Anti-Stress Potential of Aqueous Root Extract of *Cnestis ferruginea*

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**Abstract:** This study presents the results of the phytochemical screening, acute toxicity testing and anti-stress potential of aqueous root extract of *Cnestis ferruginea* in mice and rats. The forced swimming endurance test, anoxic tolerance tests and immobilization stress-induced gastric ulcer were utilized as models for the evaluation of the anti-stress property of *C. ferruginea*. The results from phytochemical tests showed the presence of alkaloids, flavonoids, saponins and glycosides as the major constituents of the root extract of *C. ferruginea*. The acute toxicity test showed a wide margin of safety with a median lethal dose (LD50 of 3.6570 g kg⁻¹) in mice. In the forced swimming test, *C. ferruginea* at a dose range of (300-500 mg kg⁻¹, p.o) significantly decreased the duration of immobility in a dose-related manner. These results showed that the extract is a potential anti-stress agent. In the anoxic tolerance test, the extract prolonged the mean time (min) before convulsion in mice in a dose-dependent manner. Also in the immobilization stress-induced gastric ulcer, the extract prevented gastric ulcer formation in rats immobilized and subjected to stress (cold) at 4°C for 2 h after pretreatment with the aqueous root extract. This further confirmed the anti-stress potential of the extract. In conclusion, the root extract of *C. ferruginea* is a potential anti-stress agent.

**Keywords:** Forced swimming endurance test, anoxic tolerance tests, immobilization stress-induced gastric ulcer, *Cnestis ferruginea*, *Panax ginseng*

**INTRODUCTION**

Stress is a biological response to aversive conditions that tend to threaten or perturb the homeostasis of the organisms (Bhattacharya and Ghosal, 2000; Piazza and Lemco, 1998; Hoffman, 2001). Stress has been shown to induce a marked rise in the brain levels of biogenic amines such as adrenaline and nor-adrenaline (Subarnas et al., 1993; Anisman and Zacharko, 1991). These chemical substances are release in response to stress signals and are meant to assist the organisms to cope with stress (Anisman and Zacharko, 1991; Bishayee and Chatterjee, 1995). However, increased utilization of the amines resulting in their depletion in prolonged severe stress is responsible for fatigue, reduced stamina, lowered mood (hopelessness) or despair seen in individuals under intense stress (Subarnas et al., 1993; Bhattacharya and Ghosal, 2000). It is has been reported that drugs with anti-stress properties induce a state of non-specific resistance against stressful conditions (Bhattacharya and Ghosal, 2000). Amphetamine, caffeine and anabolic steroids are the most widely used drugs by people to combat stress (Piazza and Lemco, 1998; Nehling et al., 1992; Sapolsky et al., 2000; Hoffman, 2001). However, the incidence of toxicity and dependence has limited the therapeutic usefulness of these drugs in the control of stressful events (Piazza and Lemco, 1998; Nehling et al., 1992; Sapolsky et al., 2000; Hoffman, 2001). The potential utility of safer and cheaper herbal medicines as anti-stress agents have been reported in literature (Ellis and Reddy, 2002; Balandrin et al., 1993; Grover et al., 1995; Josey and Tackett, 1999; Subarnas et al., 1993). Moreover, a number of plants such as *Asparagus racemosus*, *Ocimum sanctum*, *Withania somniferia*, *Phyllanthus*, *Panax ginseng*, *Hypericum perforatum* and *Ginkgo biloba* have been shown to possess anti-stress properties (Ellis and Reddy, 2002; Bhattacharya and Ghosal, 2000).

*Cnestis ferruginea* DC (Connnaraceae) root is used as laxative and the stem is used to rub on the skin and as a medicine for the throat while the bark is rubbed on the gum. No studies have shown its anti-stress or endurance promoting activity. This study reports on the phytochemical constituents and anti-stress potential of aqueous root extract of *Cnestis ferruginea* in mice and rats.

**MATERIALS AND METHODS**

**Plant material:** The dried roots of *Cnestis ferruginea* were purchased from Mushin market, Lagos, Nigeria. Identified and authenticated by Prof. D. Olowokudejo of the Department of Botany and Microbiology, University of Lagos, Nigeria.
of Lagos, Nigeria. Voucher specimen of the root was deposited in the herbarium of the Department of Pharmacognosy, College of Medicine, University of Lagos, Nigeria.

**Drug:** Korean Ginseng (white *Panax ginseng* root) (Mason vitamins Inc., Miami Lakes, FL 33014, USA) was used as reference drug in this study.

