Anti-stress Effects of Ginkgo biloba and Panax ginseng: a Comparative Study

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Abstract. Stress is a global menace fortified by the advancement of industrialization. Failure of stress management is due to lack of proper evaluation of anti-stress products. We explored the anti-stress potential of the Ginkgo biloba (G. biloba, 30 mg/kg, p.o.) and compared it with that of Panax ginseng (P. ginseng, 100 mg/kg, p.o.) against acute stress (AS) and chronic stress (CS) models in rats. Immediately after AS and CS, the rats were sacrificed, and adrenal glands and stomach were dissected out for weight determination and scoring of the ulcer index (UI), respectively, as well as changes in biochemical parameters like plasma glucose (GL), triglycerides (TG), cholesterol (CL), creatine kinase (CK), and serum corticosterone (CORT) were also estimated. AS significantly increased UI, adrenal gland weight (AGW), GL, CK activity, and CORT, whereas G. biloba significantly reduced them. P. ginseng significantly reverted GL and CK activity. In CS, a significant increase was found in the UI, AGW, plasma GL, TG, CK activity, and CORT with a decrease in the level of CL and TG. G. biloba did not produce any significant effect on CS-induced alterations. P. ginseng reduced the UI, AGW, plasma GL, TG, CK activity, and CORT level significantly. From the above study, G. biloba is more effective in AS, whereas for CS, P. ginseng will be a better option. Hence these extracts possess significant anti-stress properties and can be used for the treatment of stress-induced disorders.

Keywords: Ginkgo biloba, Panax ginseng, immobilization stress, adrenal hypertrophy, biochemical alteration

Introduction

Stress has been postulated to be involved in the etiopathogenesis of a variety of disease states, including hypertension, peptic ulcer, diabetes, immuno-suppression, reproductive dysfunctions (1), and behavioral disorders like anxiety (2) due to involvement of the central nervous system (CNS), endocrine system, and metabolic system. The resultant disturbances may vary due to the type, intensity, and duration of a particular stressor and the strain/sex differentiation of the experimental subjects (3). Various types of stressors have been used to study stress-induced alterations, among them, immobilization has been used extensively for the study of stress-related biological, biochemical and physiological responses in research. The distinct advantage of using immobilization as a stressor lies in the fact that it produces both physical as well as an in-escapable psychological stress (4, 5). Therefore in our studies, we have used an immobilization stressor in acute stress (AS) as well as chronic stress (CS) models. Furthermore, besides AS, CS alters important neurological, behavioral, and biochemical parameters possibly in a different manner than AS (6).

Since the introduction of adaptogens (7), researchers have studied several plants that were once used as tonics due to their adaptogenic and rejuvenating properties in traditional medicine (8). The drugs of plant origin are gaining increasing popularity and are being investigated for remedies of a number of disorders including anti-stress adaptogenic activity (9). Ginkgo biloba (G. biloba) is one of the widely used Chinese
plants for its medicinal properties for several thousand years. It has been used for the treatment of various common geriatric complaints including vertigo, short term memory loss, and lack of attention or vigilance. The standardized extract of *G. biloba* has been shown to have effects on cerebral vascular disorders (10) and possesses anti-oxidant (11, 12) and neuro-protective properties (13). Recently *G. biloba* has received attention as potential cognitive enhancers (14) for the treatment of Alzheimer’s disease (15). *G. biloba* was found to increase the activity of hepatic drug-metabolizing enzymes, particularly cytochrome P450 in a dose and time dependent manner (16). *Panax ginseng* (*P. ginseng*), the first clinically used adaptogen, has been extensively investigated experimentally and clinically for its stress attenuating activity (17). The anxiolytic and memory enhancing properties of *G. biloba* have been established, but its anti-stress activity still lacks convincing evidence. Moreover, comparative evaluations of the anti-stress properties of these plants have not as yet been evaluated. In the present study, we have investigated the anti-stress properties of *G. biloba* and compared it with the well-accepted adaptogenic agent *P. ginseng* in AS as well as CS models in rats.

**Materials and Methods**

**Animals**

Studies were conducted on adult male Sprague-Dawley rats (180 – 200 g) obtained from the animal house of our institute. The animals were housed in a group of 4 in polyethylene cages (38 x 23 x 10 cm) under standard housing conditions (temp., 24 ± 2°C; humidity, 60 – 65%) with 12-h light and dark cycle. The food in the form of dry pellets and water were available ad libitum. The animal experiments were performed according to the internationally accepted ethical guidelines and approved by the Research Ethics Committee of our Institute.

