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Original article

Neuropharmacological potential of various morphological parts of *Camellia sinensis* L.

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ABSTRACT

Camellia sinensis L. has long been used as a therapeutic agent for the Central nervous system (CNS) due to the presence of flavonoids. The present study aimed to evaluate the dose-dependent Neuropharmacological behavioral potential of *Camellia sinensis* seed and leaf extracts on mice. To evaluate the differential potential of leaf and seed extract various doses were prepared and examined in open field, head dip, rearing, cage cross, swimming and traction tests. One-way ANOVA set at $P^* < 0.05$ followed by POST HOC LSD ($P^* < 0.01$) was applied to evaluate the significant difference among the treatments. Herein both seed and leaf extract showed significant results at high doses. Interestingly leaf extract at high dose showed significant effect on mice CNS in open field and head dip test, while seed at high dose revealed significant stimulus on mice CNS in rearing, cage cross, swimming and traction tests. Overall results showed that seed produced more stimulant effect and less calmness as compared to leaf extract was. Tea leaves had already known as potential CNS stimulant drugs; current investigation suggests that tea seed can be used as an alternative CNS stimulant agent with more effective stimulant action.

1. Introduction

In psychiatric disorders, depression is one of the most common and foremost reasons for long-lasting disability all over the world. Antidepressant drugs commonly available in the market but most of them are either not completely effective or linked with severe adverse effects. Currently available data suggest that nutritional supplements are rich in significant phytochemicals which produce a valuable therapeutic effect in depression. Depression is among the most frequent neuropsychiatric disorder which counts about 40.5% of disabilities in the world (Whiteford et al., 2013). About 20% of the population experience the main depressive episodes like symptoms during their life span. There is an increasing trend in these symptoms as they grow old, and produce several bad effects on their family, social and professional life (Musick and Wilson 2003). It can be classified as the most commonly occurring neuropsychiatric disorder like stroke (Fazel Nabavi et al., 2015). The

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World Health Organization Global Burden of Disease states that in the year 2020 it became next reason for long term disability and the primary cause in the year 2030. Moreover, antidepressant drugs demand a longer time span for treatment with a lesser effect on the depressant symptoms of the patients (Fazel Nabavi et al., 2015). Regular use of antidepressant drugs has been also reported with several adverse effects of drugs like faintness, tremor, mental destruction, sexual dysfunction and urinary retention (Predictable, 2006) However some traditional therapies like acupuncture, exercise and the role of nutritional supplements on depressant like symptoms are developing new hope in the current scenarios (Nabavi et al., 2017).

More to the point, as mentioned before that many neurological and psychiatric disorders are related to oxidative stress which results due to an imbalance between the production of reactive oxygen species and antioxidants. Progressively more antidepressant treatments have been developed with the rising frequency of the many neurological and psychiatric diseases, but unluckily only a few have been shown to be successful. Seventy percent of the patients do not attain full reduction and 30% of the patients do not succeed to reach the current drug treatment (Kulkarni et al., 2009). Due to the increased risk of side effects associated with drug therapies patients became disappointed, and natural products have gained a promising role to reduce the mental disturbances.

There is a gradual rise in the occurrence of psychiatric and Neuropharmacological diseases. A lot of research work has been performed particularly in the field of brain-related ailments; now significant work has been performed to study how the routine life factors as diet plus exercise can affect mental health. Recent research proves that diet and exercise can play a vital role in the development and maintenance of neurons and can protect the brain from degenerative illness or diseases. There is a connection between everyday life and mental health which encourages the researchers to find out the possible use of the dietary nutrients and their predictable therapeutic effects which can boost the mental health and neurological functions. In contrast to various conventional managements, polyphenols are involved in a wide range of systems in the brain that could help in preserving the psychological and mental health, along with improvement in Neuropharmacological ailments.

Dietary polyphenols are the powerful source of the innate, cheap and non-invasive curative source to carry a healthy brain (Gomez-Pinilla and Nguyen, 2012). Polyphenols which are present in food originated from plant sources like vegetables plus fruits have gained unusual interest due to their wide range of action on brain-specific diseases. Polyphenols are linked with a brain-related neurotrophic factor that is useful in treating the neuronal atrophy and behavior discrepancy (Wollen, 2010).

