Anxiolytic activity of aqueous extract of *Camellia sinensis* in rats

Rajeshwari Shastry, Sheetal Dinkar Ullal, Shreyas Karkala, Seema Rai¹, Akash Gadgade²

Abstract:

**Objectives:** The present study was undertaken to evaluate anxiolytic effect of *Camellia sinensis* (CS) and possible mechanism on acute and chronic administration in rats.

**Materials and Methods:** Eight groups of rats with six in each group were used. Group I served as control. Group II received diazepam (1 mg/kg). Groups III, IV, and V received CS in doses of 3.3, 16.5, and 33 mg/kg, respectively. Three pharmacologically validated experimental models – elevated plus maze (EPM), light and dark box (LDB), and open field tests (OFT) – were employed. Each animal was tested initially in the EPM and then in the LDB, followed by the OFT in a single setting. In EMP, number of entries into, time spent in, and number of rears in each arm in a 5-min period were noted. In LDB, number of entries and time spent in bright arena, number of rears, and duration of immobility were noted. In OFT, number of peripheral and central squares crossed, time spent, and number of rears in central squares were observed for a 5-min period. One-way ANOVA followed by post hoc least significant difference test was performed.

**Results:** In EPM and LDB, CS at 3.3, 16.5, and 33 mg/kg (acute and chronic models) increased the number of entries and time spent and rearing in the open arms and bright arena, respectively, compared to control. In the OFT, CS at 16.5 and 33 mg/kg significantly increased the number of squares crossed, time spent, and the number of rears in the central squares compared to control. Anxiolytic effect was dose dependent in EPM and LDB and CS at 33 mg/kg showed better anxiolytic activity compared to diazepam (1 mg/kg) in all models. Flumazenil (0.5 mg/kg) and bicuculline (1 mg/kg) completely inhibited while picrotoxin (1 mg/kg) partially inhibited the anxiolytic effect of CS. Diazepam and CS at 33 mg/kg reduced the locomotor activity in rats.

**Conclusion:** CS has dose-dependent anxiolytic activity which is comparable to diazepam. Anxiolytic action of CS is likely mediated through GABA_A−benzodiazepine receptor – Cl− channel complex – since flumazenil and bicuculline inhibited the anxiolytic effect.

**Key words:**

Anxiolytic, *Camellia sinensis*, elevated plus maze, light and dark box, open field test
theophylline), amino acid (theanine: γ-ethylamide of glutamic acid), polysaccharides, volatile oils, small amounts of tannin, diphenylamine, oxalic acid, trace elements, and Vitamin C.\(^\text{[8-10]}\)

A polyherbal product NR-ANX-C has been reported to have anxiolytic activity in rodents.\(^\text{[11]}\) CS is one of the major components (33%) of NR-ANX-C. Hence, the present study was undertaken to evaluate anxiolytic effect and its possible mechanism on acute and chronic administration of aqueous extract of CS in rats.

**Materials and Methods**

**Experimental Animals**
Adult Wistar rats of either sex, weighing 150–200 g, inbred at the institutional central animal house were housed and maintained under standard laboratory conditions, fed on commercial pelleted chow and water ad libitum. Experiments were conducted between 09:00 and 17:00 hours and in accordance with the guidelines laid down by the Committee for the Purpose of Control and Supervision on Experiments on Animals. The study protocol was approved by the Institutional Animal Ethics Committee.

**Drugs**

**Standard drug**
Diazepam (1 mg/kg, oral, using a metal gavage) was obtained from Nicholas Piramal India Ltd.

**Test drug**
Aqueous extract of CS (3.3, 16.5, and 33 mg/kg, oral, using a metal gavage) was obtained.\(^\text{[11]}\)

The raw materials (leaves of CS) were obtained from M/S The Himalaya Drug Company, Makali, Bengaluru - 562123, Karnataka, India. Aqueous extract was prepared in our laboratory using Soxhlet apparatus. Raw material weighing 250 g underwent eight cycles of soxhlation, following which 30.8 g of investigational product was obtained. Hence, the yield was 8.1%.

**Antagonists**
Benzodiazepine receptor antagonist – flumazenil (0.5 mg/kg, intraperitoneally), GABA\(_\gamma\)-gated chloride channel blocker – picrotoxin (1 mg/kg, intraperitoneally), GABA\(_\delta\) receptor antagonist – bicuculline (1 mg/kg, intraperitoneally),\(^\text{[12]}\) all antagonists were obtained from Sigma-Aldrich, Bommasandra-Jigani Link Road, Bengaluru.

