Effects of *Hypericum perforatum* extract on the endocrine immune network factors in the immunosuppressed Wistar rat

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**ABSTRACT**  
The study aimed to investigate the effects of *Hypericum perforatum* extract on the endocrine immune network factors in the immunosuppressed rat. Wistar rats were used to establish an immunosuppression model. Immunosuppressed rats received *Hypericum perforatum* extract suspension for 7 days at doses of 600 mg/kg (containing hyperforin at 7.2mg/kg).Endocrine immune network factors including IL-1, DA, NE, and 5-HT were determined on day 8. The results indicated that *Hypericum perforatum* extract affects the endocrine immune network through decreasing endogenous IL-1, dopamine (DA), norepinephrine (NE), and 5-hydroxytryptamine (5-HT) levels. Hyperforin reverses immune suppression through enhancing DA levels to inhibit B cells while promoting T cell and macrophage functions to recover immune function.  

**Key words:** Dopamine (DA), Endocrine immune network factors, *Hypericum perforatum* extract, Interleukin-1 (IL-1), Norepinephrine (NE), 5-hydroxytryptamine (5-HT).

**INTRODUCTION**  
*Hypericum perforatum* L. belongs to *Hypericum* L. and is also known as *Hypericum perforatum*, Shangtianti, and Qiancengluo in China, and as St. John’s wort in Western countries (Xi and He , 2002). There are multiple effective components in *Hypericum perforatum*. Dianthrone is an extract of *Hypericum perforatum*, and other components include hypericin, pseudohypericin, pseudohypericin and ring hypericin. Hypericin is a naphthodianthrone compound with antibacterial activity. It was initially discovered, named, and the chemical structure was resolved by Russian scientists in 1975. Modern pharmacological studies have shown that hyperforin has therapeutic effects including anti-depression, anti-tumor (Xiao, 1996), anti-viral infection (Fu, 2002), anti-bacterial infection, hemostasis, and antipyretic and analgesic functions.

Hyperforin was recently found to have anti-enveloped virus activity, and it showed satisfactory therapeutic effects on hepatitis B and C virus, AIDS virus, varicella zoster virus, herpes simplex virus, and influenza virus infections. The present study investigated the effect of *Hypericum perforatum* extract on the immune-related network in experimental animals.  

**MATERIALS AND METHODS**  
**Experimental animals:** Female and male Wistar rats (gender ratio of 1:1) with a body weight of 210±10 g were purchased from the animal facility of Lanzhou University Medical college (Lanzhou, China).

**Drugs and reagents:** *Hypericum perforatum* extract (purity 1.2%) was provided by the Key Laboratory of the Chinese Academy of Agricultural Sciences, Lanzhou Institute of Husbandry and Pharmaceutical Sciences of CAAS (Lanzhou, China), and was prepared into a suspension solution using sterile water. *Hypericum perforatum* extract was irradiated for 72 hours using an ultraviolet lamp to inactive hyperforin (and named as UV-irradiated hyperforin). No hyperforin activity detected after irradiation. Detection kits for interleukin-1 (IL-1), dopamine (DA), norepinephrine (NE), and 5-hydroxytryptamine (5-HT) were purchased from Jin Bai Biological Co, Ltd (Shanghai, China).

**Experimental groups and suppression model establishment:** Forty Wistar rats were divided into 4 groups, each with 5 males and 5 females that were raised in separated cages. Group I was a control group that received a subcutaneous injection of saline at dose of 1.25 mg/kg. Group II was the suppression model group that received a subcutaneous injection of dexamethasone (Dex) at dose of 1.25 mg/kg. The peripheral white blood cell count was measured before injection, and after injection for 3, 5, and 7 days, to examine the model establishment (Table 1). Groups III and IV were hyperforin treatment group and inactivated hyperforin treatment group respectively, both groups were...
injected with 1.25 mg/kg Dex at day 1, 3, and 5 as basal injection to establish suppression model, and from day 1 to day 7 thereafter, rats in group III were treated with a dose (600mg/kg) of hyperforin (corresponding to 7.2 mg/kg), rats in group IV were treated with a dose (600 mg/kg) of inactivated hyperforin. (this is inactivated hyperforin. Then how you are getting effect? Answer: The UV-irradiated hyperforin group was just a negative control group).

Detection items and methods: A venous blood sample was taken from all the rats on day 8. Serum was separated and stored in -20°C. The concentration of interleukin-1 (IL-1), dopamine (DA), norepinephrine (NE), and 5-hydroxytryptamine (5-HT) were determined using an enzyme linked immunosorbent assay (ELISA) kit, according to the manufacturer’s instructions.

