



Review

The regulatory effect of *Xiaoyao San* on glucocorticoid receptors under the condition of chronic stress

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Abstract: In modern society, fierce competitions cause yearly increase of depression and anxiety. *Xiaoyao San* is a traditional Chinese medicine which relieves depression and nourishes liver. The active ingredients contain saikoside A, saikoside C, saikoside D, ferulic acid, ligustilide, Atractylenolide I, Atractylenolide II, Atractylenolide III, paeoniflorin, Albiflorin, liquiritin, glycyrrhizic acid and pachymic acid. In stress condition, glucocorticoid receptors participate in the hypothalamus-pituitarium-adrenal gland (HPA) axis to regulate the balance of organism. In response to stress, the HPA axis (hypothalamus-pituitarium-adrenal gland) is activated and the levels of glucocorticoid (GC) and catecholamine (CA) are increased to enhance neuroendocrine reactions. Chronic stress activates HPA axis and sustaining increase of GC, reduces the expression amount of GR and inhibits the mechanism of negative feedback on HPA. The lower negative feedback on HPA could lead to ketonemia. Several active ingredients of *Xiaoyao San* can raise the expression of GR and recover the negative feedback of HPA axis to relieve depression and illness state. In spite of the poor understanding of the current effective components in *Xiaoyao San*, this will be the focus of our further research. The study of *Xiaoyao San* could help us better understand its anti-depression mechanism and cure the patients.

Key words: *Xiaoyao San*; Chronic stress; Glucocorticoid receptor; HPA axis.

Introduction

Fierce competitions pervade in modern society, resulting in the yearly increase of morbidity of depression and anxiety, which severely affect people's life quality and social ability. Relevant studies showed that if people are exposed to stress environment, they can easily become anxious (1). Chronic stress is some special affairs in normal life including sustaining repetitive matters and accumulation of stimulation in vitro and vivo, which work smoothly and last long. Stress response is a reaction of the original to resist stress induced cell damage through regulation of gene expression and metabolic status(1). Chronic stress seriously affects people's physical and mental health, which makes the body in sub-health status, and even a variety of diseases. It has been demonstrated that chronic stress has been shown to affect the immune system, which enhances the risk of developing infectious diseases and prolong illness episodes (2). The immune response affected by chronic stress increases vulnerability to autoimmune and allergic diseases (3). The specific brain regions could be activated by chronic stress, which leads to the activation of HPA axis and sympathetic system and results in the cardiovascular diseases (4, 5). It has reported that chronic stress was linked to higher coronary heart disease risk (6).

In addition, chronic stress has become increasingly

recognized as a key factor associated with cancers including NSCLC, breast cancer and gastric cancer(7-10). The stress-related hormones and neurotransmitters secreted under chronic stress could affect the process of tumor development. Accumulated evidence indicates that the crosstalk between β 2-AR, regulated by chronic stress, and oncogene enhanced cell proliferation in cancers(11-13).

Relationship between glucocorticoid receptors and chronic stress

Structure and function of glucocorticoid receptors

GR is a kind of phosphoprotein composed of soluble single-stranded polypeptide. The gene of GR located on human chromosome 5, composing of nine exons(14). Through different ways of splicing, two forms of GR can be formed, namely GR α and GR β . Structurally, there are three functional areas, which are amino terminal with the capacity of gene transcription and activation, DNA binding zone in the central area and carboxyl terminal with hormone binding zone (15). The expression of GR α is 500 times higher than that of GR (16). GR α is widely dispersed in neurons and neuroglia, especially hippocampi, septal area, small neurons in hypothalamus which secrete CRH and AVP and adrenocorticotrophic hormone secretory cells in hypophysis. In brainstem neurons, thalamic nuclei, corpus striatum, amygdaloidal

nucleus and cortex, GR α are also very abundant. GR β only exists in the neurons of hypothalamus and hippocampi, and its amount is only 1% of GR α (16-18).

When glucocorticoid receptors combine with glucocorticoid in the target cells, hormone and its receptor compound bind with specific DNA sequence, namely glucocorticoid response element to regulate the expression of specific target genes. The glucocorticoid receptor has three functions. Firstly, it participates in the stress response of the body, raises the sensitivity of angiopathy towards catecholamine and accelerates proteolysis and gluconeogenesis; secondly, it can resist inflammation and enhance immunity; thirdly, GR can also promote apoptosis. Clinically, the occurrences of many diseases are closely related to the expression of GR.

