Green tea [Camellia sinensis (L.) O. Kuntze] extract reverses the despair behaviour in reserpinised and diabetic mice

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Green tea (C. sinensis) extract (GTE) dose dependently produced reversal of despair in normal, reserpinised and diabetic mice, thereby demonstrating an antidepressant effect. Although the exact mechanism is yet to be explored, the possible inhibition of catechol-o-methyl transferase and monoamine oxidase enzymes may be responsible for antidepressant activity of GTE.

Keywords: Antidepressant, Camellia sinensis, Depression, Despair behaviour, Diabetes, Forced swimming, Green tea extract, Polyphenols, Reserpine

Mental depression represents a major public health problem worldwide. According to World Health Organization (WHO) estimation, 100 million people worldwide suffer from clinical depression at any given time which is characterized by extreme mood swings, impairment of functioning, anger, agitation and changes in eating pattern as its primary clinical manifestation. Life time risk of developing depression in general population has been estimated to be 17% which is 8-12% for men and 20-26% for women. Patients with chronic medical illness such as diabetes mellitus are much more predisposed to depression. The presence of diabetes doubles the odds of comorbid depression¹. Reviews on treatment of depression in diabetics have revealed that classical antidepressants such as monoamine oxidase inhibitors (MAOIs) induce hypoglycemia and weight gain whereas tricyclics lead to hyperglycemia and carbohydrate craving².

Plants have always been an exemplary source of drugs and many of the currently available drugs have been derived directly or indirectly from them. Further, the use of alternative medicines is increasing worldwide especially in the West and Europe. Various plant extracts (e.g. St. John's wort extract) have shown promising results in treating depression in clinical settings³-⁶. Although many of these promising herbal agents appear to be safe, newer findings suggest serious neuropsychiatric side effects and interactions for these over-the-counter “herbal antidepressants”. Thus there is a constant need to identify newer natural antidepressants with greater efficacy, fewer side effects and to explore their potential over synthetic antidepressants.

From the time immemorial, tea is widely used as a drink of ‘rejuvenation’ in tired and sick people across the globe. Polyphenols present in green tea [Camellia sinensis (L.) O. Kuntzel] have demonstrated significant antioxidant, anticarcinogenic, anti-inflammatory, thermogenic, probiotic and antimicrobial properties in numerous human, animal and in vitro studies. Brain penetrating properties of polyphenols of green tea extract (GTE)⁷, their antioxidant⁸,⁹ and iron chelating properties as well as low side effect profile may make such compounds an important class of drugs to be developed for the treatment of neurological disorders. In vitro reports suggest that green tea extract also inhibits MAO, a major enzyme in catecholamines degradation¹⁰. Thus, the present study has been designed with an aim to investigate the antidepressant effect of GTE in mice using the model of learned helplessness by forced swimming test (FST).

Materials and Methods

Animals—Male Albino laca mice (20-30 g), bred in the Central Animal House facility of Panjab University, Chandigarh, India, were used. The animals were kept in wire cages, 12 per cage, on straw bedding in animal quarters with a natural 12:12 hr
L:D cycle. The animals had free access to standard Rodent food pellets and water. They were acclimatized to the laboratory conditions for an hour before the experiment. All the experiments were conducted between 0900 and 1700 hrs. Despair behaviour as described below was evaluated in a quite laboratory with illumination and temperature approximating those of animal quarters. The experimental protocols were approved by the Institutional Animal Ethical Committee.

**Drugs**—Green tea extract, obtained by water extraction of green tea \([Camellia sinensis (L.) O. Kuntze] leaves, was a gift from Arjuna Natural Extracts, Kerala, India. Other drugs used in the study were; imipramine (Davis Pharma, India), reserpine (Loba Chemie, Bombay, India) and streptozotocin (Sigma, USA).

Assessment of forced swimming-induced despair behaviour—The mice were forced to swim individually in a glass jar (25×12×25 cm) containing water at a height up to 15 cm at room temperature (22° ± 3°C)\(^{11, 12}\). After an initial period of vigorous activity, each animal assumed a typical immobile posture. The mice were considered to be immobile when they ceased to struggle and made minimal limb movements to keep their head above the water level. The immobility period was noted for a 6 min test session\(^{13}\). This immobility period was noted by an investigator who was unaware of the treatment.

Drug treatment—GTE and Imipramine were dissolved in distilled water and given 30 min prior the experiment. Reserpine was dissolved in one drop of glacial acetic acid, \(pH\) was adjusted to neutral and volume was made up. It was given 4 hr prior to test.

Induction of diabetes—Diabetes was induced by a single intraperitoneal (ip) injection of streptozotocin (STZ) (200 mg/kg, dissolved in 0.1N citrate buffer at \(pH\) 4.5). One group was injected with citrate buffer to serve as control. Blood samples were taken from the retro-orbital-sinus of each mouse, 4 weeks after the administration of STZ. Blood Glucose estimations were based on GOD/POD (glucose oxidase/peroxidase) and done with Enzopak glucose kit\(^{14}\). Mice with a blood glucose level greater than 300 mg/dl were selected for study after 4 weeks of STZ injection.

Study protocol—The study was divided into 3 different protocols as described below. Each group consisted of 5-6 animals.

Protocol 1: For the dose response curve, the animals were divided into 6 groups. Group 1 received distilled water, group 2-5 received GTE (10, 25, 50, 100 mg/kg ip) 60 min prior to experiment, group 6 was administered imipramine (20 mg/kg, ip) 30 min before the test.

