Research Article

Anti-stress effects of cinnamon (*Cassia zelynicum*) bark extract in cold restraint stress rat model

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ABSTRACT

The present study aims at investigating the anti-stress effects of cinnamon (*Cassia zelynicum*) bark extract in cold restrained stress (CRS) model. The cinnamon anti-stress effect was characterized using ulcer scoring, adrenal-gland weight, estimation of plasma glucose and cholesterol levels, as well as estimation of norepinephrine (NE) and corticosterone. Our results demonstrated that cinnamon is effective in minimizing stress responses by alleviating the increased level of ulcer scoring, plasma corticosterone, NE, glucose and total cholesterol levels which was induced in rats subjected to CRS. Thus, cinnamon is found to be effective in minimizing stress responses at a dose of 200 mg/kg thereby beneficial in stress therapy.

Keywords: Cinnamon; stress; hypothalamic–pituitary–adrenal axis; corticosterone, cholesterol; glucose.

INTRODUCTION

Much research has shown that many clinical disorders can either be induced, or aggravated by stress. The extreme levels of stress cause major damage to health. The neuroendocrine and emotional component of the stress reaction involves activation of limbic and hypothalamic brain structures[1]. The hypothalamic–pituitary–adrenal (HPA) axis serves as a neuroendocrine stress response system and plays a vital role in the maintenance of homeostatsis[2]. When HPA axis gets stimulated in response to a stressor, it results in the release of glucocorticoids from the adrenal cortex. These glucocorticoids minimize the long-term activation of the HPA axis through a negative feedback pathway[3]. At the central

level, HPA axis is regulated by the paraventricular nucleus (PVN)[4]. PVN produce corticotrophin releasing factor (CRF), arginine vasopressin (AVP) peptides and oxytocin. CRF and AVP intern release adrenocorticotrophic hormone (ACTH) from the anterior pituitary, while oxytocin may have a role in the mediation of the stress response[5]. Cold restraint stress (CRS) is a well-documented stressor[6] that acutely stimulates hypothalamus and initiates a transient increase in plasma ACTH and corticosterone.

A long history exists for traditional use of herbal remedies for various clinical ailments. Medicinal herbs are equally effective and also devoid of the bothersome side effects

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associated with conventional drugs. Cinnamon is one such herb that is subjected to intense research and has long been used in ayurvedic medicine. Cinnaomon was shown to have many pharmacological properties like antioxidant, antibacterial effect etc.[7,8] and perhaps, it is one of the oldest herbal medicines. Furthermore, it has been reported that the cinnamon extract has vasodilative, anti-thrombic, anti-spastic, anti-ulcerous, and anti-allergic action. However, till date anti-stress activity of cinnamon is not published. Thus, the purpose of this study was to evaluate the anti-stress activity of cinnamon bark extract repeated treatment against cold restraint stress model.

Materials and Methods
Animals

Albino rats of either sex (175-225 g) were procured from Central Drug Research Institute, Lucknow, Uttar Pradesh, India. The rats were housed in polypropylene cages (one in each cage) under standard conditions of temperature (25 ± 2 °C) and relative humidity (55-60 %). The animals were fed with a commercial pellet diet (Hindustan Lever Limited, Bangalore, India) and water *ad libitum*. Guidelines of "Principles of laboratory animal care" (NIH publication number 85-23, revised 1985) were followed for animal experiments.

Cinnamon extraction

Dried cinnamon barks were obtained from a commercial source. The barks were washed in distilled water, dried and ground to fine powder. For the preparation of cinnamon hot extract, 25 gm of this powder was suspended in 500 ml of distilled water and boiled for 1 hour (hr). This was cooled at room temperature (RT) and centrifuged at 12000 rpm for 10 min. The supernatant was filtered and the filtrate was stored at 4°C. For the concentration of the extract, its azeotropic mixture was made with methanol and acetic acid which was subjected to vacuum evaporation until dried. The dried material was re-dissolved in appropriate amount of sterile distilled water to get the appropriate concentration.

Experimental groups and drug administration

The rats (n=6) were randomly assigned to control, stress and treatment groups (cinnamon 200 mg/kg for 21 days) with or without stress (Control, CRS, CIN-200, CIN-200 + CRS). Control and CRS group rats received distilled water (DW) (3 ml/kg) as vehicle. Other two groups received 21 days repeated treatment of cinnamon (200 mg/kg, *p.o.*, 21 days). On 21st day after 1 hr of drug/vehicle treatment all groups except Control and cinnamon treated without stress group were then subjected to 2 hr CRS as described by Saxena et al (2011)[9.] Blood was collected from retro-orbital plexus of the all the rats under light halothane anesthesia using capillary tubes into microcentrifuge tubes containing heparin (10 µl, 1000 IU ml^{-1}) for corticosterone, NE, glucose and cholesterol levels estimation. Lastly, all animals were sacrificed by cervical dislocation and their stomachs and adrenal glands were collected.

Biochemical estimation

The plasma was separated by cold (4 °C) centrifugation (5 min, 5000 rpm) and corticosterone level was estimated by HPLC/PDA system (Waters, USA), according to Woodward and Emery method (1987)[10]. Norepinephrine was estimated using the flourimetric method[11,12]. Concentration of corticosterone and NE was expressed in ng /ml and g /ml, respectively. Glucose and cholesterol in the separated plasma was estimated using Span Diagnostic kit and concentration of glucose and total cholesterol was expressed in mg /dl.