Green tea has polyphenols like epigallocatechin gallate played an important role in better mental health, enhanced mood, plus protective properties against brain-specific diseases (Camfield et al., 2014). Green tea catechins play an important role in reducing the symptoms of depression in animal model possibly by the inhibition of monoamine oxidase (MAO) (Nabavi et al., 2017). According to the available data, polyphenols are gaining fame quickly due to their effective role not only in brain defense and development but also has neuroprotective effects.

This study was aimed to evaluate the neuropharmacological potential of *Camellia sinensis* leaves and seeds on the animal models. A restrained amount of scientific work as neuropharmacological aspects of locally occurring species of this family has done so far. *C. sinensis* lacks such studies in Pakistan therefore, National Tea and High-Value Crops Research Institute (NTHRI) was established in 1986 at Shinkayari, District Mansehra Pakistan and the sample were collected from here. Tea is utilized as a common beverage and Pakistan occupies 2nd position in global tea consumption. Diet and lifestyle are progressively becoming recognized for their relationship with healthy aging and up keeping of cognitive function. The main purpose of this research is to determine its cognitive action because of our best knowledge no other studies have been investigated before.

2. Materials and methods

Camellia sinensis was collected for determination of its neuropharmacological potential from National Tea and High-Value Crops Research Institute Shinkiari, Mansehra, Pakistan in the month of March 2018. The plant was identified and confirmed with the available literature flora of Pakistan as followed by (Ashfaq et al., 2019a; Bahadur et al., 2018a; Zaman et al., 2019). The voucher specimens were dried, preserved, mounted on herbarium sheets and was deposited to the pharmacognosy museum, Department of Pharmacognosy, University of Karachi following the published protocol (Bahadur et al., 2019; Bahadur et al., 2018b) Seeds and leaves were collected in different time periods depending upon the maximum quantity of active ingredients present in them. As they have a maximum amount of active ingredients in different time periods, leaves were collected in March, while seeds were collected in December. They were then kept separately in shade for 15 days. After drying the different parts of plants were pulverized to a fine powder separately and each powder was passed through sieve # 120. The fine powdered plant material was stored in amber color bottles and preserved at ambient temperature and pressure conditions.

2.1. Preparation of green tea leaves

The esteemed laboratory of National Tea and High-Value Crops Research Institute and advanced equipment were used for the preparation of green tea. Green tea is also called unfermented tea, as during processing of tea leaves the active constituents to remain unchanged. The steps involved in the processing of green tea were as follows.

2.2. Plucking

Plucking of tea leaves was carried out in the month of March. During plucking fresh green young leaflets up to 3–8 were collected either manually or by manual cutter machines. The best months for the plucking of the green tea leaves are March to November in Pakistan.

2.3. De- enzyming of the fresh leaves

A prominent feature of green tea processing was to protect the leaf from fermentation. A De-Enzymer was used for this purpose. It consisted of the roller, frame, and transmission.

2.4. Tea roller, demissing and sieving machine

After the de-enzyme process, leaves were rolled to make a slender pickle form. The rolling processes interrupted the leave tissues and blended them in a uniform shape. The rolling period is about 20–30 min. A machine was used for the separation of large-sized leaves from the small ones by shaking the sieves. It consisted of a tea bucket, chamber, transmission part, and sieves.

2.5. Tea dryer

Tea leaves were dried by exposing the moist particles to a stream of hot air. The dryer comprised of a feed-in system, drying tank, driving device shaking discharging mechanism, and an air heating furnace. The source of hot air was coal. The hot air from the furnace enters into the drying tank to dry the tea leaves.

Further heating was given to the leaves and dried directly on hot pan and twist under the pressing and rolling by the curling hand.

