EFFECT OF FRUCTUS PSORELEA ON HIGH DOSE OF EXOGENOUS SYNTHETIC GLUCOCORTICOIDS (CORTISOL) INDUCED NEURAL DEGENERATION OF HIPPOCAMPUS

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Abstract

Cortisol induced memory loss was due to disruption of hippocampal function. It negatively affects the long-term potentiation which is associated with learning and memory. Based on these observations, concern has been raised that prolonged elevation of endogenous glucocorticoids (GCs) caused by chronic cortisol or pharmacological doses of GCs commonly administered to humans with inflammatory or bronchospastic diseases might produce neuronal loss. On the other hand, studies had shown presence of potential neuroprotective plant with flavonoid and coumarin compounds plays important role against oxidative stress because it regulate the cortisol level. This infers the efficacy of flavanoid compound of Fructus Psoreleae (FP) in controlling coritsol level. So, the administration of FP not only regulate the cortisol and also reverse the elevated cortisol levels which was very much useful in the treatment of neuropsychiatric disorders such as depression, schizophrenia and alzheimers disease.

Keywords: Hippocampus, Endogenous glucocorticoids, Schizophrenia and Alzheimer’s disease

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INTRODUCTION

Cortisol is a steroid hormone, in the glucocorticoid class of hormones, and is produced in humans by the zona fasciculata of the adrenal cortex within the adrenal gland. It is released in response to cortisol and low blood-glucose concentration. Despite the other organ like heart, liver and kidney, brain is the most affected organ by cortisol. The release of cortisol is controlled by the hypothalamus, a part of the brain. The secretion of corticotropin-releasing hormone (CRH) by the hypothalamus triggers cells in the neighboring anterior pituitary to secrete another hormone, the adrenocorticotropic hormone (ACTH), into the vascular system, through which blood carries it to the adrenal cortex. ACTH stimulates the synthesis of cortisol, glucocorticoids, and dehydroepiandrosterone (DHEA). Rodent studies suggest that prolonged exposure to elevated glucocorticoid (GC) concentrations lowers the threshold for cerebral neuronal degeneration and loss. The major effects of prolonged exposure of cortisol in brain are memory loss, confusion and augmentation. It was due to increased free radical production and a resultant state of oxidative cortisol. A few studies suggest GC neurotoxic effects in primates. Older animals may be particularly vulnerable to this phenomenon. Cortisol induced memory loss was due to disruption of hippocampal function. It negatively impacts the LTP which is associated with learning and memory. Based on these observations, concern has been raised that prolonged elevation of endogenous GCs caused by chronic cortisol or pharmacological doses of GCs commonly administered to humans with inflammatory or bronchospastic diseases might produce neuronal loss. On the other hand, studies had shown presence of potential neuroprotective plant with flavonoid and coumarin compounds plays important role against oxidative cortisol. Flavonoid compound has been shown to rapidly activate adenylate cyclase, increase intracellular [Ca²⁺], activate phospholipase C to generate inositol 1,4,5-trisphosphate and diacylglycerol, stimulate nitric-oxide synthase to generate nitric oxide, and activate the extracellular regulated kinases 1/2 (ERK1/2) mitogen-activated protein kinase (MAPK) pathway.

AIM OF THIS STUDY

1. To investigate the effect of FP induced cortisol level in the hippocampal area of the rat brains by hormonal study.
2. Comparing the effect of cortisol and FP in the neurons of the hippocampal area of the rat brains by histological study.

MATERIALS AND METHODS

The study was conducted on thirty female healthy adult wistar albino rats in laboratory conditions (i.e. room temperature of 25±2°C; relative humidity 45% to 55% and a 12:12 light/dark cycle). The approval of the Institutional Animal Ethical Committee (IAEC) of Saveetha University (IAEC No.Anat.002/2009) was taken prior to the experiments. All the protocols and the experiments were conducted in strict compliance according to ethical principles and
guidelines provided by Committee for the Purchase of Control and Supervision of Experiments on Animals (CPCSEA).

**Experimental design**
The experiment was carried out for a period of 2–4 months with 30 healthy adult wistar albino rats. Before starting the experiment, the rats were made to acclimatize to the laboratory environment for one week. Then rats were randomly assigned into 4 groups of 6 animals with age of 2 months.