**Drugs**

*P. ginseng* root extract powder was purchased from Sigma (St. Louis, MO, USA), and the standardized extract of *G. biloba* leaf was obtained from Meckel-Gmbh (Germany). The extract of *G. biloba* (30 mg/kg) and the extract of *P. ginseng* (100 mg/kg) were administered orally daily as aqueous suspensions using gum acacia (0.5%) as surfactant to different groups of animals. The doses of the extracts used were determined on the basis of the initial pilot studies.

In our pilot studies, increasing doses of *G. biloba* (15, 30, and 60 mg/kg, p.o.) and *P. ginseng* (50, 100, and 200 mg/kg, p.o.) were studied in the non-stress group and AS group of rats in order to evaluate their effect per se and to find out the effective dose of *G. biloba* and *P. ginseng* for further use in experimental studies. Rats were exposed to the AS protocol and immediately after that the various parameters were studied to find the best effective dose.

**Experiment procedure**

The rats were divided into control non-stress groups, AS, and CS groups, with 7 rats in each group; and the drug-treated groups were also divided into the AS and CS groups, with 7 rats in each group. The AS drug groups were fed with plant extracts of *G. biloba* and *P. ginseng* for 3 days. On the 2nd day after drug feeding, they were fasted for overnight with free access to water. On the 3rd day, 45 min. after drug feeding, the rats were stressed. In CS, the drugs were feed continuously 45 min prior to the stress regimen up to seven consecutive days except that the rats were kept fasted overnight on the 6th day after drug feeding and stress exposure.

The stress was produced by restraining the naive animals inside an adjustable acrylic hemi-cylindrical plastic tube (4.5-cm diameter, 12-cm-long). The rats were confined individually and exposed continuously for a period of 150 min once only in AS and once daily for seven consecutive days for CS (18). The rats were sacrificed immediately after stress by decapitation, and the blood was collected in EDTA-coated tubes kept on ice and in propylene tubes to collect blood plasma and blood serum, respectively. The blood was centrifuged (3000 rpm x 20 min at 4°C) and plasma and serum were separated out and stored at –20°C for biochemical and hormonal assays. The plasma was used to analyze the following biochemical parameters: glucose, triglycerides, cholesterol, and creatine kinase using autoanalyzer (Synchron Cx-5; Beckman Coulter, Inc., Brea, CA, USA) with their respective kits (Beckman Coulter International, Nyon, Switzerland). The serum corticosterone was estimated using an RIA kit. The adrenal glands were dissected out and weighed. The stomach was dissected out and cut open along the greater curvature for scoring the incidence of ulcer. Ulcer index (UI) was scored according to the method of Gupta et al. (19).

**Statistical analyses**

Statistical analysis was done using one way ANOVA followed by the Student-Newman-Keuls multiple comparison test; *P* values <0.05 were considered significant. The statistical analysis for the change in the UI was done by nonparametric ANOVA (Kruskal Wallis test) followed by Dunn’s multiple comparison test; *P*<0.05
was considered significant.

Results

In our pilot studies, graded doses of the *G. biloba* (15, 30, and 60 mg/kg, p.o.) and *P. ginseng* (50, 100, and 200 mg/kg, p.o.) were studied on the AS and non-stress group of rats. It was observed that *G. biloba* and *P. ginseng* produced the maximum effect at a dose of 30 and 100 mg/kg, p.o., respectively, in the AS group (Table 1). The 60 mg/kg, p.o. dose of *G. biloba* and 200 mg/kg, p.o. dose of *P. ginseng* was not found to bring any significant changes in comparison to its immediate lower dose. Therefore, we used these doses in our experiment. Moreover, *G. biloba* (30 mg/kg, p.o.) and *P. ginseng* (100 mg/kg, p.o.) did not produce any significant changes in the above-mentioned parameters in the non-stress group and the values were within the normal physiological ranges.

Effect of pretreatment of extracts of *G. biloba* and *P. ginseng* on AS and CS induced alterations in UI and adrenal gland weight

AS and CS exposures resulted a significant increase (*P*<0.01) in the UI. Pretreatment with *G. biloba* (30 mg/kg, p.o.) decreased the AS-induced UI significantly (*P*<0.01), but was not significantly effective in the CS-induced UI. Pretreatment with *P. ginseng* (100 mg/kg, p.o.) was not effective in reversing the AS-induced UI to a significant level, but reversed the CS-induced UI significantly (*P*<0.01) in comparison to the CS group of rats (Fig. 1).