Protocol 2: To find out the possible mechanism of catecholamines in the reversal of immobility by GTE, the animals were divided into 4 groups. All the groups were administered reserpine (2 mg/kg, ip) 4 hr before the test. Group 1 received the distilled water 30 min after reserpine (i.e. 3.5 hr before the test), group 2-4 received GTE (25, 50, 100 mg/kg, ip) 30 min after reserpine (i.e. 3.5 hr before the test).

Protocol 3: To find out the effect of GTE in reversing diabetic depression, 4 groups of diabetic animals were used. Group 1 diabetic animals received distilled water 30 min before the test, group 2-3 received GTE (25 and 50 mg/kg, ip) 60 min prior to the test, group 4 received imipramine (20 mg/kg, ip) 30 min before the test. Group 5 served as control which had received the citrate buffer instead of STZ and they were administered distilled water 30 min prior to FST.

Statistical analysis—Results were measured in seconds and expressed as mean±SE. The intergroup variation was measured by one way analysis of variance (ANOVA) followed by Fischer’s LSD test. Statistical significance was considered at \(P<0.05\). The statistical analysis was done using the Jandel Sigma Stat Statistical Software version 2.0.

Results

Effect of GTE on forced swimming-induced immobility period—GTE significantly reduced the immobility period in a dose dependent manner. At lower dose (10 mg/kg), there was no effect but at the dose of 100 mg/kg, the antidepressant effect of GTE (\(P<0.05\) as compared to control) was comparable with that of imipramine (20 mg/kg) (Fig. 1).

Effect of GTE on reserpine-induced immobility period—Reserpine (2 mg/kg, ip) significantly increases the immobility period as compared to the control (\(P<0.05\) after 4 hrs of administration. All the doses of GTE (25, 50, 100 mg/kg, ip) administered 30 min after the reserpine treatment reversed the reserpine induced immobility (Fig. 2).

Effect of GTE on immobility period in diabetic mice—The diabetic mice (blood sugar level 467.3±8.33 mg/dl as compared to 125.1±5.8 mg/dl of control) when tested after 4 weeks of STZ injection,
showed significantly longer duration of immobility as compared with control mice ($P<0.05$). GTE markedly attenuated the increased immobility time of diabetic mice (Fig. 3).

**Discussion**

The positive effects of depression management go beyond improved mood. It has been noted that people with untreated depression are likely to have poorer outcome from treatment for coexisting medical complications such as hypertension, diabetes, heart disease and cancer. The unique importance of depression management in medically ill population may be its potential to improve the medical condition itself. Though several neurotransmitters have been implicated in its pathogenesis but all theories suggest that it is not a single neurotransmitter disorder.

Traditional beverages, as represented by tea have been demonstrated to be beneficial to the human health. The polyphenols found in the tea (C. sinensis) are more commonly known as flavonoids or catechins and comprise 30-40% of extractable solids of dried green tea leaves. The main catechins in green tea are epicatechin (EC), epicatechin-3-gallate (ECG), epigallocatechin (EGC) and epigallocatechin-3-gallate (EGCG). The major activity of GTE is due to its polyphenols especially EGCG. In the present study, the antidepressant effect of GTE was investigated using forced swimming induced immobility test model. The model, which was initially developed by Porsolt et al. and modified by Mehta and Kulkarni, is a well accepted model of behavioral depression. Green tea polyphenols have been shown to sufficiently cross the blood brain barrier and hence the present study has been aimed to study the acute effect of GTE in behavioural despair test. In an earlier study, GTE has shown attenuation of lipopolysaccharide-induced immobility period in mice. Here again GTE significantly reversed the immobility caused by forced swimming in naïve mice in a dose dependent manner (10, 25, 50, 100 mg/kg, ip). The effect of GTE on immobility was comparable to that of imipramine which is known to act through stabilization of sympathetic system. Though caffeine, which is also a constituent of the green tea, per se can also decrease the immobility time as shown in various reports but caffeine concentration in GTE used in the present study is <5% (Refs 17, 18). thereby nullifying its contribution in the observed antidepressant effect of GTE seen.

Green tea has been shown to activate sympathoadrenal axis in humans through its catechin-polyphenols and caffeine. Such a control over sympathetic mechanism in brain might be involved in mediating the antidepressant effect of GTE. The
catecholamines in the body are mainly degraded by two enzymes, viz Catechol-O-methyl transferase (COMT) and MAO. *In vitro* studies done by Zhu *et al.* 20 showed COMT inhibitory activity of Green tea polyphenols. GTE and its constituents, catechin and EGCG have shown a MAO inhibiting effect also. Apart from the *per se* effect of GTE, it also reversed the increase in immobility caused by the catecholamine depletion action of reserpine. Reduced brain tryptophan levels, and decreased brain turnover of catecholamines and serotonin in diabetic rats suggests involvement of decreased monoamine activity in the genesis of diabetes associated depression 21-23.

Recently there is an emphasis on the role of dietary antioxidants in controlling the prognosis of diabetes 24. Medicinal herbs with antihyperglycemic activities are increasingly sought by diabetic patients and health care professionals 25. Some studies have also shown antidiabetic potential of GTE and its effective role in reducing the oxidative stress produced by diabetes 26, 27. The antioxidant potential of GTE might be involved in stabilization on sympathoadrenal axis of diabetic mice. We did not explore the mechanism of action of GTE but we strongly speculate that the catecholamine modulating activity of GTE by MAO or COMT inhibition might be responsible for its antidepressant activity.

In conclusion, the present study demonstrated the beneficial effect of GTE in reversing the despair behaviour in naïve, reserpinised and diabetic mice. These preliminary findings warrant detailed investigations on GTE such that it can be employed as an antidepressant in a clinical setting.

References