2.6. Extraction procedure for leaves

The 60 g of *Camellia sinensis* leaves were crushed into powder form and are passed through the sieve number 120 and soaked for three days in 1000 ml of methanol, occasional shaking was done. Filtration was carried out using Whatman filter no 1. Rotary vacuum evaporator (Rotavapor R-200, Buchi) was used for evaporation of filtrate at temperature 50 °C with rotation 40 rpm and pressure 0.09MPA. The dried material was weighted, labeled and stored in the refrigerator at 4 °C. These condense extracts were then used for the determination of neuropharmacological activity.

2.7. Extraction procedure for seed:

Seeds were collected in December. They were then kept in shade for 15 days. When they were sufficiently dried, pulverized to a fine powder and were passed through sieve # 70. The powdered seeds were stored in amber color bottles and preserved at ambient temperature and pressure conditions. This seed powder is used for the oil extraction procedure.

2.8. Oil extraction

The Soxhlet extraction procedure was used for oil extraction (Wang et al., 2011). Ten grams of the tea seeds sample were weighed and were put in the extraction thimble while 300 ml of petroleum ether was used as a solvent. According to the post-extraction practice, oil was extracted persistently for 8 h at temperature 60–80 °C. The solvent was then evaporated by drying the extract at 103 °C in order to remove the remaining solvent. It was then cooled in the desiccator for 30 min and then weighed. The triplicates of this experimentation were conducted.

2.9. Animals

Albino mice of both sexes were used as test animals of 20-22 g weight as test animals in order to conduct the experiments. All these animals were purchased from Dow University Karachi. Standard laboratory conditions were maintained as 25 °C temperature; light and dark cycles of 12 h were maintained. Animals were provided with standard food and water ad libitum. The experimental procedure was approved by Karachi University.

In this study 24 mice were used for activity and were divided randomly into four equal groups as follows:

1. The control group without receiving any medication.
2. The treated group receiving a low dose of medication (seed and leaf) 300 mg/kg.
3. The treated group receiving a high dose of medication (seed and leaf) 600 mg/kg.
4. The group receiving standard medicine Caffeine (10 mg/kg).

2.10. Neuropharmacological activity

Various tests were used to access the neuropharmacological activity like head dip, open field, rearing, cage cross, forced swimming and traction test. A peaceful environment was required to perform these tests. For every test, animals were divided into four groups, each group comprised of 6 mice. For the standard values, Caffeine was used at a dose of 10 mg per kg. The standard drugs were also administered by the oral route. Just like the crude extract, the same volume of the saline was administered to the control animals. Readings were observed right after 30 min as the test substance administered orally (Riaz et al., 2014).

2.11. Head dip test

For this study, a specially designed box was used which was square-shaped and had three holes on either side. For a specified period of time the number of head dip through these holes was calculated (Kennett et al., 1985; Riaz et al., 2014). Readings were noted for 30 min both for the controlled, treated and standard mice. This test is used to evaluate the CNS stimulant effect.

2.12. Open field test

The apparatus intended to assess the open field activity in the mice was comprised of 76 × 76 cm square areas with a dense wall of about 42 cm height. As discussed earlier this experiment was performed in a peaceful room (Kennett et al., 1985; Riaz et al., 2014). In a sequential manner, each mice was taken from its cage and placed in the middle of the open field. Now very carefully the numbers of squares crossed by the mice were calculated for 30 min. The same experiment was performed on standard, treated drug mice and on the controlled group mice at the same time. After the exposure of the mice, the apparatus was moped with 10% alcohol. CNS stimulant effect can be evaluated by this test.

2.13. Rearing test

For this study, a 1L beaker was used which was covered with white paper at the bottom. Mice were placed in beaker and numbers of upward movement of the mice were calculated while keeping the body in erect position (Wyttenbach et al., 1998; Zia et al., 2013). This test was used to evaluate the CNS effect of the drug.

2.14. Cage cross test

This test was also used to evaluate the CNS effect of the drug. An especially designed rectangular shape apparatus was used. Individual movement of both treated, standard and (Sanchez-Mateo et al., 2002; Riaz et al., 2014) controlled mice for 30 min was noted and their comparative difference was evaluated (Sakina and Dandiya 1990; Crestani et al., 2000).