Exposure to AS and CS significantly increased the adrenal gland weight in comparison to the control group of rats (*P*<0.01). Pretreatment with *G. biloba* (30 mg/kg, p.o.) significantly decreased the AS-induced adrenal gland weight in comparison to the AS group of rats (*P*<0.05). Pretreatment with *P. ginseng* (100 mg/kg, p.o.) was considered significant.

Table 1. Changes in ulcer index, adrenal gland weight, plasma glucose, triglyceride, cholesterol, and creatine kinase activity under control, acute stress, and drug-treated groups (graded dose)

<table>
<thead>
<tr>
<th></th>
<th>Non-stress Control</th>
<th>Vehicle + AS</th>
<th><em>G. biloba</em> (15 mg/kg) + AS</th>
<th><em>G. biloba</em> (30 mg/kg) + AS</th>
<th><em>G. biloba</em> (60 mg/kg) + AS</th>
<th><em>P. ginseng</em> (50 mg/kg) + AS</th>
<th><em>P. ginseng</em> (100 mg/kg) + AS</th>
<th><em>P. ginseng</em> (200 mg/kg) + AS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ulcer index</td>
<td>0</td>
<td>21.43 ± 1.43**</td>
<td>18.57 ± 3.401</td>
<td>2.857 ± 1.844**</td>
<td>5.71 ± 2.02**</td>
<td>15.71 ± 2.97</td>
<td>10.0 ± 2.18</td>
<td>8.57 ± 3.401**</td>
</tr>
<tr>
<td>Adrenal gland weight</td>
<td>0.0127 ± 0.0013**</td>
<td>0.0164</td>
<td>0.0136</td>
<td>0.0143</td>
<td>0.0162</td>
<td>0.0155</td>
<td>0.0152</td>
<td></td>
</tr>
<tr>
<td>Plasma glucose</td>
<td>108.0 ± 12.49**</td>
<td>146.0</td>
<td>115.0</td>
<td>120.0</td>
<td>146.0</td>
<td>125.14</td>
<td>132.54</td>
<td></td>
</tr>
<tr>
<td>Plasma triglyceride</td>
<td>71.67 ± 14.33</td>
<td>55.62</td>
<td>51.4</td>
<td>57.14</td>
<td>52.31</td>
<td>54.86</td>
<td>56.76</td>
<td></td>
</tr>
<tr>
<td>Plasma cholesterol</td>
<td>55.5 ± 4.48</td>
<td>60.16</td>
<td>51.4</td>
<td>57.61</td>
<td>58.66</td>
<td>50.86</td>
<td>52.57</td>
<td></td>
</tr>
<tr>
<td>Plasma creatine kinase</td>
<td>167.4 ± 12.34</td>
<td>582.57</td>
<td>471.11</td>
<td>203.22</td>
<td>254.82</td>
<td>544.58</td>
<td>308.43</td>
<td>415.0</td>
</tr>
</tbody>
</table>

The stress group was compared with the control group, and the drug-treated groups were compared with the stress group. The effect of *G. biloba* at 60 mg/kg, p.o. and *P. ginseng* at 200 mg/kg, p.o. were compared with the acute stress and with their respective lower doses for significant response. Results are represented as the mean ± S.E.M. with *n* = 7 in each group. ** *P*<0.01, as compared with the non-stress control group; *P*<0.05, *** *P*<0.01, as compared with acute stress (AS) group.
Ginkgo biloba as a Potent Adaptogen

Fig. 2. Changes in adrenal gland weight (hypertrophy) under acute stress and chronic stress of the control, stress, and drug-treated groups. The control group was compared with the control group, and the drug-treated groups were compared with their respective stress group. Results are represented as the mean ± S.E.M. with n=7 in each group. **P<0.01, as compared to the control group; *P<0.05, as compared to the acute stress (AS) group; ††P<0.01, as compared to the chronic stress (CS) group. GB: G. biloba, PG: P. ginseng.

Effect of plant extract treatment on AS- and CS-induced biochemical alterations

AS resulted in a significant (P<0.01) increase in the plasma glucose level (hyperglycemia) as compared to the control. CS resulted in a significant (P<0.01) decrease in the level of plasma glucose level as compared to the control, but the value was within the normal physiological range. Pretreatment with G. biloba (30 mg/kg, p.o.) and P. ginseng (100 mg/kg, p.o.) significantly (P<0.01) reverted the AS-induced hyperglycemia in comparison to the AS group of rats. No significant change was observed after pretreatment with G. biloba (30 mg/kg, p.o.) on plasma glucose level during CS, but pretreatment with P. ginseng (100 mg/kg, p.o.) further reduced the plasma glucose level (P<0.01) as compared to the CS group of rats, but the value of plasma glucose was within the normal physiological level range (Fig. 3).