Statistical analysis: Statistical software SAS9.0 was used for statistical analysis, this software released by SAS Institute Inc. (Beijing, China). Data is presented as the mean±standard deviation. New repolarization was used for multiple comparisons. P<0.05 was considered to be a significant difference, and P<0.01 was considered to be a very significant difference. The statistical graphs were plotted using EXCEL2010 which developed by microsoft in combination with the SAS9.0 statistical analysis results.

RESULTS AND DISCUSSION

Immune suppression rat model establishment: Rats in Group II received subcutaneous injections of Dex to establish the immune-suppressed model. The peripheral white cell count in group II rats was significantly decreased compared to the control group (group I) (P<0.05; Table 1).

Effect of Hypericum perforatum extract on IL-1 in the immunosuppressed rat: Figure (1) show that IL-1 in the immunosuppressed group was significantly higher than that of the control group (P<0.01), indicating that the immunosuppressed rat model was successfully established. The IL-1 level in the hyperforin treatment group was significantly different from immunosuppressed group (P<0.01), but not different from the control group. This indicates that hyperforin treatment could decrease the immune suppression-induced IL-1 elevation into the normal range. However, IL-1 levels in the UV-irradiated Hyperforin group (Please tell me how inactivated Hyperforin effect on immunosuppression? Answer: The UV-irradiated Hyperforin group was just a negative control group) were significantly different from the control group (P<0.01) and not different from the immunosuppressed group. This suggests that hyperforin treatment group cannot decrease the IL-1 elevation caused by immune suppression.

Effect of Hypericum perforatum extract on dopamine (DA) in the immunosuppressed rat: Figure (2) show that the DA level was significantly increased in immunosuppressed rats compared to the control group (P<0.01), indicating that the immunosuppressed rat model was successfully established. The DA level in the hyperforin treatment group (group III) was significantly different from that in the immunosuppressed group (P<0.01), but not different from the control group. This indicates that hyperforin effectively decreased the DA elevation caused by immune suppression. However, the DA level following UV-irradiated Hyperforin treatment (no active hyperforin, Answer: The UV-irradiated hyperforin group was just a negative control group) was not different from the immunosuppressed group, but it was significantly different from the control group (P<0.01). This indicates that UV-irradiated Hyperforin cannot effectively decrease the DA level elevation caused by immune suppression.

Effect of Hypericum perforatum extract on noradrenaline (NE) in the immunosuppressed rat: Figure (3) show that the NE level in the immunosuppressed group was significantly higher than that in control group (P<0.01),
indicating that the immunosuppressed model was successfully established. The NE level in the hyperforin treatment group was significantly different from both the immunosuppressed group and the control group ($P<0.01$). This indicates that hyperforin effectively decreases the immune suppression-induced NE elevation into the normal range. Conversely, UV-irradiated hyperforin treatment group (No active hyperforin?, Answer: UV-irradiated hyperforin treatment group is corresponding to the hyperforin treatment group) was also significantly different from both the immunosuppressed group and the control group ($P<0.01$). This indicates that UV-irradiated hyperforin treatment group ( ) can also decrease the NE elevation, which is caused by immune suppression, although not into the normal range.

**Effect of Hypericum perforatum extract on 5-hydroxytryptamine (5HT) on the immunosuppressed rat:**

Figure (4) show that the 5-HT level in the immunosuppressed group was significantly higher than that of the control group ($P<0.05$), indicating that the immunosuppressed rat model was successfully established. The 5-HT level in the hyperforin treatment group was significantly different from the immunosuppressed group ($P<0.05$), but not different from the control group. This indicates that hyperforin treatment group effectively decreases the immune suppression-induced increase in the 5-HT level. However,
the 5-HT level in the UV-irradiated hyperforin treatment group (no active hyperforin)? Answer: UV-irradiated hyperforin treatment group is corresponding to the hyperforin treatment group, it is the group IV was studied by KP (2005), and also down-regulated P-2013) used hypericin-treatment (2014) and they showed that the LC 50 experiments of leukemia treatment and 2013) showed that 5-HT synthesis in dose-dependent manners, and was attenuated by pCPA and also accompanied by upregulated 5-HT synthesis in dose- and time-dependent manners, and was attenuated by pCPA or carbidopa, but exacerbated by clorgiline, inhibiting

In the current research, We showed that IL-1, DA, NE, and 5-HT in immunosuppressed rats were all significantly different from that in the control rats. IL-1 significantly increased in immunosuppressed rats, indicating the immunosuppression status, which is consistent with other studies (Besedovsky and Sorkin, 1977) showing that IL-1 secretion inhibits immune responses. Hypericum perforatum extract treatment resulted in a significant decrease in IL-1 levels, indicating that hypericum enhances immunity, and thereby antagonizes the immunosuppression caused by Dex. However, UV-irradiated hyperforin group did not decrease the IL-1 level significantly, suggesting that UV-irradiated hyperforin group might not be able to enhance humoral immunity.