2.15. Forced swimming test

Forced swimming test was to determine the CNS depressant activity. A swimming water bath was used with dimensions like 45 × 19 × 19 cm (Sanchez-Mateo et al., 2002; Riaz et al., 2014). The water glass bath was filled with water up to the marked level with water at room temperature of about 25 ± 2 °C. Before evaluating the forced swimming test all mice were trained for swimming. One by one all mice were placed in a water bath for swimming for 6 min in order to train them. The swift movement of the front and hind paws of the mice was beginning as they were placed in the water. With the help of a stopwatch, their activity

time was noted throughout their total of 6 min. When they stopped movements or showed minimum activity just to keep them floating this time was noted and it was recorded as an immobile phase. In order to assess the depression this test is mainly used.

2.16. Traction test

A one-meter rod was used for this test and animal was trained to travel on this rod. Time taken by the animal to move from one end of the rod to another end was noted. Time spends by both controlled mice and drug-treated mice was measured and compared. The increase or decrease in the time taken by the mice to travel the rod indicates the stimulant or sedative effect of the drug (Sanchez-Mateo et al., 2002; Chattopadhyay et al., 2003).

2.17. Statistical analysis

The results were expressed like the mean ± standard error of the mean (SEM). One-way analysis of variance (ANOVA) was used for comparison tests of significant differences among groups, followed by POST HOC LSD. After obtaining the information from different groups, the results were analyzed at ($P < 0.05$) as an indicator of significance. SPSS software was used for data analysis.

3. Results

3.1. CNS stimulant effect

Exploratory effects of the mice were evaluated by the head dip, open field, rearing, and cage cross tests. Results were compared with the negative control group, in which mice were taking no drugs and the positive control group in which mice were taking the standard drug Caffeine i.e. 2 mg/kg. Results were presented the mean observations ±SEM in their respective Tables.

In control group head dip values for seed and leaf extracts was 15.67±0.67, an increased motor function was observed for both seed and leaf extracts for dose 300 mg/kg and 600 mg/kg with head dip value i.e. 28.17±2.06, 40.5±2.4 and 55.33±0.88, 82.83±2.21 respectively. While the standard dose of Caffeine i.e., 2 mg/kg showed the values 31.33±0.49 for a head dip. Here the low dose of leaf extract is giving significant results than the standard while seed at high dose gives the significant result as shown in Table 1 and Fig. 1.

3.2. Open field

In open field test control group values for seed and leaf extracts was 124±1.06, while for doses 300 mg/kg and 600 mg/kg of seed and leaf extract motor function was increased in dose-dependent manners values were 206.83±17.76, 259±2.28 and 288±3.4, 348±4.43. Here, the standard dose of Caffeine i.e., 2 mg/kg showed the result, 213.83±0.79 for the open field. The leaf extract was giving significant results even at a low dose when compared with the standard while seed at high dose gives the significant result as shown in Table 2 and Fig. 2.

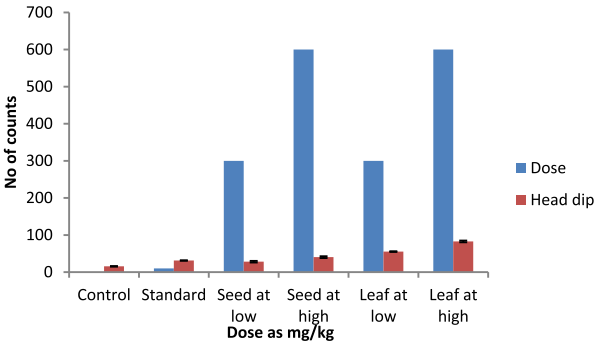

Fig. 1. Showing Head dip effect seed and leaf extract.

3.3. Rearing

In rearing test control group values for seed and leaf extracts was 33±1.59, while for doses 300 mg/kg and 600 mg/kg motor activity was increased in case of seed extract in dose-dependent function while leaf extracts showed different response as shown by values 49.67±1.96, 61.83±1.70 and 27.67±0.49, 13.67±0.76. While the standard dose of Caffeine i.e., 2 mg/kg showed the values 45.33±0.42 for rearing as shown in Table 3 and Fig. 3.

