A STUDY ON ANTIDEPRESSANT ACTIVITY OF MARKETED TEA EXTRACTS OF Camellia sinensis

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ABSTRACT:
Ethanolic extract and water extract of marketed tea of Camellia sinensis have shown reduction in total immobility time in mice in forced swim test and tail suspension test at the different doses. However, antidepressant activity was as follows, ethanolic extract > water extract. Ethanolic extracts of marketed tea showed antidepressant activity was comparable to standard drug i.e. Imipramine (10 mg/kg). Tea plant contains more than 4000 bioactive compounds. The major parts of these compounds are flavonoids, polyphenols and catechins. Tea is containing bioactive compounds of which one third is polyphenols. Polyphenols found in tea are mostly flavonoids. The polyphenols, a large group of plant chemicals that includes the catechins, are thought to be responsible for the health benefits. The antidepressant activity of the deferent extract may be due to polyphenols (flavonoids and catechin) compounds.

Keywords: Camellia sinensis, Marketed Tea, Antidepressant, Forced swim test, Tail suspension test and phytochemical investigation

INTRODUCTION
Camellia sinensis is commonly known as Tea, Hindi – Chai. Tea plant is recognized as Camellia sinensis by botanists. They are small bushy plants about 3 o 4 feet high. Tea leaves are picked three to four times between spring and fall of each year. According to Chinese history, about 47 centuries ago, Emperor Sheng-Nong reported that a daily cup of tea could dissolve many poisons in the body.

Tea is reported to contain nearly 4000 bioactive compounds of which one third is contributed by polyphenols. Compounds are alkaloids, amino acids, carbohydrates, proteins, chlorophyll, volatile organic compounds, fluoride, aluminium, minerals and trace elements. Polyphenols found in tea are mostly flavonoids. The polyphenols, a large group of plant chemicals that includes the catechins, are thought to be responsible for the health benefits that have traditionally been attributed to tea, especially green tea.

Major catechins are (−)-epicatechin gallate (ECG), (−)-epicatechin (EC), (−)-epigallocatechin (EGC) and (−)-epigallocatechin gallate (EGCG). The most active and abundant catechin in green tea is epigallocatechin-3-gallate (EGCG). Oolong tea contains a mixture of simple polyphenols, such as catechins and complex Polyphenols.

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In traditional Chinese and Indian medicine, practitioners used green tea to improving mental processes and health. Dating back more than 4,000 years, according to tradition, Chinese green tea could cure anything from headaches, body aches, and pains to constipation and depression.

Literature survey reveal that large number of plants and their constituents showed antidepressant activity in various animal models. *Abies pindrow*, *Annona muricata*, *Aquilaria agollocha*, *Apocynum venetum*, *Areca catechu*, *Bacopa monniera*, *Bajitian oligosaccharide (Indian mulberry)*, *Ballota larandana* and *Ballota nigra*, *Cimicifuga racemosa*, *Cissampelos sympodialis*, *Citrus fragrance / lemon odour*, *Curcuma longa*, *Euphobia hirta*, *Gentianella nevadensis*, *Muccu pruriens* (Kapikacchu) and *Withania somnifera (Aswagandha)*, *Ocimum sanctum*, *Panax ginseng*, *Perilla frutescens*, *Prunus spinosa* were reported to have antidepressant activity.

In the present study, marketed tea of *C. sinensis* was evaluated for antidepressant activity. Literature shows that traditionally this plant is being use in the treatment of depression but no scientific and research data is available / reported to treat depression using this plant. Our attempt is to establish the scientific data of this plant as common, cheap and affordable, safe, effective, readily available alternative antidepressant agent.

**MATERIALS AND METHODS:**

This research work embodies the results of antidepressant activity of marketed tea extracts of *C. Sinensis* L. The marketed tea of *C. Sinensis* used for the present studies were commercially procured from local market of Indore, Madhya Pradesh, India.