- **Group I (Control group):** This group rats were housed and maintained for two months from the start of the experiment. After two months, the rats were sacrificed.
- **Group II:** Orally treated with FP in a daily dose of 25 mg/kg orally by gavage for 2 months.
- **Group III:** Induced with synthetic glucocorticoids by intraperitoneal injection of hydrocortisone sodium succinate at 15 mg/kg, respectively, for 20 days (cortisol) daily.
- **Group IV:** Induced with synthetic glucocorticoids (cortisol) 15 mg/kg BW and orally treated with FP 25 mg/kg by gavage method for 2 months.

**Cortisol Assay**
The blood from each animal of all the groups were collected and analyzed for cortisol assay using ADVIA Centaur System. The ADVIA Centaur cortisol assay is a competitive immunoassay using direct chemiluminescent technology.

**RESULTS**

**Cortisol level result**
The blood from all the rats were collected and analyzed for cortisol assay using ADVIA Centaur System.

| Group   | Cortisol level (pg/ml) |
|---------|------------------------|
| Group I | 8.7±0.29               |
| Group II| 6.2±0.26               |
| Group III| 14.4±0.41            |
| Group IV| 10.2±0.56             |

This above results showed that the mean cortisol level was increased in group III as when compared with other groups. But in group II, it was decreased as compared to all the other groups. So, this showed that FP regulating the cortisol level. The ANOVA test done for cortisol level showed that the difference in cortisol level between the groups were statistically significant (F= 62.37; P<0.01). Post hoc test revealed that comparing the mean cortisol level of all the five groups, group II had significant value. This showed that cortisol induced the cortisol level in group IV but in it was regulated by FP. So FP treated group was better than other groups. Cortisol level of FP treated group was significantly different from other group (n=6; F= 62.37; P<0.01).
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Figure 1: Bar diagram: Mean ± SEM of Cortisol (Pg/ml) of all the groups

Figure 2: Topography of toluidine blue stained hippocampus (400x magnification)

GI - Blue arrow shows the Pyramidal Shaped Neurons
GII, GIV - Red arrow shows the Increased Pyramidal Shaped Neurons
GIII - Black arrow shows the Inactive Circular Shaped Neurons
DISCUSSION

This study showed that cortisol, a hormone produced by the adrenal gland within the HPA-axis in response to synthetic glucocorticoids, was high level in cortisol treated group. Mainly it produces cortisol related loss of neurons and decreases long-term potentiation (LTP) in the hippocampus. The present study reveals that increased cortisol impairs the hippocampal dependent memory, which was proved by the above date (Table 1). So elevation of cortisol must necessarily be associated with learning deficits in rats. These findings were consistent with previous research that cumulative exposure to cortisol had functional and structural effects on the hippocampus and finally end up with decrease hippocampal integrity and hippocampal dependent cognitive task. On comparing group II (9.7±0.28) with group IV (14.4±0.41), cortisol had raised in group IV. So the synthetic glucocorticoids induced elevation of cortisol was due to the activation of HPA which produce more complex effects on cognitive behaviour and also attenuates the normally occurring neurogenesis in hippocampal dentate gyrus, a process that was thought to be necessary to sustain a constant level of neuron density in this region. Prolonged exposure to glucocorticoids was also associated with several adverse effects on brain morphology, particularly in the hippocampus. The exposure may ‘endanger’ neuronal integrity and cause atrophy of apical dendrites in the Ammon’s horn. It has been proposed that also these neurodegenerative changes may induce some of the deleterious behavioral effects. The hippocampus was enriched with two classes of corticosteroid receptors, type I, mineralocorticoid receptors (MRs); and type II, glucocorticoid receptors (GRs). Chronic exposure to glucocorticoids induces changes in adrenal steroid receptor density and or affinity that may account for some of the cognitive effects. This effects of glucocorticoids (cortisol) on the hippocampus depends on the concentration GRs. The adverse effects of cortisol on the hippocampus seems to be mediated largely by the lower-affinity GRs, which become heavily occupied with corticosteroids in response to cortisol. In the rat hippocampus, corticosterone binding to GRs had been shown to adversely affect neuronal metabolism, cell survival, physiological functions and neuronal morphology. Moreover, changed receptor densities induced by prolonged glucocorticoid exposure may alter autonomic, neuroendocrine and
behavioral responsiveness during learning, which may affect memory consolidation. MR/GR imbalances can occur and the blockade of MR by a specific antagonist increases circulating levels of cortisol under basal and cortisol condition. So the secretion of high level of cortisol was known to affect learning and memory. But in group II and IV, the cortisol level was decreased (6.2±0.26; 10.2±0.56) compared with group III (14.4±0.41). This was mainly due to the effect of FP. It had been suggested that action of FP on dysregulation and normalization of the HPA axis system plays an important role in the pathophysiology of cognition.