Here in case of rearing for seed extract low, high and standard drugs give significant and effective results when compared with control. For leaf extract, both control and treated groups give insignificant results, which showed that seed extract produced a more effective response for rearing as compared with leaf extract.

3.4. Cage cross

In the cage, cross-test control group values for seed and leaf extracts was 33.83 ± 1.82, while for doses 300 mg/kg and 600 mg/kg of seed and leaf extract cage cross-test results were 54.67±1.36, 64.33±0.71 and 35±0.97, 40±1.18 noted which showed the dose-dependent increase in motor function. While the standard dose of Caffeine i.e., 2 mg/kg showed the values 55.33±0.88 for cage cross. Here only seed at higher dose gives comparatively good results when compared with standard as shown in Table 4 and Fig. 4.

3.5. Antidepressant effect

Mobility time was recorded in the forced swimming test to evaluate it's increased or decreased struggling or carefree activity. Results were presented the mean observations ±SEM.

Table 1
Head dip effect of seed and leaf extract.

No of Obs	Groups	Dose	Mean + SEM
1	Control	–	15.67±0.67
2	Standard	10 mg/kg	31.33 ± 0.49*
3	Seed at low dose	300 mg/kg	28.17 ± 2.06
4	Seed at high dose	600 mg/kg	40.50 ± 2.40*
5	Leaf at low dose	300 mg/kg	55.33 ± 0.88*
6	Leaf at high dose	600 mg/kg	82.83 ± 2.21**

These values are the mean of observation obtained from 6mice (n = 6) ± SEM.
Significant at P* < 0.05.
Significant at P** < 0.01.

Table 2
Open field effect of seed and leaf extracts.

No of Obs	Groups	Dose	Mean + SEM
1	Control	–	124.00 ± 1.06
2	Standard	10 mg/kg	213.83 ± 0.79*
3	Seed at low dose	300 mg/kg	206.83 ± 17.76*
4	Seed at high dose	600 mg/kg	259.67 ± 2.28**
5	Leaf at low dose	300 mg/kg	288.00 ± 3.40*
6	Leaf at high dose	600 mg/kg	348.00 ± 4.43**

These values are the mean of observation obtained from 6mice (n = 6) ± SEM.
Significant at P* < 0.05.
Significant at P** < 0.01.

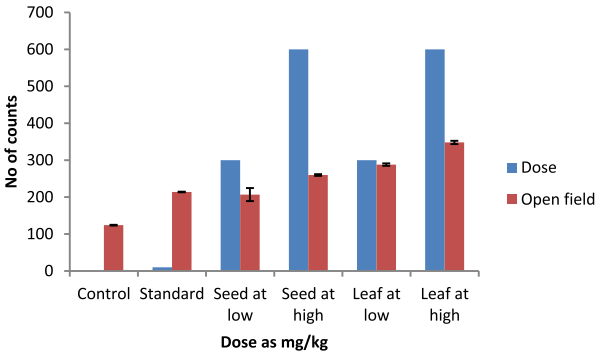

Fig. 2. Showing Open field effect seed and leaf extract.

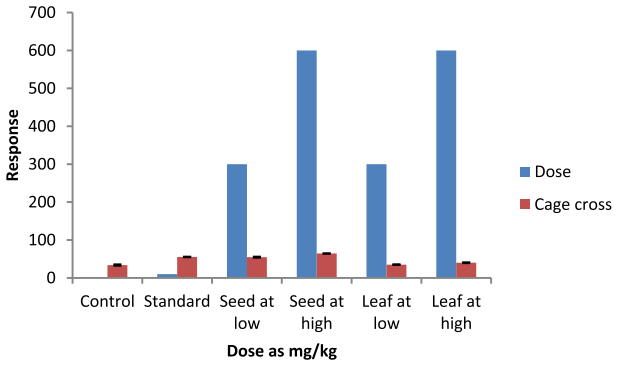

Fig. 4. Showing Cage cross effect of seed and leaf extracts.