Each drug solution was freshly prepared before administration. Doses of diazepam and CS were selected on the basis of earlier findings with NR-ANX-C.\(^\text{[11]}\)

Eight groups of animals were used and each group had six animals. Group I received normal saline, served as control. Group II received diazepam (1 mg/kg). Groups III, IV, and V received CS in doses of 3.3, 16.5, and 33 mg/kg, respectively. Group VI, VII, and VIII were cotreated with flumazenil (0.5 mg/kg), picrotoxin (1 mg/kg), and bicuculline (1 mg/kg), respectively, along with CS (33 mg/kg). In the acute study, all oral drugs were administered 60 min before the experiment. In the chronic study, drugs were administered once daily for 14 days and last dose was given on the 14th day 60 min before the experiment. Intrapерitoneal drugs were given 15 min before the experiment. In Groups VI, VII, and VIII, rats were pretreated with the antagonist to identify the site/mechanism of action of CS; hence, the dose of CS which showed maximum anxiolytic activity was chosen for these groups.

**Behavioral Testing**
Three pharmacologically validated experimental models, elevated plus maze (EPM),\(^\text{[13]}\) light and dark box (LDB),\(^\text{[14]}\) and open field test (OFT),\(^\text{[15]}\) were employed.

Each animal was tested initially in the EPM and then in the LDB, followed by the OFT in a single setting. Each animal was placed in the central square of EPM, facing one of the open arms. Number of entries into and time spent in the open and closed arms and number of rears in each arm in a 5-min period were noted. In the LDB, animals were placed at the center of brightly lit arena and number of re-entries into the bright arena; time spent in the bright arena, number of rears, and duration of immobility were noted. In the OFT, each animal was placed in one of the peripheral corner squares of the open field, and number of peripheral and central squares crossed, time spent and number of rears in central squares were observed for a 5-min period. Following each trial, apparatus was cleaned with spirit to mask the odor left by the animal in the previous experiment.

**Statistical Analysis**
Data obtained were coded and analyzed in a blind manner by a statistician who was not aware of the coding. One-way ANOVA was used with drug treatment as the independent factor. Post hoc comparisons were performed by applying least significant difference test. \(P < 0.05\) was considered statistically significant.

**Results**

**Acute Study**
In the EPM [Table 1], diazepam and CS at the dose of 33 mg/kg showed a significant increase in the entries (\(P = 0.004\) and \(P < 0.001\), respectively), time spent (\(P < 0.001\)), and rears (\(P = 0.025\) and \(P < 0.001\), respectively) in the open arm compared to the control group. CS at the dose of 33 mg/kg also significantly increased the time spent (\(P < 0.001\)) and number of rears (\(P = 0.003\)) in the open arm compared to the diazepam treated group.

Coadministration of flumazenil and bicuculline decreased the number of entries (\(P < 0.001\), time spent (\(P < 0.001\), and rears (\(P < 0.001\)) in the open arms as compared to CS at 33 mg/kg alone. Picrotoxin coadministration reduced the time spent (\(P = 0.03\)) and rears (\(P = 0.002\)) but had no effect on the number of entries into the open arm compared to CS at 3 mg/kg alone.

In the LDB [Table 2], diazepam and CS at all the three doses (3.3, 16.5, and 33 mg/kg) significantly increased the number of entries (\(P < 0.001, P = 0.014, P < 0.001,\) and \(P < 0.001,\) respectively), time spent (\(P < 0.001, P = 0.008, P < 0.001,\) and \(P < 0.001,\) respectively), and rears (\(P < 0.001, P = 0.002, P < 0.001,\) and \(P < 0.001,\) respectively) in the bright arena compared to control. Moreover, for each, CS at the dose of 33 mg/kg significantly increased the number of entries (\(P < 0.0001\) and
Table 1: Effect of drugs on behavior of rats in elevated plus maze on single dose administration (acute study)