Glucocorticoid receptors and chronic stress

Stress is a non-specific adaptive reaction when the organism receives strong or harmful stimuli, which causes the psychological and physiological imbalance of internal environment. Stress response is involved in many biological systems, mainly including sympathetic-adrenal medullary system (SAS) and hypothalamic-pituitary-adrenal (HPA) axis. In response to stress, the high levels of glucocorticoid (GC) and catecholamine (CA) are considered as the symbols(19, 20). Its main feature is the enhancement of neuroendocrine reactions, manifested as the excited HPA axis (hypothalamus-pituitary-adrenal gland) and the increase of endogenous stress hormone and glucocorticoid. Its purpose is reinforcing immunity, maintaining and recovering homeostasis. GR can only be activated in high GC concentration due to low appetency. GR mainly gives negative feedback to HPA axis. In hippocampus, pituitary and anterior ventricles, glucocorticoid receptor is the main regulator of HPA axis negative feedback, especially in the hippocampus (21) (Figure 1). Research shows that when the body initiates stress reaction, HPA will make adrenal gland secrete large amount of adrenocortical hormone which will further down-regulate glucocorticoid receptor in hippocampus to inhibit the excitement of HPA axis (22). GR is relevant with many kinds of disease, such as Cushing syndrome, depression, diabetes

and angiocardopathy, etc. Many sicknesses related to stress, such as burn, scald and shock are found to be abnormal in GR(23).

GR protein expression was down-regulated in the hippocampus of rat with chronic stress, but MR protein expression was increased(24). It has been observed that the mRNA level of GR α decreased and expression of GR β increased in gastric mucosal cells of multiple injuries induced gastric mucosal damage mouse model. The decrease of GR is an important factor of the damage of irritable gastric mucosa after multiple injuries(25). In the chronic stress T2DM rats, the GR expression of the islet cells rises, while the GR expression in the liver cell goes down(26).

The effects of *Xiaoyao San* on diseases

In traditional Chinese medical science, it was considered that aberrant emotion makes people disorder of blood and imbalance of Yin and Yang. The abnormal condition of body is associated with disease morbidity and its mechanism in traditional Chinese medical science has a lot in common with stress theory. Stress induced homeostasis maybe a major cause of related disease(27). *Xiaoyao San*, firstly reported in Tai Ping Hui Min and JiJu Fang, has function of clearing liver, removing stress, nourishing blood and strengthening spleen(28, 29).

This is the comprehensive review outlining recent updates on association of *Xiaoyao San* with nervous system and its pharmacologic mechanism. In this paper, we provide an overview of *Xiaoyao San* and focus on its effects on chronic stress. The comprehension of bioactive constituents and mechanism of *Xiaoyao San* will help us choose appropriate drugs and cure depression patients under chronic stress.

Active ingredients involved in *Xiaoyao San*

Xiaoyao San plays essential roles to clear liver, strengthen spleen, nourish blood and regulate menstruation, used widely in clinic. *Xiaoyao San* mainly contains thirteen active ingredients, which are saikoside A (C₄₂H₆₈O₁₃), saikoside C (C₄₈H₇₈O₁₈), saikoside D (C₄₂H₆₈O₁₃), ferulic acid (C₁₀H₁₀O₄), ligustilide (C₁₂H₁₄O₂), Atractylenolide I (C₁₅H₁₈O₂), Atractylenolide II (C₁₅H₂₀O₂), Atractylenolide III (C₁₅H₂₀O₃), paeoniflorin (C₂₃H₂₈O₁₁), Albiflorin (C₂₃H₂₈O₁₁), liquiritin (C₂₁H₂₂O₉), glycyrrhizic acid (C₄₂H₆₂O₁₆), pachymic acid (C₃₃H₅₂O₅)(30). The active ingredients and their functions are showed in Table 1.

Saikosaponins can protect patients against depression. Its mechanism might be related to neural protection mechanism and resistance of endoplasmic reticulum (ER) associated stress pathways and mitochondrial apoptosis pathways(31). Saikosaponins can also regulate inflammatory mediator by inhibiting MAPK and NF- κ B pathways in stimulus RAW 264.7 cells model and plays anti-inflammatory roles(32). Using gas chromatography-mass spectrography, Zhou's study showed that the volatile and lipophilic constituents in *Xiaoyao San* can resist depression, mainly including ligustilide, palmitic acid, Atractylenolide I and Atractylenolide II(33). In *Angelica sinensis*, anti-depression active