Table 3
Rearing effect of seed and leaf extracts.

No of Obs	Groups	Dose	Mean + SEM
1	Control	–	33.00 ± 1.59
2	Standard	10 mg/kg	45.33 ± 0.42*
3	Seed at low dose	300 mg/kg	49.67 ± 1.96*
4	Seed at high dose	600 mg/kg	61.83 ± 1.70**
5	Leaf at low dose	300 mg/kg	27.67 ± 0.49
6	Leaf at high dose	600 mg/kg	13.67 ± 0.76

These values are the mean of observation obtained from 6mice (n = 6) ± SEM.
Significant at P* < 0.05.
Significant at P** < 0.01.

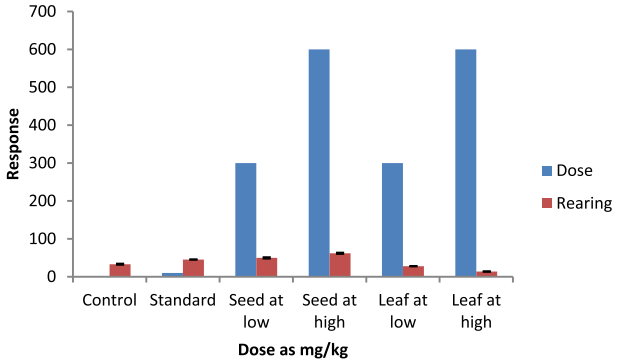

Fig. 3. Showing Rearing effect of seed and leaf extracts.

Table 4
Cage cross effect of seed and leaf extracts.

No of Obs	Groups	Dose	Mean + SEM
1	Control	–	33.83 ± 1.82
2	Standard	10 mg/kg	55.33 ± 0.88*
3	Seed at low dose	300 mg/kg	54.67 ± 1.36*
4	Seed at high dose	600 mg/kg	64.33 ± 0.71**
5	Leaf at low dose	300 mg/kg	35.00 ± 0.97
6	Leaf at high dose	600 mg/kg	40.00 ± 1.18

These values are the mean of observation obtained from 6mice (n = 6) ± SEM.
Significant at P* < 0.05.
Significant at P** < 0.01.

Mobility time in forced swimming test in seconds was recorded for control group values for seed and leaf extracts was 266.17 ±4.75, while for doses 300 mg/kg and 600 mg/kg forced swimming test results were 312 ±2.38, 347.33 ±1.87 and 315.83 ±3.68, 335.33 ±1.50 noted which showed the dose-dependent increase in mobility time. While the standard dose of Caffeine i.e., 2 mg/kg showed the values of 314±0.96 for swimming tests. Here both seed and leaf at high dose give better results. An increased in the mobility time showed the corresponding increased swimming and struggling effect as shown in Table 5 and Fig. 5.

3.6. Stimulant effect

Motor coordination activity was evaluated with the help of a traction test. Mice were first trained to cross the rod, then the time taken for crossing the rod in seconds and the number of falls was observed. The evaluation was established by comparing the control, treated and standard drugs. Results were presented the mean observations ±SEM.

Traction test for control group values for seed and leaf extracts was 265.33±10.90, while for doses 300 mg/kg and 600 mg/kg the results were 315±9.21, 385±0.29 and 319±1.83, 355.5±8.76. While the standard dose of Caffeine i.e., 2 mg/kg showed the values 310±0.56 for the traction test. Here both seed and leaf extract at high dose level are giving a significant result. This fact can be seen with POST HOC LSD as shown in Table 6 and Fig. 6.

4. Discussion

For neuropharmacological evaluation different doses of seed and leaf extracts were fed to mice and then their behavior for different activities was noted, previous studies prove that head dip test can evaluate the anxiolytic behavior of the animal model. Here the increased number of head dip activity showed the anxiolytic state of the animal (Doukkali et al., 2015). The results showed that it is a predictive parameter that measures the motor function of the animal model after the ingestion of seed and leaf extracts, and results are compared with control and standard group. Here increased numbers of count expect the increased number of motor functions. The results showed that seed extract showed significant effect at high dose at P*< 0.05 while leaf extract at high dose showed highly significant at P**< 0.01.