**Laboratory animals:** Sprague Dawley rats (150-200 g) and Swiss albino mice (17-25 g) of either sex used in the study were purchased from the Laboratory Animal centre, College of Medicine, University of Lagos, Nigeria. They were kept in a well-ventilated and hygienic environment, with free access to standard feed pellet and water *ad libitum*. The ethical guidelines for the handling of experimental animals were followed in the study.

**Extraction procedure:** The dried roots of *C. ferruginea* were cut into smaller pieces and ground into fine powder. Two hundred eighty grams of the powdered roots were soaked in 650 mL of distilled water for 48 h. The solution thereafter filtered after 48 h and the filtrate was evaporated to a dark-brownish sticky residue in an oven at 38°C. The yield of the extract was 14% with reference to the powdered roots. Four hundred milligram of the residue was dissolved in 10 mL of distilled water for the study.

**Experimental procedure:** Forced swim test: The modified method of Subbarao *et al.* (1993) was followed in this study. Mice (10 per group) were treated with the extract (300-500 mg kg⁻¹, p.o) in Group I-III, Group IV-VI (Experimental control) were pretreated with *P. ginseng* (50-200 mg kg⁻¹, p.o). Normal saline (5 mL kg⁻¹, p.o.) as control test. All drugs were administered 1 h before the test.

**Anoxic tolerance test:** Mice (10 per group), drugs were administered in the same pattern as described above, before mice were forced to enter a 250 mL conical flask that was made airtight by covering the opening with cotton wool and adhesive tape. The mean time to convulsion was recorded and the animal was removed at onset of convulsion.

**Immobilization stress-induced gastric ulcer:** The method described by Bhattacharya *et al.* (1987) was used for this test. Rats (5 per group). Group I-III received (300-500 mg kg⁻¹ of extract p.o). Group IV received 100 mg kg⁻¹ of ginseng orally. Group V received 5 mL kg⁻¹ of normal saline and Group was neither treated nor subjected to any condition of stress. After 2 h in cold condition, the rats were sacrificed by cervical dislocation and the stomach was eviscerated and split open along the greater curvature for gross and histopathological findings.

**Phytochemical screening:** The extract of *C. ferruginea* was screened for the presence of alkaloids, reducing sugars, flavonoids, phenols, tannins, saponins, steroids, anthraquinones and glycosides (Trezise and Evans, 2000).

**Statistical analysis:** Results were analyzed statistically by means of Student’s t-test. p-values less than 0.05 were considered statistically significant in all statistical tests.

**RESULTS**

**Forced swim test:** Results depicted in Table 1 clearly show a significant decreased in duration of immobility in a dose dependent manner, similar effects were observed in mice pretreated with ginseng compare to saline treated mice.

**Anoxic tolerance test:** *C. ferruginea* at a dose range of (400-500 mg kg⁻¹, oral) significantly prolonged the mean time (min) before convulsion in mice in a dose dependent manner (Table 2).

**Immobilization stress-induced gastric ulcer:** The normal untreated, unstressed rats serve as normal control. The normal saline group developed about 60% severe gastric ulcer. The rats pretreated with 300-500 mg kg⁻¹ of extract and 100 mg kg⁻¹ *P. ginseng* group produced no gastric ulcer.

**Table 1:** Effect of aqueous root extract of *C. ferruginea* on the duration of immobility in mice.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Dose (mg kg⁻¹)</th>
<th>Duration of immobility (sec)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal saline control</td>
<td>-</td>
<td>207.40±1.69</td>
</tr>
<tr>
<td><em>P. ginseng</em></td>
<td>50</td>
<td>117.00±1.84*</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>28.40±1.44*</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>0.00±0.00</td>
</tr>
<tr>
<td><em>C. ferruginea</em></td>
<td>300</td>
<td>4.40±0.40*</td>
</tr>
<tr>
<td></td>
<td>400</td>
<td>0.00±0.05*</td>
</tr>
<tr>
<td></td>
<td>500</td>
<td>0.00±0.05*</td>
</tr>
</tbody>
</table>

Each value represents the Mean±SEM of 10 animals per group, *p<0.05* compared with saline-control group (Student t-test).