Statistical analysis

Values are expressed as Mean $\pm$ SEM. The data was analyzed with GraphPad Prism 4 (San Diego, CA). Statistical analysis of data was done by One-way ANOVA, followed by Tukey's test. A level of $P<0.05$ was accepted as statistically significant.

Results

The effect of repeated treatment of cinnamon on the ulcer index, adrenal gland weight, corticosterone, norepinephrine, glucose and total cholesterol level in CRS model is shown in Figure 1. One-way ANOVA upon repeated treatment with cinnamon showed significant differences in ulcer index [$F_{(3, 23)} = 363.3$, $P<0.0001$], plasma corticosterone level [$F_{(3, 23)} = 123.1$; $P<0.0001$], plasma norepinephrine level [$F_{(3, 23)} = 67.93$; $P<0.0001$], plasma glucose concentration [$F_{(3, 23)} = 26.96$; $P<0.001$] and plasma total cholesterol level [$F_{(3, 23)} = 22.01$; $P<0.001$] while there is no significant difference in adrenal gland weight among the groups. Post-hoc analysis of the study showed that CRS, significantly,
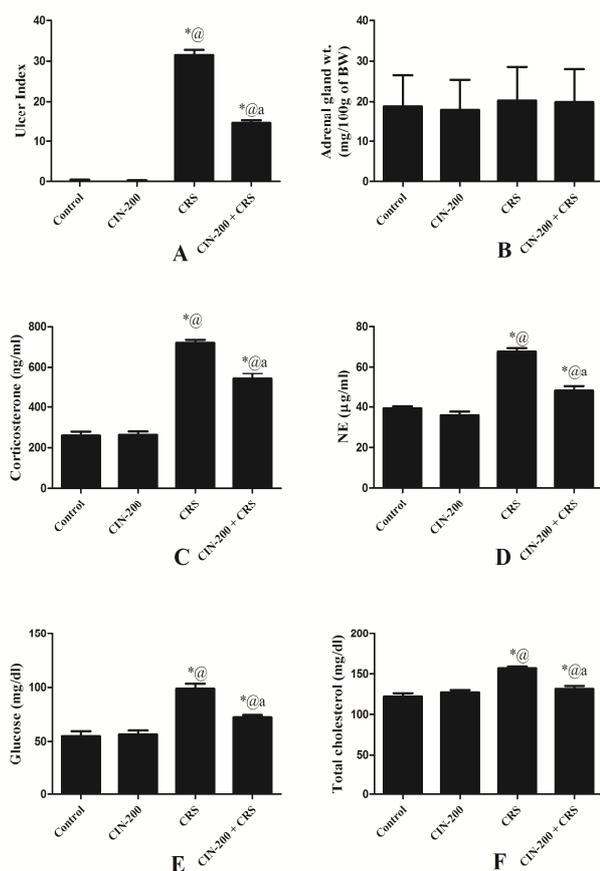
Fig.1. Effect of cinnamon treatment on ulcer index (A), adrenal gland weight (B), plasma corticosterone (C), Norepinephrine (D), glucose level (E), total cholesterol (F) in CRS model. Results are expressed in each column as mean ± SEM (n = 6).*P < 0.05 and @P < 0.05 and ⁰P < 0.05 compared to control, cinnamon 200 mg/kg (CIN-200) and stress groups respectively (one way ANOVA followed by Tukey's test)

increased ulcer index, corticosterone, corticosterone, norepinephrine, glucose and total cholesterol levels in plasma. There is no alteration in adrenal gland weight on stress exposure. Repeated treatment with 200 mg/kg of cinnamon significantly, mitigated stress induced increase in ulcer index, corticosterone, norepinephrine, glucose and total cholesterol levels in plasma. These results indicate the anti-stress effect of the drug.

Discussion

The rats subjected to chronic restraint stress showed significant increase in ulcer scoring, corticosterone, norepinephrine, glucose and total cholesterol levels in plasma

while there is no significant change in adrenal weight. Cinnamon mitigated the increased level of ulcer scoring, corticosterone, glucose and total cholesterol levels in plasma. The CRS model is used to evaluate agents that can inhibit the gastric ulcers development by virtue of its anti-stress effect[6]. Rats subjected to CRS showed significant increase in corticosterone level in plasma due to stimulation of hypothalamo-pituitary axis (HPA)[13]. Increase in corticosterone level causes immobilization of lipids and synthesis of cholesterol[14], thus lead to increase plasma total cholesterol levels. The increase in corticosterone and total cholesterol levels in stress in our studies (Fig.1) also support the above statement. Cinnamon treatment mitigates the increased level of corticosterone and total cholesterol levels in plasma due to its anti-stress effect.

Stress also results in the stimulation of sympathetic nervous system which results in increase in norepinephrine level which in turns increases glucose level[13]. In our studies also CRS increases the norepinephrine and glucose level. Cinnamon treatment decreased the stress induced increase in norepinephrine and glucose level (Fig.1).

As the cinnamon action is delayed one we can assume that the 21 day treatment with cinnamon promote adaptation to stress. However, the involvement of an adaptive mechanism in the anti-stress effect of cinnamon needs further elucidation.

Conclusion

Cinnamon is found to be effective in minimizing stress responses at a dose of 200 mg/kg thereby beneficial in stress therapy.

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