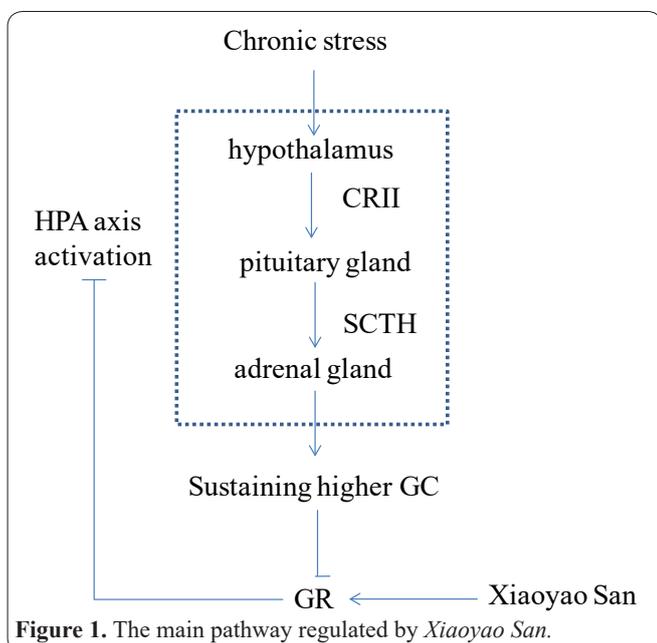
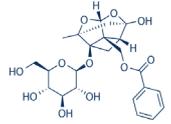
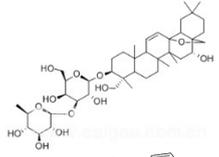
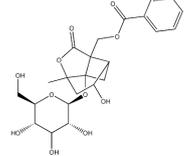
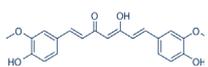
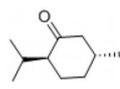
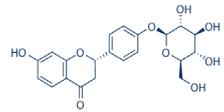
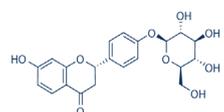


Table 1. The active ingredients and their functions.

Active ingredients	Functions	Reference	Structural formula
Saikosaponins	be related to neural protection mechanism and resistance of endoplasmic reticulum (ER) associated stress pathways and mitochondrial apoptosis pathways	(31)	
Paeoniflorin	nourishing blood and liver might be related to HPA axis and monoamine neurotransmitter	(36)	$C_{23}H_{28}O_{11}$ 
Saikosaponin d	strong bioactivity with wide biological activity, such as anti-cancer, immune regulation, sedation, anti-epileptic and anti-depression	(46)	$C_{42}H_{68}O_{13}$ 
Albiflorin	related to the inhibition of HPA axis' hyperfunction, resist depression without activate or inhibit the central nervous system	(67)	$C_{23}H_{28}O_{11}$ 
Curcumin	potential regulation function on central nervous system	(52)	$C_{21}H_{20}O_6$ 
L-menthone	be related to increase of the expression of GR mRNA and inhibition of over-activity of HPA axis	(55)	$C_{10}H_{18}O$ 
Liquiritin	relieve the depression syndrome	(56)	$C_{21}H_{22}O_9$ 
Sodium Ferulate	prevent DNA from damage, inhibit cell apoptosis, protect endothelial cells, promote vascular smooth muscle cells proliferation and improve the local blood supply	(57)	$C_{10}H_9NaO_4$ 

constituents include coniferyl ferulate, ligustilide and Z-butylidenephthalide(34), Liquiritin and isoliquiritin can up-regulate the 5-HT and NE in the hippocampus, hypothalamus and cerebral cortex of the stressed rats, help in relieve depression(35).

Paeoniflorin, the effective constituent of radix paeoniaealba, its mechanism of nourishing blood and liver might be related to HPA axis and monoamine neurotransmitter. Paeoniflorin can increase number of peripheral erythrocytes and hemameba, consumption of carbohydrate and weight of body in the chronic stressed induced rats. Its mechanism might be involved in up-regulate ACTH in the serum of HPA axis, 5-HT and DA in the hippocampus of the rats. Paeoniflorin produces protective effect against glutamate induced neurotoxicity in PC 12 cells through regulating the membrane potential of chondriosome and Bcl-2/Bax pathway(36). Total glycosides of peony's neural protective effects against neurovirulence induced corticosterone in PC 12 cells might be related to its anti-oxidation effects(37-39).