| Treatment (n=6) | Number of open arm entries | Number of total arm entries | Time spent in open arm (sec) | Time spent in closed arm (sec) | Number of rears in open arms |
|----------------|---------------------------|-----------------------------|----------------------------|-------------------------------|----------------------------|
| Control        | 4.00±0.58                 | 8.17±0.70                   | 71.67±6.87                 | 228.33±6.87                   | 7.17±0.60                   |
| Diazepam 1 mg/kg | 7.17±0.60a               | 9.83±0.79                   | 205.5±1.40b                | 94.5±1.14c                    | 12±1.36i                    |
| CS (mg/kg)     |                           |                             |                            |                               |                            |
| 3.3            | 3.17±0.30                 | 12.5±0.61d                  | 62.33±1.89                 | 237.67±1.89                   | 5±1.06                      |
| 16.5           | 5±0.36                    | 8.50±0.42                   | 87.67±1.89                 | 212.33±1.89                   | 10.17±1.58                  |
| 33             | 9.17±1.27b               | 20.17±1.99a                 | 241.5±2.94b                | 58.5±2.94b                    | 18.67±2.10e                 |
| Flumazenil + CS 33 mg/kg | 3.33±0.42a   | 6.67±0.558b                 | 43.17±4.71b                | 256.83±4.71b                  | 2.83±0.60b                  |
| Bicuculline + CS 33 mg/kg | 3.50±0.84b | 7±1.94b                     | 43.50±7.19c                | 256.50±7.19c                  | 3±0.57b                     |
| Picrotoxin + CS 33 mg/kg | 7±0.63        | 9.83±0.87b                  | 216.83±12.73c              | 83.17±12.73c                  | 12.17±1.35c                 |
| F              | 11.748                    | 241.5±2.94                  | 212.33±1.89                |                               |                            |
| df             | 4                         |                             |                             |                               |                            |

Values are mean±SEM. *P<0.004 as compared to control, †P<0.001 as compared to control; diazepam and CS33. ‡P=0.008 as compared to control, ¶P=0.025 as compared to control, ‡‡P=0.001 as compared to diazepam, ‡‡‡P=0.037 as compared to CS33. CS=Camellia sinensis, SEM=Standard error of mean

Table 2: Effect of drugs on behavior of rats in light and dark box on single dose administration (acute study)

| Treatment groups (n=6) | Number of entries in bright arena | Time spent in bright arena (sec) | Rears in bright arena | Rears in dark arena | Immobility (sec) |
|------------------------|-----------------------------------|----------------------------------|----------------------|-------------------|-----------------|
| Control                | 1±0.0                             | 7.33±0.88                        | 0.33±0.21            | 4.50±0.56         | 7.17±2.43       |
| Diazepam 1 mg/kg       | 3.50±0.43a                        | 25±3.00a                         | 3.17±0.60a           | 12.67±1.43a       | 18.33±2.09a     |
| CS (mg/kg)             |                                   |                                  |                      |                   |                 |
| 3.3                    | 2.33±0.21b                        | 14.50±0.95b                      | 2.17±0.30b           | 2.67±0.33         | 0.17±0.16h      |
| 16.5                   | 3.50±0.22a                        | 19.17±0.79a                      | 2.67±0.33a           | 5.67±0.56         | 0.83±0.83h      |
| 33                     | 5.83±0.60b                        | 30.50±2.01b                      | 3.83±0.31b           | 11.67±0.88b       | 1.17±1.16h      |
| Flumazenil + CS 33 mg/kg | 2±0.36e                          | 9.67±1.11e                       | 1.17±0.16e           | 2.50±0.42         | 6±4.45          |
| Bicuculline + CS 33 mg/kg | 1.50±0.22                        | 9.83±2.67f                      | 1.17±0.30            | 2.33±0.61         | 7.50±6.73       |
| Picrotoxin + CS 33 mg/kg | 3.83±0.30b                       | 26.50±1.96c                      | 3.50±0.76            | 3.50±0.76         | 0.8±0.78        |
| F                      | 24.848                            | 25.802                           | 12.461               | 27.995            | 23.923          |
| df                     | 4                                 |                                  |                      |                   |                 |

Values are mean±SEM. *P<0.004 as compared to control, †P<0.014 as compared to control, ‡P=0.008 as compared to control, ¶P=0.002 as compared to control, ††P=0.004 as compared to control, †‡P=0.009 as compared to control, ‡‡P=0.012 as compared to control, ‡‡‡P=0.001 as compared to diazepam, ¶¶P=0.038 as compared to diazepam, †††P<0.001 as compared to CS33, ††‡P=0.002 as compared to CS33. CS=Camellia sinensis, SEM=Standard error of mean

Time spent (P = 0.038) in the bright arena compared to the diazepam-treated group. Diazepam-treated rats showed significant increase in the duration of immobility (P < 0.001) compared to control. CS-treated rats at all the three doses (3.3, 16.5, and 33 mg/kg) significantly decreased immobility compared to control (P = 0.004, P = 0.009, and P = 0.012, respectively) and diazepam-treated group (P < 0.001).