A significant decrease (P<0.01 and P<0.05) was found in the level of plasma triglyceride and cholesterol after CS exposure, respectively, whereas no significant change was observed after AS exposure in the level of plasma triglyceride and cholesterol level in comparison to the control group of rats. Pretreatment with P. ginseng (100 mg/kg, p.o.) further significantly reduced the plasma triglyceride level (P<0.05) as compared to the CS group of rats (Fig. 3).

Exposure to AS and CS resulted in a significant increase (P<0.01) in the plasma creatine kinase activity in comparison to the non-stressed control group of rats. Pretreatment with G. biloba (30 mg/kg, p.o.) (P<0.01) and P. ginseng (100 mg/kg, p.o.) (P<0.05) significantly reversed the AS-induced increased creatine kinase activity in comparison to the AS group of rats. Pretreatment with P. ginseng (100 mg/kg, p.o.) (P<0.01) significantly reduced the creatine kinase activity in the CS experiment in comparison to the CS group of rats. The decrease in the CS-induced increased creatine kinase activity in G. biloba (30 mg/kg, p.o.) pretreated rats was not found to be significant (Fig. 4).

Exposure to AS and CS resulted in a significant increase (P<0.01) in the serum corticosterone level in comparison to the non-stressed control group of rats. Pretreatment with G. biloba (30 mg/kg, p.o.) resulted in a significant (P<0.01) decrease in the serum corticosterone level in comparison to the AS group of rats, whereas no significant change was observed with P. ginseng (100 mg/kg, p.o.) pretreatment. CS pretreatment with P. ginseng (100 mg/kg, p.o.) significantly (P<0.05) decreased the serum corticosterone level in comparison to the AS group of rats, whereas the G. biloba (30 mg/kg, p.o.) pretreatment was not found to be significantly effective (Fig. 5).
Stress is a global menace fortified by the advancement of industrialization and elicited by a variety of factors, viz., environmental, social or pathological phenomenons of life. Considerable evidences published in the last decade have focused on a constellation of neurochemical, biochemical, and molecular effects caused by stress in the CNS, endocrine system, and immune system (20). Normally stress-induced changes are self-limiting and adaptive until and unless events that override “threshold” limits become irreversible and pathological (21).

Advancements in the understanding of processes leading to the etiopathogenesis of stress-induced disorders can not obscure the simple fact that the exhaustion of energy supply is still the basis for triggering the disorders and collapse of energy metabolism following glucose deprivation in the circulation (22). The desire to augment the coping mechanism has led to the emergence of the science of adaptation that focuses on elucidating mechanisms that may help to counteract excessive and unnecessary responses to stress. A number of plants extracts has been studied for their rejuvenating and adaptogenic or anti-stress properties (8, 23). The anti-stress properties of the G. biloba are still unexplored. P. ginseng is a well-known adaptogen and was shown to be effective in attenuating stress-induced adverse effect in astronauts and soldiers (24). Therefore we selected these plant extracts to explore and compare the anti-stress activity of G. biloba with P. ginseng.

The G. biloba and P. ginseng were found most effective in the 30 mg/kg, p.o. and 100 mg/kg, p.o. dose, respectively. Therefore these doses were selected to study the anti-stress activities of these extracts. The study by other authors have also used P. ginseng at a dose of 100 mg/kg, p.o. for studying its adaptogenic activity (25). The previous studies with the G. biloba for its learning and memory effects have also shown significant facilitatory effects at 15, 30, and 60 mg/kg, p.o. (26).

Exposure to AS and CS resulted in adrenal hypertrophy and gastric ulceration, indicating the active involvement of the hypothalamic-pituitary-adrenal (HPA) axis which is highly responsive to stress (27, 28). The hyper-activation of the PVN of the hypothalamus during stress causes a decrease in mucosal blood flow and hyper-contractility through descending projections that induces pathogenesis of gastric ulcers (29). The adrenal hypertrophy takes place in response to the secretion of ACTH from the pituitary for increased corticosterone from cortical cells to combat stress (30). The level of corticosterone was found to be more elevated during the AS rather then the CS in the experiment, indicating that CS alters important biochemical and endocrinological parameters in a different fashion than AS, as habituation occurs during CS (6). The CS has been reported as a model of aging, which mimics the truncated glucocorticoid negative feed back to the gluco-

**Fig. 4.** Changes in plasma creatine kinase activity under acute stress and chronic stress of the control, stress, and drug-treated groups. The stress group was compared with the control group, and the drug-treated groups were compared with their respective stress group. Results are represented as the mean ± S.E.M. with n = 7 in each group. **P<0.01, as compared to the control group; *P<0.05, "P<0.01, as compared to the acute stress (AS) group; †P<0.01, as compared to the chronic stress (CS) group. GB: G. biloba, PG: P. ginseng.