DA is a neurotransmitter that suppresses B cell function and enhance T cell and macrophage functions. Andrzejewski et al. (2016) showed that the impact of degeneration of the nigrostriatal dopaminergic pathway on resting breathing and hypoxic ventilatory response in conscious rats, a dopamine deficiency in the striatum and an increased turnover of DOPAC/DA in the brainstem. In the current study using an immunosuppressed rat model, the DA level increased significantly, but after hyperforin treatment, the DA level decreased significantly. This indicates that hyperforin restores immune function through inhibiting B cell and enhancing T cell and macrophage function.

NE is a neurotransmitter that activates the α receptor, and has little effect on the β receptor. Sigurdardottir et al. (2016) showed that the role of genetic influence of the NE system on NET binding to be perturbated in ADHD. It is generally believed that β receptor activators inhibit immunity, whereas α receptor activators stimulate immune responses. In our immunosuppressed rat model, the NE level was significantly increased, indicating the immune suppressed status. After treatment with hyperforin, the NE level was significantly decreased indicating that hyperforin inhibits NE secretion, and thereby immunity was stimulated.

5-HT is a neurotransmitter that may also be inhibited by hyperforin. However, the mechanism of 5-HT on immune regulation is not known, Li et al's research showed (2016), in the BRL-3A cells, dexamethasone-induced IR was also accompanied by upregulated 5-HT-synthesis in dose- and time-dependent manners, and was attenuated by pCPA or carbidopa, but exacerbated by clorgiline, inhibiting monoamine oxidase-A to further increase 5-HT level. Dexamethasone also enhanced 5-HT 2A and 2B receptor expressions in both tissues and BRL-3A cells. Additionally, blocking 5-HT transporter with fluoxetine significantly suppressed 5-HT-induced IR in BRL-3A cells.

Judge et al. (2016) research showed that acute organophosphate exposure inhibits dorsal raphe nucleus cholinesterase leading to acetylcholine accumulation, the acetylcholine activates nicotinic receptors on 5-HT neurones and also on glutamatergic neurones, thus releasing glutamate and activating 5-HT neuronal AMPA/kainate receptors, the increase in 5-HT neuronal activity, and resulting 5-HT release, may lead to 5-HT1A autoreceptor down-regulation (2016).

In vitro experiments of leukemia treatment and pathogenesis of chronic lymphocytic leukemia (CLL) showed that purified hyperforin had effects on regulating leukemia cell proliferation, survival, apoptosis, migration, and angiogenesis; hyperforin also down-regulated P-glycoprotein expression, a protein involved in leukemia cell resistance to chemotherapy (Novelli et al., 2014). In addition, Semelakova et al. (Billard et al., 2013) used hypericin-mediated photodynamic therapy (hy-pdt) to investigate the effect of sub-optimal hyperforin doses at the cellular level. They found that hyperforin stimulates hy-pdt-exposed HT-29 colon cancer cell apoptosis, inhibits cell cycle progression, and suppresses matrix metalloproteinase-2 and metalloproteinase-9 expression and their mediated cell adhesion, which affected the potential to form neoplastic colonies. The embryotoxic or teratogenic effects of hyperforin have not yet been thoroughly evaluated. Experiments on mouse embryonic stem cell proliferation and differentiation by Nakamura et al. (2013) showed that hyperforin is currently a safe and effective antidepressant. Normal doses of hyperforin have little risk of embryo toxicity for pregnant women; however, the risk would be increased if a large amount of hyperforin was administrated. The insecticidal effect of hyperforin was studied by KP Mitsopoulou et al. (2014) and they showed that the LC50 value was 26.72 mg/L for mosquitoes (Diptera: Culicidae). Hyperforin is currently used to treat basal cell carcinoma, pancreatic cancer, bladder cancer, and brain tumors (Zhang, 2007; He, 2006) in clinical practice.

Our previous studies indicated that hypericin has special therapeutic and prophylactic effects in animal diseases, especially the viral infections. Hypericin can eliminate avian influenza virus (AIV) including both the low pathogenic H9N2 subtype and the highly pathogenic H5N1 subtype (Shan et al., 1998) inhibit proliferation of foot-and-mouth disease virus (FMDV) (Ling et al., 2005), and antagonize PRRSV activity (Liang et al., 2009). It has also demonstrated strong inhibitory effects on infectious bursal disease virus (IBDV) and canister distemper virus. and further investigation is needed.
REFERENCES