1. **Extraction method and conditions of marketed tea of *C. sinensis***

The marketed tea was dried in hot air oven at 37°C. Marketed tea was passed through a sieve #10. The coarsely powdered of marketed tea (200 gms) were extracted separately with petroleum ether (60-80°C), chloroform, ethanol (95 % v/v) successively using soxhlet apparatus till few drops of the last portion of the elute did not leave perceptible residue on drying. The ultimate dried mark of these three parts were macerated with warm distilled water and filtered. Then the extracts were obtained on evaporation of solvent under reduced pressure by ‘Rotavapour Apparatus’. Water extracts were obtained by evaporation of water extract on hot plate in china dish. The extract thus obtained from petroleum ether, chloroform, ethanol and water were examined and their colour, phytochemical and antidepressant activities were noted.

2. **Photochemical investigations:**

Phytochemical examinations were carried out as per the standard methods for extracts as mentioned below.

1. **Detection of alkaloids:** Extracts were dissolved in dilute Hydrochloric acid and filtered.

*Mayer’s Test*: Filtrates were treated with Mayer’s reagent (Potassium Mercuric Iodide). Formation of a yellow coloured precipitate indicates the presence of alkaloids.
Wagner’s Test: Filtrates were treated with Wagner’s reagent (Iodine in Potassium Iodide). Formation of brown/reddish precipitate indicates the presence of alkaloids.

Dragendorff’s Test: Filtrates were treated with Dragendorff’s reagent (solution of Potassium Bismuth Iodide). Formation of red precipitate indicates the presence of alkaloids.

Hager’s Test: Filtrates were treated with Hager’s reagent (saturated picric acid solution). Presence of alkaloids confirmed by the formation of yellow coloured precipitate.

II. Detection of carbohydrates: Extracts were dissolved individually in 5 ml distilled water and filtered. The filtrates were used to test for the presence of carbohydrates.

Molisch’s Test: Filtrates were treated with 2 drops of alcoholic α-naphthol solution in a test tube. Formation of the violet ring at the junction indicates the presence of Carbohydrates.

Benedict’s Test: Filtrates were treated with Benedict’s reagent and heated gently. Orange red precipitate indicates the presence of reducing sugars.

Fehling’s Test: Filtrates were hydrolysed with dil. HCl, neutralized with alkali and heated with Fehling’s A & B solutions. Formation of red precipitate indicates the presence of reducing sugars.

III. Detection of glycosides: Extracts were hydrolysed with dil. HCl, and then subjected to test for glycosides.

Modified Borntrager’s Test: Extracts were treated with Ferric Chloride solution and immersed in boiling water for about 5 minutes. The mixture was cooled and extracted with equal volumes of benzene. The benzene layer was separated and treated with ammonia solution. Formation of rose-pink colour in the ammonical layer indicates the presence of anthranol glycosides.

Legal’s Test: Extracts were treated with sodium nitropruside in pyridine and sodium hydroxide. Formation of pink to blood red colour indicates the presence of cardiac glycosides.

IV. Detection of saponins:

Froth Test: Extracts were diluted with distilled water to 20ml and this was shaken in a graduated cylinder for 15 minutes. Formation of 1 cm layer of foam indicates the presence of saponins.

Foam Test: 0.5 gm of extract was shaken with 2 ml of water. If foam produced persists for ten minutes it indicates the presence of saponins.

V. Detection of phytosterols:

Salkowski’s Test: Extracts were treated with chloroform and filtered. The filtrates were treated with few drops of Conc. Sulphuric acid, shaken and allowed to stand. Appearance of golden yellow colour indicates the presence of triterpenes.

Liebermann Burchard’s test: Extracts were treated with chloroform and filtered. The filtrates were treated with few drops of acetic anhydride, boiled and cooled. Conc. Sulphuric acid was added. Formation of brown ring at the junction indicates the presence of phytosterols.

VI. Detection of phenols:

Ferric Chloride Test: Extracts were treated with 3-4 drops of ferric chloride solution. Formation of bluish black colour indicates the presence of phenols.
VII. Detection of tannins:
    Gelatin Test: To the extract, 1% gelatin solution containing sodium chloride was added. Formation of white precipitate indicates the presence of tannins.