Coadministration of flumazenil and bicuculline reduced the number of entries (P < 0.001), time spent (P < 0.001), and rears (P < 0.001) in the bright arena compared to CS at 33 mg/kg alone. Picrotoxin coadministration reduced the number of entries (P = 0.002) into the bright arena but had no effect on the time spent and rears in the bright arena. None of the antagonists had any effect on immobility.

In the open field paradigm [Table 3], both diazepam and higher doses of CS (16.5 and 33 mg/kg) significantly increased the number of squares crossed (P < 0.001), rears (P < 0.001), and time spent (P < 0.001) in the central squares compared to the control group. CS at 33 mg/kg also significantly increased the time spent (P < 0.001) and number of rears (P = 0.001) in the central squares compared to the diazepam-treated group. Treatment with diazepam and CS at 33 mg/kg significantly reduced the total number of squares crossed (P < 0.001) compared to control. CS at 33 mg/kg showed significantly higher open arm entries compared to CS at 3.3 mg/kg (P < 0.001). Time spent in the open arms was significantly higher in the CS at 33 mg/kg treated group compared to CS at 16.5 and 3.3 mg/kg (P < 0.001). Time spent in the open arms was also significantly higher in the CS at 16.5 mg/kg treated group compared to 3.3 mg/kg (P = 0.008).

Coadministration of flumazenil, bicuculline, and picrotoxin significantly reduced the number of squares crossed (P < 0.001), rears (P < 0.001), and time spent (P < 0.001) in the central squares compared to the CS 33 mg/kg treated group. All three antagonists also significantly increased the total number of squares crossed compared to the CS 33 mg/kg treated group (P < 0.001).

Chronic Study
In the EPM [Table 4], diazepam and CS at the dose of 16.5 and 33 mg/kg showed a significant increase in the entries (P = 0.001, P = 0.012, P < 0.001, respectively), time spent (P < 0.001) and rears (P = 0.026, P = 0.04, P < 0.001, respectively) in the open arm compared to the control group. CS at the dose of 33 mg/kg also significantly increased the number of entries (P = 0.017) and time spent (P < 0.001) in the open arm compared to the diazepam-treated group.

In the LDB [Table 5], diazepam and CS at all the three doses significantly increased the number of entries, time spent, and IL.
Shastry, et al.: Anxiolytic activity of Camellia sinensis

### Table 3: Effects of drugs on behavior of rats in open field paradigm on single dose administration (acute study)

| Treatment (mg/kg) | Number of central squares crossed | Total number of squares crossed | Time spent in central squares (sec) | Number of rears in central squares |
|-------------------|----------------------------------|--------------------------------|------------------------------------|-----------------------------------|
| Control           | 2.17±0.65                        | 56.17±6.62                    | 11.67±3.23                        | 0.50±0.22                         |
| Diazepam 1 mg/kg  | 15.83±1.79                       | 32.67±2.01                    | 142.67±6.64                       | 7.83±0.91                         |
| CS                 |                                  |                                |                                    |                                   |
| 3.3               | 4.83±0.30                        | 68.83±1.81                    | 16.83±1.55                        | 2.0±0.36                          |
| 16.5              | 11.67±0.95                       | 53.17±1.51                    | 86.67±1.66                        | 6.66±0.49                         |
| 33                | 17.33±0.71                       | 33±0.57                      | 165.83±1.97                       | 10.67±0.49                        |
| Flumazenil + CS 33 mg/kg | 3.17±0.47                       | 55.33±5.42                    | 13.67±2.94                        | 1.33±0.42                         |
| Bicuculline + CS 33 mg/kg | 3.50±0.42                       | 75.33±3.16                    | 16±3.44                           | 1.67±0.76                         |
| Picrotoxin + CS 33 mg/kg | 3.3±0.66                       | 66.33±3.40                    | 91.67±14.08                       | 3.50±0.76                         |
| **F**             | 43.010                           | 28.278                       | 390.099                           | 58.954                            |
| **df**            | 4                                |                               |                                    |                                   |

Values are mean±SEM. *P<0.001 as compared to control, †P<0.001 as compared to diazepam, ‡P<0.001 as compared to CS33. CS=Camellia sinensis, SEM=Standard error of mean.