Table 5
Forced swimming effect of seed and leaf extracts.

No of Obs	Groups	Dose	Mean + SEM
1	Control	–	266.17 ± 4.75
2	Standard	10 mg/kg	314.50 ± 0.96
3	Seed at low dose	300 mg/kg	312.00 ± 2.38
4	Seed at high dose	600 mg/kg	347.33 ± 1.87**
5	Leaf at low dose	300 mg/kg	315.83 ± 3.68*
6	Leaf at high dose	600 mg/kg	335.33 ± 1.50**

These values are the mean of observation obtained from 6mice (n = 6) ± SEM.
Significant at P* < 0.05.
Significant at P** < 0.01.

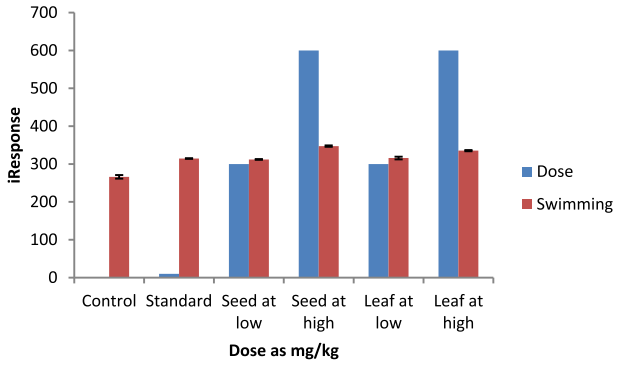

Fig. 5. Showing Forced swimming effect of seed and leaf extracts.

Table 6
Traction test effect of seed and leaf extracts.

No of Obs	Groups	Dose	Mean + SEM
1	Control	–	265.33 ± 10.09
2	Standard	10 mg/kg	310.50 ± 0.56*
3	Seed at low dose	300 mg/kg	315.67 ± 9.21*
4	Seed at high dose	600 mg/kg	385.67 ± 9.21**
5	Leaf at low dose	300 mg/kg	319.00 ± 1.83*
6	Leaf at high dose	600 mg/kg	355.50 ± 8.76**

These values are the mean of observation obtained from 6mice (n = 6) ± SEM.
Significant at P* < 0.05.
Significant at P** < 0.01.

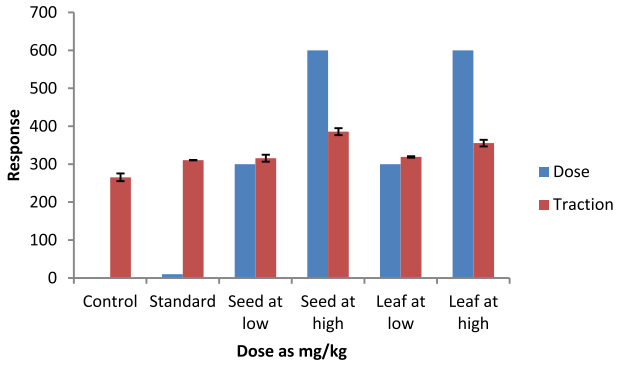

Fig. 6. Showing Traction test effect of seed and leaf extracts.

While in the case of open field and cage cross the corresponding increments of motor function in the animal model after ingestion of both seed and leaf extracts, are in the same dose-dependent manners. Here again, highly significant results are obtained at P**< 0.01 by leaf extract, at the high dose.

The open field is the best test to separate the individual on the basis of their slow and fast exploration behavior. It is the best possible tool to assess the personality of the animal. It is also used to evaluate the locomotor and stimulant effect of the mice. It is also helpful for predicting the behavior of the animal in stressful situations.