VIII. Detection of flavonoids:
    Alkaline Reagent Test: Extracts were treated with few drops of sodium hydroxide solution. Formation of intense yellow colour, which becomes colourless on addition of dilute acid, indicates the presence of flavonoids.
    Lead acetate Test: Extracts were treated with few drops of lead acetate solution. Formation of yellow colour precipitate indicates the presence of flavonoids.

IX. Detection of proteins and aminoacids:
    Xanthoproteic Test: The extracts were treated with few drops of conc. Nitric acid. Formation of yellow colour indicates the presence of proteins.
    Ninhydrin Test: To the extract, 0.25% w/v ninhydrin reagent was added and boiled for few minutes. Formation of blue colour indicates the presence of amino acid.

X. Detection of diterpenes:
    Copper acetate Test: Extracts were dissolved in water and treated with 3-4 drops of copper acetate solution. Formation of emerald green colour indicates the presence of diterpenes.

XI. Detection of fixed oils and fats:
    A small quantity of petroleum ether or benzene extract is pressed separately between two filter paper. Oil stain on the paper indicates presence of fixed oils.
    Few drops of 0.5 N alcoholic potassium hydroxide is added to a small quantity of petroleum ether or benzene extract along with a drop of phenolphthalein. The mixture is heated on water bath for 1-2 hours. Formation of soap or partial neutralization of alkali indicated the presence of fixed oils and fat.

XII. Detection of volatile oils:
    About 50 gm of powdered drug material is taken in a volatile oil estimation apparatus and subjected to hydro-distillation for the detection of volatile oil. The distillate is collected in the graduated tube of the assembly in which aqueous portion is automatically separated from the volatile oil, if it is present in the drug and returned back to the distillation flask.

3. Biological study
All animal experiments were approved by Institutional Animal Ethics Committee (IAEC) of Pinacle Bio-medical Research Institute, Bhopal, India (Registration No. 1283/c/09/CPCSEA). Study protocol approval reference no. was PBRI/13/IAEC/PN-335.

3.1 Animals
Albino mice (Laca strain) weighing 20 - 25 gm, breed Central Animal House of Pinacle Bio-medical Research Laboratories, Bhopal (M.P), India were used. The animals were housed under standard 12 ± 1 hours light / dark cycle with food (Golden feed, New Delhi, India) and tap water ad libitum the animals were selected at random (male and female). The experiments were conducted between 9.00 am to 5.00 pm.

3.2 Drug treatment
Dried extracts suspended in Tween 80 (2-5%) and then suspended in distilled water, to disperse the dose of the extracts and standard drug. Imipramine (Intas Pharmaceutical Limited, Ahmadabad) (10 mg/kg) taken as the standard drug. All the
drugs prepared afresh at the beginning of each experiment.

3.3 Statistical analysis

Each experiment consisted of a group of minimum 6 animals. The data expressed as average immobility time ± Standard Error of Mean. All the extracts have been compared with control and imipramine (standard) separately using one way analysis of variance (ANOVA) followed by Dunnett's Method. Results at P<0.001 were considered statistically significant.

3.4 Animal model for antidepressant activity

**Forced swim test (FST)**

The mice were divided into 3 groups (n=6). First group (control) which received Tween 80 suspended in distilled water (10 ml/kg) orally and second group which received reference drug 10 mg/kg (orally) of Imipramine and third group which received extracts at 100, 200, 300 and 400 mg/kg (orally). The FST was performed on mice by individually mice forced to swim in an open glass cylindrical jar (Height: 25 cm and Diameter: 10 cm), containing 15 cm of water at 25 ± 1°C. The total duration of immobility during the six minutes of test was recorded. Decrease in the duration of immobility during the FST taken as a measure of antidepressant activity.  