### Table 4: Effect of diazepam and Camellia sinensis on behavior of rats in elevated plus maze on administration for 14 days (chronic study)

| Treatment     | Number of open arm entries | Total number of arm entries | Time spent in open arms (sec) | Time spent in closed arms (sec) | Number of rears in open arms |
|---------------|-----------------------------|----------------------------|--------------------------------|--------------------------------|-------------------------------|
| Control       | 4.83±0.31                   | 9.50±0.72                  | 76.33±4.30                    | 222±4.73                       | 7.67±1.02                     |
| Diazepam 1 mg/kg | 8.67±0.55*                  | 11.33±0.61                | 211.83±9.0*                   | 88.17±9.0*                     | 11.83±1.39                    |
| CS (mg/kg)    |                             |                            |                                |                                |                               |
| 3.3           | 4.50±0.34                   | 12.17±0.47*               | 87.50±1.14                    | 215.17±1.9                     | 6.5±1.08                      |
| 16.5          | 7.50±0.56*                  | 10±0.73                   | 144.33±1.74*                  | 155.67±1.74*                   | 11.33±0.8*                    |
| 33            | 11.17±1.24†                 | 18.33±1.20†               | 250.33±0.84†                  | 49.67±0.84†                    | 14.83±1.78†                   |
| **F**         | 16.03                       | 20.354                    | 276.89                        | 262.589                        | 7.258                         |
| **df**        | 4                           |                            |                                |                                |                               |

Values are mean±SEM. *P<0.001 as compared to control, †P=0.012 as compared to control, ‡P<0.001 as compared to control, §P=0.017 as compared to diazepam, ¶P=0.024 as compared to control and diazepam, ‡P=0.002 as compared to control, ¶P=0.048 as compared to control. CS=Camellia sinensis, SEM=Standard error of mean.

### Table 5: Effect of diazepam and Camellia sinensis on behavior of rats in light and dark box on administration for 14 days (chronic study)

| Treatment            | Number of entries in bright arena | Time spent in bright arena (sec) | Rears in bright arena | Rears in dark arena | Immobility (sec) |
|----------------------|----------------------------------|---------------------------------|----------------------|--------------------|------------------|
| Control              | 1.0±0.00                         | 7.50±1.05                      | 0.33±0.21            | 5.17±0.47         | 1.17±3.12        |
| Diazepam 1 mg/kg     | 3.17±0.31*                      | 25.17±2.01*                    | 3.00±0.77*           | 13.00±1.31*       | 24.50±2.89*      |
| CS (mg/kg)           |                                  |                                |                      |                    |                  |
| 3.3                  | 2.33±0.21*                      | 16.33±0.76*                    | 1.50±0.22*           | 2.50±0.22         | 4.00±2.64*       |
| 16.5                 | 3.00±0.26*                      | 22.33±1.11*                    | 2.33±0.21*           | 2.67±0.21         | 0.67±0.66*       |
| 33                   | 4.83±0.31*                      | 30.00±1.07*                    | 3.50±0.22*           | 3.50±0.22         | 0.83±0.83*       |
| **F**                | 32.69                           | 46.524                        | 9.982                | 45.891            | 18.729           |
| **df**               | 4                               |                                |                      |                    |                  |

Values are mean±SEM. *P<0.001 as compared to control, †P=0.001 as compared to control, ‡P<0.001 as compared to diazepam, §P=0.013 as compared to diazepam, ¶P=0.048 as compared to control. CS=Camellia sinensis, SEM=Standard error of mean.

Rears in the bright arena compared to control. Moreover, CS at the dose of 33 mg/kg significantly increased the number of entries (P < 0.001) and time spent (P = 0.013) in the bright arena compared to the diazepam-treated group. The diazepam-treated rats showed significant increase in duration of immobility compared to control. CS-treated groups in all the three doses (3.3, 16.5, and 33 mg/kg) significantly reduced the immobility (P < 0.001) compared to the diazepam-treated groups. Number of entries to the bright arena was significantly higher in the CS at 16.5 and 33 mg/kg treated groups compared to CS at 3.3 mg/kg (P = 0.03, <0.001). Number of entries to the bright arena was also significantly higher in the CS 16.5 mg/kg compared to CS at 33 mg/kg treated groups (P < 0.001). Time spent in the bright arena was significantly higher in the CS 33 mg/kg treated group compared to CS 3.3 at mg/kg and 16.5 mg/kg treated groups (P < 0.001).

In the open field paradigm Table 6, both diazepam and higher doses of CS (16.5 and 33 mg/kg) significantly increased the number of squares crossed (P < 0.001), rears (P < 0.001, P < 0.001, and P = 0.014, respectively), and time spent (P < 0.001) in the central squares compared to the control group. CS at 33 mg/kg also significantly increased the number of squares crossed (P = 0.001) and time spent (P < 0.001) in the central squares compared to the diazepam-treated group. Treatment with diazepam and CS at 33 mg/kg significantly reduced
the total number of squares crossed compared to control. However, the total number of squares crossed in the CS at 3.3 and 16.5 mg/kg treated groups was significantly more than the diazepam-treated group (P < 0.001).

**Discussion**

In the present study, anxiolytic effects of CS have been studied in three experimental models of anxiety – EPM, LDB, and open field paradigm. These models are based on the fact that an unfamiliar, brightly lit, and open environment induces stress which provokes anxiety, thereby inhibiting normal behavior in rats.[17]

In the EPM, animals find the open arms to be unprotective and more anxiogenic than the closed arms. Animal hence prefers to spend more time and shows normal rearing behavior in the closed arm. Anxiolytic drugs increase the number of entries, time spent, and rearing in the open arms.[16] Following both acute and chronic treatment, CS in all three doses increased the number of entries, time spent, and rearing in the open arms compared to control. There was no difference between the acute and chronically treated groups. Behavioral changes were comparable to diazepam and CS at 33 mg/kg increased the time spent and rears in open arm more than diazepam-treated group. This suggests that anxiolytic effect of CS was comparable to diazepam at low doses and better than diazepam at a higher dose. A dose-dependent anxiolytic action was also observed in this model.

In the LDB, rodents tend to avoid entry into and reduce spontaneous exploratory behavior in the brightly illuminated area; a natural tendency when a rat is exposed to an unfamiliar environment.[13] Anxiolytics tend to increase the number of entries, time spent, and rears in the bright arena. In the present study, CS, at different doses on both acute and chronic therapy, increased the number of entries, time spent, and rears in the bright arena compared to control indicating anxiolytic effect. There was no difference in the acute and chronically treated groups. A better anxiolytic effect of CS than that of diazepam was observed at a higher dose. A dose-dependent anxiolytic effect was observed in this model also.

The open field paradigm is used with the dual purpose of assessing not only the anxiolytic activity but also the locomotor activity in rodents.[17] After both acute and chronic treatments, CS at 16.5 and 33 mg/kg showed a significant increase in the number of squares crossed, time spent, and the number of rears in the central squares compared to control. At 33 mg/kg, a better anxiolytic effect than that of diazepam was also observed. Treatment with diazepam reduced the locomotor activity. This was observed at CS 33 mg/kg but not at 16.5 mg/kg. Hence, CS at 16.5 mg/kg showed anxiolytic activity without affecting the locomotor activity.

An earlier study by Heese et al.[18] using L-theanine which is one of the components of CS did not show anxiolytic activity when used alone but showed anxiolytic activity in combination with midazolam. However, anxiolytic effect of CS, observed in the present study, suggests the presence of other components in CS extract which may be responsible for anxiolytic effect. Our findings are similar to the study done by Tabassum et al.[19] who found that CS increased number of entries and time spent in the open arm in EPM and increased ambulation in OFT; however, the dose (100 mg/kg) used was much higher than in our study (33 mg/kg). Indian traditional medicine too is a proponent of the idea that whole parts of plants are better than individual constituents since the combined synergistic action of constituents produces final beneficial effect.

Anxiolitics such as benzodiazepines act through the GABA<sub>A</sub>-benzodiazepine receptor – Cl<sup>-</sup> channel complex. CS also appears to mediate its anxiolytic action predominantly through this complex since flumazenil and bicuculline completely inhibited while picrotoxin partially inhibited the anxiolytic effect of CS. Flumazenil, bicuculline, and picrotoxin pretreatment also antagonized the effect of CS on locomotor activity. Both these effects appear to be mediated through the GABA<sub>A</sub>-benzodiazepine receptor – Cl<sup>-</sup> channel complex. Further studies are required to confirm site of action of CS.

Foodstuffs can be considered as functional if it satisfactorily demonstrates functional action on target functions in the body. Tea is the most consumed beverage in the world. Beneficial health effects of green tea are increasingly proved, and it should be considered in the group of beverages with functional properties. Anxiolytic effect of CS extract needs further evaluation in large-scale studies.

**Conclusion**

CS has dose-dependent anxiolytic potential, likely mediated through the GABA<sub>A</sub>-benzodiazepine receptor – Cl<sup>-</sup> channel complex. Anxiolytic effect is comparable to diazepam and needs evaluation in further studies.
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Conflicts of Interest
There are no conflicts of interest.

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