***Xiaoyao San's* effective constituents regulate the glucocorticoid receptors under the condition of chronic stress**

The most important feature of stress response is activation of HPA axis and increase of GC secretion(40). When the stress induced signals in central nervous reaches to paraventricular nucleus (PVN), CRH is secreted and promotes adrenocorticotrophic hormone (ACTH) synthesize and secrete. ACTH promotes adrenal cortex zona fasciculateneticularis synthesize and secret GC to induce stress response reaction. Through binding with glucocorticoid receptor (GR), GC plays its biological effects in body(27, 40). PVN-CRH/HPA axis interacts with LC-NE/SAS axis and form a positive feedback loop. Activation of a system often active another system(27). In chronic stress state, HPA axis' constant excessive activation can lead to the dysfunction of neural-endocrine-immune system, but *Xiaoyao San* and its modifications can improve this state at different levels. The study of Sun *et al.*(41) finds that *Xiaoyao San* can down-regulate the active state of HPA axis of the CUMS depression model significantly, and also glucocorticoid receptor is the target of *Xiaoyao San's* anti-depression function. Danggui Shaoyao Powder can significantly reduce the activity of G-6-Pase in the liver of the chronic stress rat and the level of CRH, ACTH, CORT and

INS. *Xiaoyao San* regulate the balance of expression of GR in hippocampal neuron, especially synergistic effect with MK-801. It is speculated that the synergistic effects of *Xiaoyao San* and MK-801 can recover the negative feedback of hippocampus in chronic stress condition, but the mechanism need more validation. *Xiaoyao San* treatment corrects the imbalance expression state of subunits of NR, promotes recovery or maintaining of normal ratio of NR2A and NR2B, keeps the calcium homeostasis in cells and resists down-regulated iGR level induced by chronic stress. These function play key roles in keep normal state of hippocampus nerve cells to protect neuron and relieve the stress induced injury(27). Only MK-801 can't block calcium overload and low level of iGR in chronic stress microenvironment. MK-801 and *Xiaoyao San* jointly suppress NR activation, keep number and activation of NR, inhibit calcium overload, maintain balance of calcium level and suppress down-regulated iGR(27). *Xiaoyao San* maintains steady state of iGR in hippocampus nerve cells through multi-pathways, especially Glu-NR-Ca²⁺ + -cAMP-iGR signaling pathway(27).

Xiaoyao San protects nerve cells through two ways: inhibition of stress induced synapse or synapse connection atrophy and promotion new synapse germination, to resist chronic stress damage structure of nerve synapse(42, 43).

Sustaining over-excitation of HPA axis in chronic stress treated rats increase the concentration of serum corticosterone aberrantly. RU-38486, a GR inhibitor, can resist this chronic stress induced high blood corticosterone and decreased serum corticosterone level. *Xiaoyao San* can repair chronic stress caused rats hippocampus injury and regulate the negative feedback loop. The constituents of *Xiaoyao San* are complex, the biological active constituents, biological mechanism and molecular targets are still unclear(44, 45).

Saikosaponin d

Saikosaponin is the main bioactive constituent of radix bupleuri. Based on chemical structure, it has been classified as Saikosaponin a, b, c, d, all being pentacyclic triterpene oleanane ramification. Saikosaponin d has strong bioactivity with wide biological activity, such as anti-cancer, immune regulation, sedation, anti-epileptic and anti-depression, etc. It can partially reverse the dysfunction of mitochondria and inhibit the mitochondria apoptosis pathway to protect PC12 cell from being traumatized by corticosterone through regulating the operation of mitochondria and glucocorticoid receptor in the cell nucleus(46). Some studies show that saikosaponin d can raise the expression of GR mRNA in HL60 cells and inhibit the growth of cells (47).

Albiflorin

It believes that albiflorin can resist depression through regulate the central nervous system(48). Albiflorin can significantly reverse the increase of corticosterone in serum and adrenocorticotrophic hormone in the rats of olfactory bulbectomy model. In addition, Western blot shows that albiflorin can increase the expression of glucocorticoid receptor in hippocampus. It

exemplifies that albiflorin has obvious anti-depression function towards the rats, the mechanism of which is related to the inhibition of HPA axis' hyperfunction(49).

Curcumin

In recent years, more and more traditional Chinese medicines are paid attention and used to cure physical and mental illnesses, such as curcumin. Curcumin is a major biological active constituent of another Chinese herbal compound, Jieyu pill(50, 51). In ancient China, Jieyu pill was used to promote qi circulation and relieve depression and was effective to treatment of neurological and psychotic disorders. In American, curcumin has been approved by the FDA and used as the 3th generation anti-tumor drug. Due to its low toxic and side effect, it has broad application prospects(50, 52). In recent studies, curcumin can relieve the poor cognitive function caused by Alzheimer's Disease, which suggests that its potential regulation function on central nervous system(52, 53).