**Tail suspension test (TST)**

The mice were divided into 3 groups (n=6). First group (control) which received Tween 80 suspended in distilled water (10 ml/kg) orally and second group which received reference drug 10 mg/kg (orally) of Imipramine and third group which received extract at 100, 200, 300 and 400 mg/kg (orally). Mice both acoustically and visually isolated were suspended 50 cm above the floor by adhesive tape placed approximately 1 cm from the tip of the tail. Immobility time recorded during a 6 minutes test. 

The antidepressant activity of extracts and drug was evaluated by administering drug and extracts before 30 minutes of the evaluation of activity.

**OBSERVATIONS AND RESULTS**

1. **Chemical study**

The colours of extracts (Table – 1) and results of phytochemical investigations (Table – 2) were noted.

2. **Antidepressant activity study**

The extracts of marketed tea of *C. sinensis* were subjected to forced swim test (FST) and tail suspension test (TST) for evaluation of antidepressant activity. Results of antidepressant activity of extracts were reported (Figure – 1 to 8).

<table>
<thead>
<tr>
<th>Marketed tea extract</th>
<th>Colour of extracts</th>
</tr>
</thead>
<tbody>
<tr>
<td>Petroleum Ether Extract</td>
<td>Dark green</td>
</tr>
<tr>
<td>Chloroform Extract</td>
<td>Dark green to yellowish-brown</td>
</tr>
<tr>
<td>Ethanol Extract</td>
<td>Dark brown</td>
</tr>
<tr>
<td>Water Extract</td>
<td>Brownish-black</td>
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</tbody>
</table>
Table – 2, Results of Phytochemical evaluation of marketed tea extract of *C. sinensis*

<table>
<thead>
<tr>
<th>Test performed for</th>
<th>Petroleum Ether Extract</th>
<th>Chloroform Extract</th>
<th>Ethanol Extract</th>
<th>Water Extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloids</td>
<td>---</td>
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<td>✓</td>
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</tr>
<tr>
<td>Carbohydrates</td>
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<tr>
<td>Glycosides</td>
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<tr>
<td>Saponins</td>
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<tr>
<td>Phytosterols</td>
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<tr>
<td>Tannins</td>
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<tr>
<td>Flavonoids</td>
<td>---</td>
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<tr>
<td>Proteins and Amino acids</td>
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<tr>
<td>Diterpenes</td>
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<tr>
<td>Fixed oils and fats</td>
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<td>Volatile oils</td>
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</tr>
</tbody>
</table>

Figure - 1, Antidepressant activity of petroleum ether extract of marketed tea of *C. Sinensis* in FST

Figure - 2, Antidepressant activity of chloroform extract of marketed tea of *C. Sinensis* in FST
Figure - 3, Antidepressant activity of ethanol extract of marketed tea of *C. Sinensis* in FST

Figure - 4, Antidepressant activity of water extract of marketed tea of *C. Sinensis* in FST

Figure - 5, Antidepressant activity of petroleum ether extract of marketed tea of *C. Sinensis* in TST

Figure - 6, Antidepressant activity of chloroform extract of marketed tea of *C. Sinensis* in TST
Summary:

Ethanol extract and water extract of marketed tea have shown significant reduction in total immobility time in mice in both of the animal models at the all doses. However, antidepressant activity was as follows, ethanolic extracts > water extracts. Ethanolic extracts of marketed tea shown antidepressant activity was comparable to standard drug i.e. Imipramine (10 mg/kg).

As reported earlier, tea plant contains more than 4000 bioactive compounds. The major parts of these compounds are flavonoids, polyphenols and catechins. All these are the biologically active compounds.

Tea is reported to contain bioactive compounds of which one third is contributed by polyphenols. Polyphenols found in tea are mostly flavonoids. The polyphenols, a large group of plant chemicals that includes the catechins, are thought to be responsible for the health benefits that have traditionally been attributed to tea, especially green tea. Major catechins are epicatechin gallate, epicatechin, epigallocatechin and epigallocatechin gallate. The most active and abundant catechin in green tea is epigallocatechin-3- gallate.

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REFERENCE:


