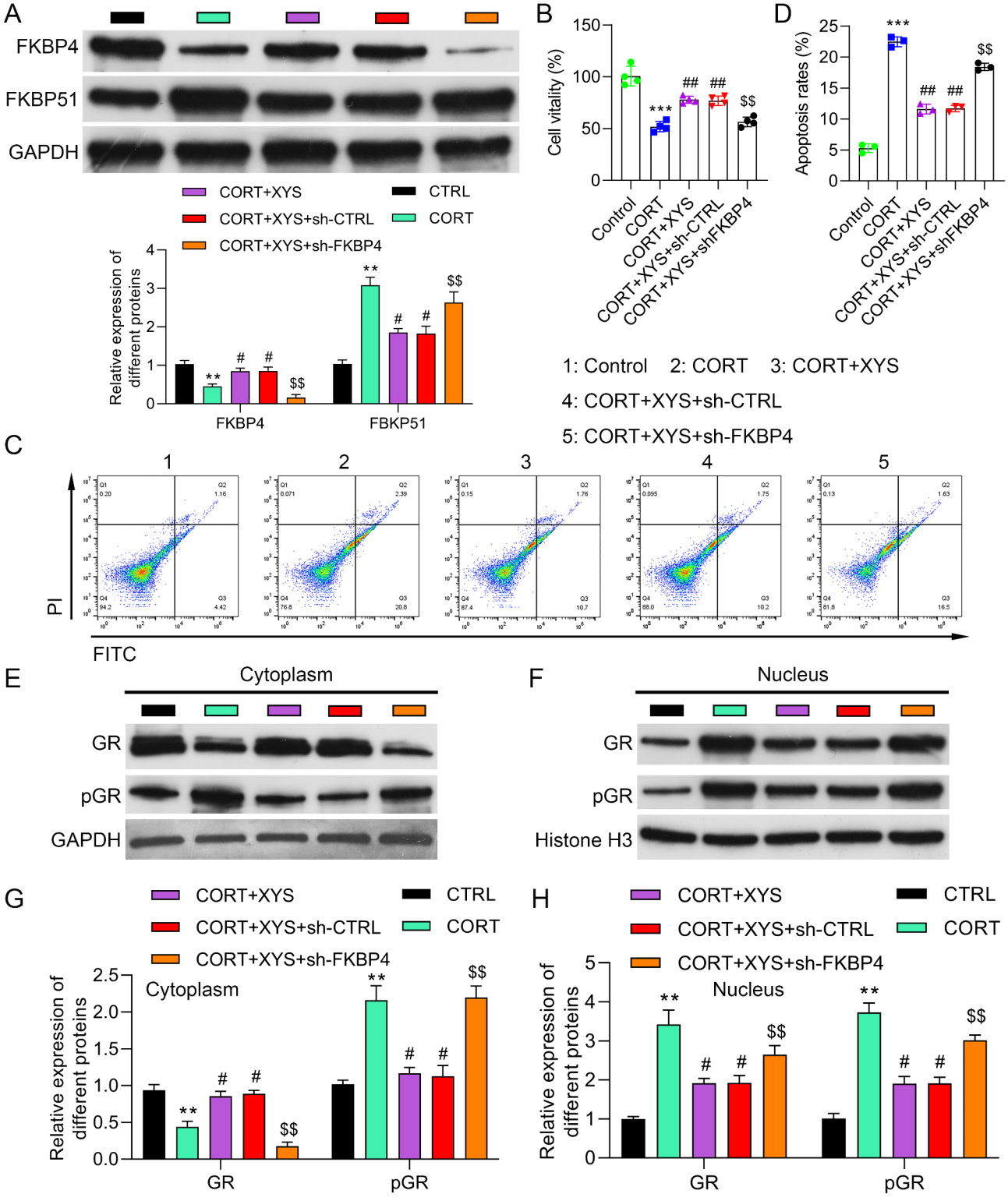

Fig. 5 Knockdown of FKBP4 Abrogates Xiao Yao San's Protective Effect. (**A**) FKBP4 knockdown in corticosterone-treated neurons and immunoblotting for FKBP4 and FKBP51 expression. (**B**) CCK-8 assay assessing cell viability in different groups. (**C**, **D**) Flow cytometry analysis of apoptosis in different treatment groups. (**E**, **G**) GR and p-GR expression in the cytoplasm of different groups. (**F**, **H**) GR and p-GR expression in the nuclei of different groups. The samples were derived from the same experiment, and the gels/blots were processed in parallel

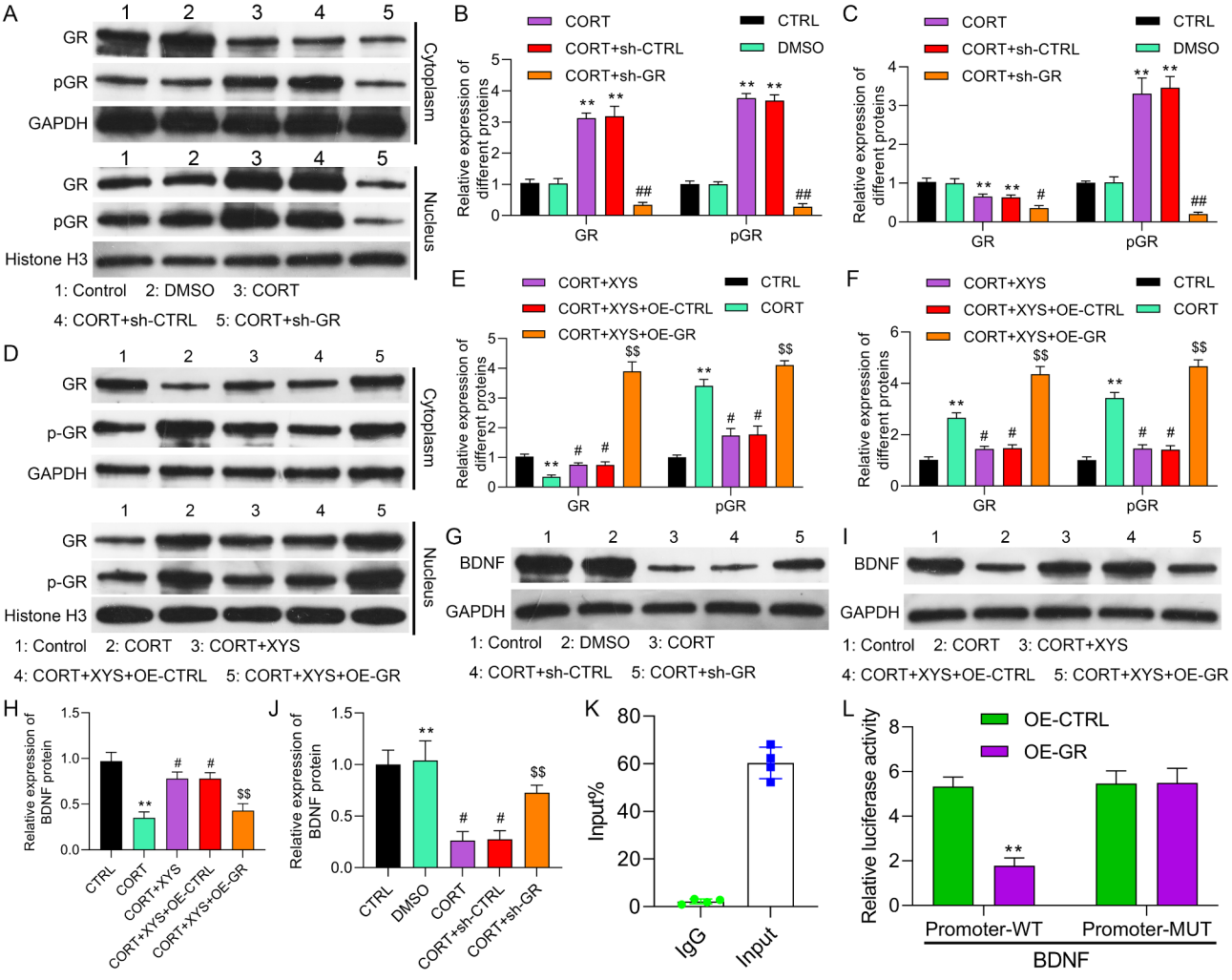

Fig. 6 GR Targets and Negatively Regulates BDNF (**A**, **B**, **C**) Knockdown of GR in corticosterone-treated neurons and immunoblotting for GR/p-GR expression in the nucleus and cytoplasm. (**D**, **E**, **F**) Overexpression of GR in Xiao Yao San-treated neurons, immunoblotting for GR/p-GR expression in the nucleus and cytoplasm. (**G**, **J**) GR expression was knocked down in corticosterone-treated neurons, and immunoblotting was used to measure BDNF expression. (**I**, **H**) GR overexpression in Xiao Yao San-treated neurons, immunoblotting for BDNF expression. (**K**, **L**) Chromatin immunoprecipitation (ChIP) and luciferase assays were used to assess GR binding to the BDNF promoter. The samples were derived from the same experiment, and the gels/blots were processed in parallel

and found that sh-GR treatment significantly prevented the upregulation of GR/p-GR and nuclear translocation induced by corticosterone treatment. Conversely, we overexpressed GR (Fig. 6D–F) and found that GR overexpression counteracted the effect of Xiao Yao San treatment. The data demonstrated that corticosterone treatment significantly downregulated BDNF expression, whereas GR knockdown notably suppressed the effect of corticosterone (Fig. 6G and J). Moreover, GR overexpression abolished the protective effect of Xiao Yao San on BDNF expression (Fig. 6I and H). Chromatin immunoprecipitation confirmed the negative regulatory effects of GR on the promoter of BDNF (Fig. 6K and L).