Calcium flux through NMDARs was thought to be critical in synaptic plasticity, a cellular mechanism for learning and memory. Corticosterone modulates NMDA receptor–mediated Calcium influx in cultured hippocampal neurons and NMDA-dependent long-term potentiation and these effects were mediated by glucocorticoid receptors. In addition, NMDA receptor plays a critical role in dendritic plasticity and dendritic reorganization occurring in frontal cortex after cholinergic differentiation. NMDA receptors mediate corticosterone’s effects on dendritic morphology in hippocampal CA3 neurons. Thus, glutamatergic transmission at NMDA receptors may play a role in cortisol-induced dendritic reorganization in hippocampus. Consistent with this hypothesis, chronic administration of corticosterone down regulates expression of the NR2B subunit of the NMDA receptor in hippocampus. Given that NMDA receptor activation was crucial for producing remodeling in the hippocampus due to cortisol and for reorganization in frontal cortex due to cholinergic differentiation. NMDA receptors may also play a role in alcoholic cortisol-induced dendritic remodeling in medial prefrontal cortex. So by blocking NMDA receptors during chronic cortisol prevents cortisol-induced dendritic retraction of hippocampus. So FP may act as NMDA receptor blocker, which blocks sodium and T-type calcium channels. Normal level of cortisol facilitate hippocampal plasticity and promote the survival of dentate gyrus granule cells through activation of MR-mediated effects which stabilize neuronal transmission and appear critical for neuronal integrity of a sub-region of the hippocampal dentate gyrus. This finding suggests that one of the MR-mediated effects of cortisol was the suppression of the activity of the HPA axis by means of increase hippocampal plasticity. In contrast, the elevated cortisol levels of group III (14.4±0.41),...
not only to negate the effects of MR activation but also to promote the debilitating GR effects on hippocampal function, including a dampening of LTP. So this proposed that decreased hippocampal volume was associated with high cortisol level. Intake of FP which regulate the cortisol (group II = 6.2±0.26) resulting in improved short-term memory, working memory processes such as selective attention, memory consolidation, and LTP. This result clearly explains that FP regulate the cortisol level in group II and IV (10.2±0.06). This infers the efficacy of FP in controlling cortisol level caused due to cortisol. So, the administration of FP not only regulate the cortisol and also reverse the elevated cortisol levels which was seen in group V rats. Thus, FP was very much useful in the treatment of neuropsychiatric disorders such as depression, schizophrenia and Alzheimer’s disease.

Activation of the HPA axis was considered to be a characteristic physiological response to alcoholic cortisol. Oestrogen is thought to play a causal role in the gender or menstrual cycle-dependent differential HPA-axis responses, because ovariectomy (OVX) reduces basal CORT levels, whereas estrogentic replacement restores basal plasma CORT concentrations. In addition, these data provide valuable insight into the mechanisms mediating decreased HPA responsiveness, which in-turn regulate the cortisol level. There was a strong relationship between HPA function and monoamine oxidase (MAO) activity that induces oxidative cortisol. Therefore, it had been suggested that MAO inhibitors used for treating cortisol induced alcoholics. Finally, the effect by FP was consistent with previous result that clorgyline, MAO inhibitor, inhibited HPA axis function. Therefore, inhibition of MAO and HPA axis may be responsible for the therapeutic effects of FP to treat depression and Parkinson’s disease. It seems likely that FP exhibit anticortisol activity by inhibiting MAO-A and MAO-B activities. In fact, it had been shown that psoralen and isopsoralen suppressed MAO-A and MAO-B activities in rat brain mitochondria in vitro. It was hypothesized that the FP anti-cortisol acts, at least in part, by inhibition of MAO activity.

CONCLUSIONS:
So administration of FP not only regulate the cortisol and also reverse the elevated cortisol levels which was very much useful in the treatment of neuropsychiatric disorders such as depression, schizophrenia and alzheimer’s disease.

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