Locomotion tests and rearing tests are conventionally used to measure learning in the open field. The locomotor activity can be used as a parameter to assess alertness or sadness. Increase locomotor activity can be associated with alertness and vice versa. The locomotor activity of mice can be predicted by the Open field test. Seed and leaf extracts showed increased stimulant effect, however the comparative study showed that leaf extract has more stimulant effect as compared to the seed extract

Exploration in the animal model was defined as sniffing the object with the help of nose or touching the object with the help of forepaws, grooming or turning around the object was not considered in the definition. Rearing activity can be assessed by exploration.

In the case of seed extract there is significant effect in rearing and upward movement of mice, while in the case of seed and leaf extracts, as the dose increased there was gradual decrease in the rearing which showed that seed and leaf extracts, produced calmness at higher dose and seed extract produced more stimulant effect and less calmness.

Rearing test results are quite different when compared with the control and standard group. In the case of seed, there is a gradual increment of stimulant effect, but leaf proved contrasting effect that is less rearing and more calmness when compared with control and standard group. The seed extract showed significant results as stimulant, while leaf, showed highly significant results as producing more calmness and mode moderation at high dose at P**< 0.01.

A cage cross-test is used to evaluate locomotion. Here seed showed highly significant results at P**< 0.01 results at high dose when compared with the same dose of leaf extract, which showed that seed extract produced a more anxiolytic effect than leaf extract. Cage cross and rearing tests are the predictive tools for the anxiolytic effects of the drugs. Significant results are obtained at high dose of seed extract, while leaf extract at both dose level did not give the significant results when compared with standard, this result can be interpreted differently that as we increase the dose of leaf extract it produces more calmness and mode moderation in animal model in contrast to the stimulant effect.

These results suggest that seed extract at high dose produced more stimulant effect while leaf extract at high dose produced more calmness and mode moderation. Hence different plant parts of the same plant produce different effects. Seed extract produces a powerful stimulant effect while leaf extract produces a powerful mental calming effect (Singal et al., 2004).

Mobility time in seconds was noted for the swimming test. Both seed and leaf extracts showed the same results as shown in open field and cage cross-test that's there is a dose-dependent increment of motor function when compared with control. Here not only motor function is increased but also antidepressant effects are improved without producing sleep. Here seed extract showed highly significant at P**< 0.01 at high dose when compared with leaf extract, at the same dose level.

Forced swimming test is used to evaluate the stimulant effect of the drugs. Forced swimming test is used to assess the state of behavioral despair in mice. It is also reported that foods and beverages rich in polyphenolic compounds play a key role in the prevention of depression-like behavior (McCreath and Delgoda 2017).

Both with seed and leaf extracts, with the rising dose there is a gradual rise in immobility time. It is suggested that increased amount of membrane protein kinase and systolic fraction in the hippocampus of mice in the learning site is caused by EGCG which results in the enhancement of exploratory behavior and consequently increased no of counts in open field, forced swimming test (Clifford et al., 2013)

In the case of the traction test, more effective results are produced with both seed and leaf extracts, taking lesser time (second) is consumed for crossing the rod in traction test. Highly significant at P**< 0.01 were obtained for both extracts at a high dose, which showed that they had about almost same effect. The traction test is also the indicator test for the assessment of locomotor activity.

Cage cross, swimming and traction tests also produced different results as expected. Here the seed extract at higher doses produced more stimulant effect as compared to the leaf extract, at the same dose level. This proved that seed extract has a more stimulant

effect as compared to the leaf extract. Furthermore, investigation of micro-morphological features of *Camellia sinensis* using light and scanning electron microscopy is recommended for the authentication and correct identification. As foliar epidermal (Ashfaq et al., 2019b; Gul et al., 2019a; 2019b) and pollen features (Ayaz et al., 2019; Gul et al., 2019c; Naz et al., 2019; Sufyan et al., 2018) have been proved significantly in the correct identification of various plant taxa.

5. Conclusion

In light of our observations, it would be concluded that both leaves and seeds are active as a neuropharmacological agent. The crude extract of leaves and seeds exhibited the protruding results when compared with standard Caffeine 10 mg/kg which endorse its usage in traditional system of medicine as cognitive action but more research is necessary particularly in seeds extract to access mode of action and discovery of new phytochemicals of clinical effectiveness.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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