**Table 2:** The effect of *Cnemias ferruginea* on anoxic tolerance in mice.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Dose (mg kg⁻¹)</th>
<th>Mean time before convulsion (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal saline control</td>
<td>-</td>
<td>17.62±0.36</td>
</tr>
<tr>
<td><em>P. ginseng</em></td>
<td>50</td>
<td>19.81±0.21</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>35.90±0.57*</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>67.11±0.38*</td>
</tr>
<tr>
<td><em>C. ferruginea</em></td>
<td>300</td>
<td>18.79±0.21</td>
</tr>
<tr>
<td></td>
<td>400</td>
<td>42.52±0.71*</td>
</tr>
<tr>
<td></td>
<td>500</td>
<td>90.70±1.00*</td>
</tr>
</tbody>
</table>

Each value represents the Mean±SEM of 10 animals per group, *p<0.05* compared with saline-control group (Student t-test).
ulceration, respectively. This result showed the ability and efficacy of the extract to prevent gastric ulcer formation in stressed rats. The phytochemical screening showed that the major active constituents of the aqueous root extract of *C. ferruginea* are alkaloids, flavonoids, saponins and glycosides.

**DISCUSSION**

The results of the study showed that the extract is a potential anti-stress agent due to its ability; to reduce the duration of immobility in forced swim test, prolonged the mean time before convolution in anoxia tolerance test and prevented ulcer formation in rats immobilized and subjected to cold.

The forced swimming test is the most widely used paradigm for the evaluation of anti-stress and antidepressant property of a novel compound (Subarnas et al., 1993; Anisman and Zacharko, 1991). This paradigm is based on the observation that animals forced to swim in water eventually assumed a characteristic immobile posture, devoid of any activity (Subarnas et al., 1993). The appearance of immobility therefore, reflects a state of tiredness, fatigue, reduced stamina or a lowered mood (hopelessness) (Subarnas et al., 1993; Bhattacharya and Ghosal, 2000). These signs represent the core symptoms observed in depressed patients and in individuals under intense stress (Anisman and Zacharko, 1991). It is well known that drugs with anti-stress properties reduce the duration of immobility in animals (Subarnas et al., 1993). The ability of the extract of this plant to reduce the duration of immobility, therefore suggests an anti-stress property.

Central neurotransmitters are functionally involved in the regulation of stress responses (Bishayee and Chatterjee, 1995). These chemical substances are released in response to stress and are meant to strengthen the organisms by resisting against the stressful events, a process known as adaptation (Anisman and Zacharko, 1991; Bishayee and Chatterjee, 1995). However, prolonged severe stress creates ineffective adaptation, which results in reduced stamina or mood (Anisman and Zacharko, 1991). Previous studies have shown reduced brain levels of adrenaline and nor-adrenaline in animals exposed to stress such as the swimming test, immobilization stress (Bishayee and Chatterjee, 1995; Anisman and Zacharko, 1991; Subarnas et al., 1993). It is well established that under stressful conditions, utilization and synthesis of these amines are increased in various regions of the brain (Anisman and Zacharko, 1991; Bishayee and Chatterjee, 1995). However, if the stress persists and becomes uncontrollable, the utilization of the amines exceeds synthesis thereby resulting in their depletion (Anisman and Zacharko 1991; Subarnas et al., 1993).

The results of the study showed that the extract did prolonged the moment before convolution, which therefore demonstrate anti-stress property. Prolongation of mean time to convolution could be as a result of its powerful anti-oxidant and free radical scavenging activities (Oke and Hamburger, 2002).

Experimental stress-induced gastric ulceration has been suggested to results from autonomic nervous system hyperactivity (Parasympathetic hyperactivity) leading to vascular stasis of gastric mucosa and altered gastric mucosal microcirculation (Athey and Iam, 1981). Gastric ulcerations induced by a large numbers of stressors which is at present, one of the most widely used paradigms to evaluate anti-stress activity (Bhattacharya and Ghosal, 2000). The gastric pathology produced under stress is a continuous from gastric to ulceration, sometimes with perforation (Crawford and Chen, 1999).

The Ginseng prevented, immobilization stress induced gastric ulcer, this is in line with earlier finding on anti-stress properties of Ginseng (Ellis and Reddy, 2002). This reflects the anti-stress activity of this extract at different doses.

The acute toxicity studies revealed that *C. ferruginea* has a wide margin of safety profile especially when administered orally. Phytochemical screening revealed the presence of alkaloids, flavonoids, saponins and glycosides in *C. ferruginea*. Plants possessing flavonoids have been found to exhibit anti-inflammatory, antioxidant and membrane stabilizing properties (Perenz et al., 1995; Levy, 1976). Furthermore, they have been reported to offer protection of cellular components or organs against injurious substances (Rastrelli et al., 1998; Mascolo et al., 1998).

It is likely that the presence of these active ingredients especially flavonoids and saponins may perhaps account for the pharmacological effects demonstrated by *C. ferruginea* in this study.

**CONCLUSIONS**

The results obtained from this study suggest that the extract is a potential Antistress agent.

**REFERENCES**