Discussion

Corticosterone stimulation injury poses significant challenges to both patients and clinicians because of the limitations of current therapeutic interventions, which often involve issues of addiction, tolerance, and clinical risk. However, the historical formula Xiao Yao San, documented in the Song Dynasty's "Tai Ping Hui Min He Ji Ju Fang," has emerged as a promising candidate for addressing changes in corticosterone stimulation-related neuroplasticity. Owing to its well-established reputation for liver-soothing and depression-relieving effects, Xiao Yao San has considerable clinical and research value.

The intricate mechanisms underlying glucocorticoid action through glucocorticoid receptors (GRs) have substantial implications for understanding the

neurobiology of corticosterone stimulation [24–26]. By binding to the GR, glucocorticoids orchestrate gene transcription and impact various physiological processes [27, 28]. The distribution of GR throughout the central nervous system, notably in the hippocampus, underscores its role in stress regulation. The activation of GR by glucocorticoids initiates the formation of complexes that modulate gene expression, contributing to feedback regulation of the hypothalamic–pituitary–adrenal (HPA) axis [29]. Notably, corticosterone stimulation disrupts this intricate balance by inducing sustained GR reductions, fueling a cycle of hippocampal damage and HPA axis overactivity.

At the molecular level, FK506 binding protein 51 (FKBP51, or FKBP5) has emerged as a critical mediator of the GR signaling pathway in response to corticosterone stimulation [30–32]. By acting as a cochaperone for heat shock protein 90 (Hsp90), FKBP5 has a negative regulatory effect on GR activity [33, 34]. It influences ligand binding, GR information transfer, nuclear translocation, and DNA binding to target genes. The interplay between GR and FKBP5 serves as a crucial nexus in the context of corticosterone stimulation-induced neuroplasticity alterations [35]. In this study, we found that Xiao Yao San inhibited the expression of FKBP51 and its binding with GR, thereby alleviating hippocampal stress and exerting a certain protective effect on nerve cells. In addition, FKBP4 inhibited these effects.

Brain-derived neurotrophic factor (BDNF) has emerged as a potent protective factor against changes in neuroplasticity triggered by corticosterone stimulation [36, 37]. The role of BDNF in neuronal growth, development, and differentiation underscores its importance in maintaining neuronal health. Reduced BDNF levels are implicated in the changes in neuroplasticity observed in neurodegenerative conditions [38]. By promoting axonal and dendritic growth, BDNF contributes to hippocampal neuroplasticity in response to corticosterone stimulation. Interestingly, the knockdown of BDNF is associated with diminished dendritic complexity and stress responsiveness. In subsequent research, we can start from the macroscopic phenotype of Xiao Yao San and BDNF, gradually explore their related pathways, and build a clearer relationship network. In addition, the pharmacological effects of Xiao Yao San in other systems should also be taken seriously, which is conducive to its clinical application. In summary, our work not only clarifies the target and metabolic pathway of Xiao Yao San in the nervous system but also provides a standardized workflow for the research of other traditional Chinese medicines, laying a good foundation for the development of traditional Chinese medicine.

Conclusion

In conclusion, the historical formula Xiao Yao San stands out as a potential therapeutic avenue for mitigating nerve damage induced by corticosterone stimulation. By targeting critical elements such as GR and FKBP5, Xiao Yao San may hold promise in restoring the disrupted neurobiological balance and alleviating the negative impact of corticosterone stimulation on neuroplasticity. Future clinical studies and rigorous scientific validation are essential to fully understand the multifaceted mechanisms and therapeutic potential of Xiao Yao San in corticosterone stimulation-related conditions.

Abbreviations

XYS	Xiao Yao San
GR	Glucocorticoid receptor
FKBP51	FK506-binding protein 51
FKBP4	FK506 binding protein 4
BDNF	Brain-derived neurotrophic factor

Acknowledgements

None.

Author contributions

Songjun Lin conceived and designed the study and provided administrative support. Xuedi Kang and Ting Wang performed the experiments and analyzed data. Songjun Lin, Xuedi Kang and Haiping Wan analyzed and interpreted the data. Ting Wang and Wenjun Fu wrote the manuscript. All authors read and approved the final manuscript.

Funding

The work was supported by the Research Fund of ShenZhen Science and Technology Planning Project (JCYJ20190812163201666), Guangzhou Science and Technology Planning Project (202201011643), and Sanming Project of Medicine in Shenzhen (SZZYSM202111011).

Data availability

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

Declarations

Ethics approval and consent to participate

The use of hippocampal neurons from E18 mouse embryos and the animal experiments were in compliance with the approval of the Fourth Clinical Medical College of Guangzhou University of Chinese Medicine's institutional animal care and use committee. All methods were performed in accordance with the relevant guidelines and regulations.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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Received: 21 November 2023 / Accepted: 10 April 2025
Published online: 23 April 2025

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