**Fig. 5.** Changes in plasma corticosterone level under acute stress and chronic stress of the control, stress, and drug-treated groups. The stress group was compared with the control group, and the drug-treated groups were compared with their respective stress group. Results are represented as the mean ± S.E.M. with n = 7 in each group. **P<0.01, as compared to the control group; *P<0.01, as compared to the acute stress (AS) group; †P<0.05, as compared to the chronic stress (CS) group. GB: G. biloba, PG: P. ginseng.

**Discussion**

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corticoid receptors in the hippocampus in aged subjects (31).

The exposure to AS resulted in an increase in the plasma glucose, whereas creatine kinase activity was increased in AS as well as in CS. The more prominent effect was found in AS in a comparison for which again the adaptation or habituation to the stress seems responsible. During AS, the increased level of glucose and creatine kinase activity was important for maintaining the ATP availability to muscles, CNS, and the organ of demand, which reduces during the CS episodes due to redirection of the energy substrates to the specific stress demanding site, which was a non-specific and non target oriented during the AS response (3, 32). Other authors (3, 33) have also reported that the hyperglycemic effect of epinephrine and corticosterone is due to increased glycogenolysis of glycogen in liver during acute stress. The depletion of stored glycogen during CS initiates gluconeogenesis and utilizes reserve fats as a secondary substrate in response to corticosterone (34) for which the level of triglyceride and cholesterol (35) was decreased during the CS episodes. The reduction in the plasma triglyceride in stressed rats may be secondary to the effect of catecholamines on the triglyceride lipase activity in the adipose tissue.

The pretreatment with *G. biloba* decreased the AS-induced UI, adrenal hypertrophy, plasma glucose, creatine kinase, and circulating corticosterone. The decrease in the UI, adrenal gland weight, and corticosterone indicated its action on the HPA axis during the AS as these parameters are related with the markers of HPA axis regulation. The hyperglycemia and creatine kinase activity seems to be mediated through the circulating corticosterone level and the glucose, and creatine kinase activity can be the peripheral covariant of corticosterone. *P. ginseng* was able to reverse the plasma glucose and creatine kinase activity during the AS only, which may be due to the peripheral effect of *P. ginseng*, but it was unable to reverse the markers of HPA.

The pretreatment of *G. biloba* was not found to reverse any of the parameters to significant extent in the CS experiment. This also indicates that CS alters the selected parameters in a different way than AS. The pretreatment with *P. ginseng* reversed the UI, adrenal gland weight, creatine kinase and circulating corticosterone. The decrease in the level of UI, adrenal gland weight, and corticosterone indicates its HPA axis attenuating properties. This indicates that *P. ginseng* attenuates the HPA axis by different pathways during the CS, which is absent or remains inactive during the AS. The further decrease in the level of plasma glucose and triglyceride may be the outcome of continuation of increased glucose utilization and gluconeogenesis even after *P. ginseng* treatment.

The ginkgolide B has been proposed to inhibit steroid synthesis by transcriptional suppression of the adrenal cortical peripheral-type benzodiazepine receptor gene, which plays an important role during the flight-fight response of AS (36). During CS, the attenuated feed back response to the glucocorticoid receptors is found to be responsible for the higher level of circulating corticosterone. The *P. ginseng* has been found to restore this attenuated negative feed-back and thus augmented the circulating corticosterone. The corticosteroid-like action of *ginseng* is strongly suspected to be responsible for the adaptogenic properties of *ginseng*. Previous endocrinological studies also supports that *ginseng* may attenuate adrenal steroidogenesis via an indirect action on the pituitary gland and ACTH secretion (37).

From the above results, it is evident that *G. biloba* will be a better option for treating the AS, whereas *P. ginseng* will be a better option for CS treatment. In conclusion, the extracts of *G. biloba* and *P. ginseng* have potent adaptogenic activity that is mediated by regulation of cortical cells of adrenal and pituitary ACTH secretion, respectively, to target stress.

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