Xu *et al.* (54) adopted multiple constant and unpredictable ways of stimulation for 20 days to build the chronic stress depression model of the rats. They used this model to conduct experiment and found that the experiment group has higher ratio of the weight of adrenal gland to that of body, higher density of adrenal cortex, higher level of corticosterone in serum and lower expression of GR mRNA. Small amount of curcumin can reverse these changes and meanwhile reserve the reduction of the amount of brain derived neurotrophic factor (BDNF) caused by stress.

l-menthone

Some studies(55) show that 15 mg/kg of l-menthone can significantly shorten the time of forced swimming and tail suspension for the rats, and reduce the expression of GR mRNA and BDNF. Thus, it can be seen l-menthone's mechanism of anti-depression can be related to increase of the expression of GR mRNA and inhibition of over-activity of HPA axis.

Liquiritin

Zhao *et al.* (56) used liquiritin to treat chronic stress rats for three weeks and found that it can relieve the depression syndrome, such as active degree, weight, food intake, forced swimming time. The effective dosage is 20 mg/kg. With the increase of liquiritin, the concentration of GR receptors in hippocampus of the rats decreases. However, there is no significant difference between experimental group and control group.

Sodium Ferulate (SF)

Sodium ferulate is a steady, low-toxic, water-soluble endothelin receptor inhibitor, which can prevent DNA from damage, inhibit cell apoptosis, protect endothelial cells, promote vascular smooth muscle cells proliferation and improve the local blood supply(57). Sodium ferulate can relieve the symptom of glucocorticoids-induced osteoporosis which may be involved in its inhibition function to GR(58, 59). The clear function

of sodium ferulate in *Xiaoyao San* is unknown, but its function in depression needs more attention.

Application of *Xiaoyao San* in chronic stress

It has accumulated studies to explore the causes of depression, such as single amine neurotransmitter and receptor theory, immune system-cytokines theory, glutamate in nervous system theory, endocrine-stress theory. Traditional Chinese medicine believes that disorders of emotions is related with liver's catharsis, thus the treatment should focus on liver. *Xiaoyao San* firstly was reported in *Tai Ping Hui Min and JiJu Fang*, composed of radix bupleuri, atractylodes, Angelica sinensis, peony, Poriacocos, mint, dried ginger and liquorice. Radix bupleuri and peony are the main ingredients of *Xiaoyao San*. It is mainly used to soothe the liver, remove the stress, nourish blood and strengthen spleen. In modern clinical practice, the original formula and modified ones are used to treat chronic stress, anxiety and depression (28, 29). In *Xiaoyao San*, radix bupleuri and mint relieve depression and nourish liver, radix paeoniaealba and angelica sinensis astring yin and nourish blood, atractylodes, pahymeliquorice and gingers strengthen spleen. *Xiaoyao San* soothes the liver and relieves remove the stress through regulating hippocampi, amygdaloidal nucleus, HPA Axis, immune system and each target organ. Obviously, *Xiaoyao San* can resist the behavioral change caused by chronic stress. *Xiaoyao San* can regulate AMPA receptor subunit mRNA expression in hippocampal CA1 region and amygdale increase stress induced decrease 5-hypothalamic (5-HT), dopamine (DA) and norepinephrine (NE) levels, downregulate the contents of leptin and leptin receptor (ob-R) and neuropeptide Y (NPY) in the hypothalamus of stressed rats, regulating the symptoms such as appetite decrease and bodyweight loss (60), increase postsynaptic density protein 95 (PSD-95) and synaptophysin (SYP) on the hippocampal nerve synapses and improving synaptic plasticity, reversing CIS induced learning and memory deficit of rats with chronic stress (61). The effects of *Xiaoyao San* on depression may be involved in regulation of the dysfunctions of energy metabolism, amino acid metabolism and gut microflora changes (62).

Regulation of the metabolism of amino acids, energy and glycogen might be one mechanism of *Xiaoyao San* induced chronic stress resistance (63). It can also ease anxiety, which might be related with the up-regulation of amygdaloidal nucleus's CRF1R and BDNF in the stressed rats (64).

Conclusions

Glucocorticoid receptors is a key factor of chronic stress (65). Most studies show that the decrease of GR expression can reduce the feedback of HPA axis mediated by GR for chronic stress patients(66). Chronic stress activates HPA axis, reduces the expression amount of GR and inhibit the mechanism of negative feedback on HPA. *Xiaoyao San* can raise the expression of GR and recover the negative feedback of HPA. Its main active constituents include saikoside, albiflorin, curcumin, and l-menthone. The opposite effects of liquiritin need to be further studied.

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Conflicts of interest

The authors declare that there is no conflict of interests regarding the publication of this paper.

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