Research Article

Pharmacological Screening of Decaffeinated Tea Extract to Explore Potential Adjuvant Therapy in Parkinsonism.

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ABSTRACT
Parkinson's disease (PD) is a consequence of specific progressive neurodegeneration of substantia nigra pars compacta dopaminergic neuron resulting depletion in Dopamine (DA). L-dopa is first line DA replacement therapy. Caffeine and Antimuscarinic agents are effective adjuvant in PD management. Caffeine is given to counteract the adverse effects of anticholinergics. Caffeine is considered as a very safe psychostimulant. Contrary, higher doses of Caffeine associated with tachycardia, nausea and vomiting. Anticholinergics at therapeutic doses produce amnesia and cognitive impairment, particularly intense in elderly with preexisting neurodegenerative disease. Little is known about association between caffeine use, toxicity and dependence and risk of common psychiatric and substance use disorder. In present study, Decaffeinated Tea Extract (DTE) has been screened for antimuscarnic and anticataleptic activity. The results indicate that DTE significantly inhibited Ach- induced contractions and Haloperidol-induced catalepsy. The effects are comparable to Atropine & Caffeine. As per our study findings, decaffeinated tea is an effective option as an adjuvant as compared to Caffeine and Atropine.

1. INTRODUCTION

Parkinson's disease (PD) is second most common neurodegenerative disorder affecting approximately 1% of population older than 60 years [1]. PD is considered as disease of motor system; diagnosis is based on occurrence of set of cardinal motor signs viz: tremor, rigidity, akinesia and postural reflux disturbances. Etiology is attributable to consequences of neurodegeneration in substantia nigra pars compacta and results consequent reduction in dopamine (DA) levels in the striatum [2]. Since the incidences of PD increases with age (most important risk factor), possibly number of people suffering from PD will rise steadily in future. The neurodegenerative process that lead to sporadic PD begins many years before the appearance of characteristic motor symptoms; additional neuronal mechanisms and neurotransmitter systems are also involved in PD. Pathogenesis of PD is contribution of several etiological factors and cumulative effect of associated complex mechanisms. Pathogenesis of PD is extended to the additional fields in the brain including anterior olfactory structures, dorsal motor nucleus of vagus, caudal raphe nuclei, locus coreruleus, the autonomic nervous system, hippocampus and cerebral cortex [3]. Nowadays considerable scientific evidences show that non-dopaminergic degeneration also occurs in other brain areas which seem to result the deficit in olfactory, emotional and memory functions that precede the classical motor symptoms in PD [4]. Oxidative stress is regarded as main factor contributing to the etiology of PD. Subtle changes in DA synthesis or metabolism may influence production of free radicals but glutamate overflow seems to be critical for oxidative stress development in late stage of PD. Excessive release of glutamate from nerve terminal causes overstimulation of glutamate receptors, calcium overload in cytosol and stimulation of excitotoxicity. Increased Ca^{2+} influx into cytosol disturb mitochondrial function and mitochondrial respiratory chain. This leads to leakage of superoxide radicals from mitochondrial complex after cellular defense system's failure to oxidative stress [5]. The functional interplay between DA & Acetyl choline (Ach) in the brain is well established [6, 7,8]. A disinhibition of cholinergic neurons in the striatum has been reported after treatment with neuroleptic drugs [9]. As a result of this homeostatic imbalance, cholinergic activity is enhanced in the striatum that causes dyskinesia and tremors. Catalepsy induced by Haloperidol is considered a rodent model of PD - like side effects caused by typical antipsychotics in humans [10]. Haloperidol- induced catalepsy in rodent is due to blockade of striatal dopamine D_2 receptors [11]. Catalepsy is thought to be the good predictor of extra pyramidal symptoms in humans. Neuroleptic- induced catalepsy is a behavioral test widely used to screen the potential of antiparkinsononian drugs [12]. Muscarinic antagonists were the first pharmacological treatment for PD [13]. Their usefulness is seriously limited by various systemic and CNS side effects. These side effects are more severe in elderly patients with the neurodegenerative disease. At therapeutic doses anticholinergics often cause amnesia and cognitive impairment [14, 15, 16] that exacerbate the subcortico- frontal syndrome usually associated with PD. Thus dose reduction in anticholinergics is necessary in management of PD [17]. The non- selective muscarinic antagonist e.g. Scopolamine does not inhibit Haloperidol- induced catalepsy in mice lacking the M_4 receptor subtype [18] suggests that blockade of M_4 receptors must underlie the anticataleptic effect of anticholinergics in rodents [19].

DA replacement of depleted stores in substantia nigra pars compacta is the first line therapy in management of PD. L- DOPA with Carbidopa is used in different proportion to alleviate the classical symptoms of PD. In fact, L- DOPA's Neuroprotective versus toxic effects depends on stage of neuronal damage, amount of DA synthesized and rate of its metabolism. DA itself is a source of free radicals that are formed during enzymatic metabolism of DA by Monoamine Oxidase (MAO) or by its non- enzymatic auto- oxidation that leads to production of very reactive DA- quinines and semi quinine [20]. Caffeine is the most widely consumed psychostimulant substance being self administered legally throughout the wide range of conditions and present in numerous dietary products. Recent experimental findings have indicated the potential of Caffeine in management of non- motor symptoms (eg. depression, olfactory and memory dysfunction) of PD which has failed to improve with current dopaminergic drugs [4]. Several reports show that Caffeine produce discriminative stimuli and reinforcing effects similar to those produced by classical stimulants such as Cocaine [21]. Repeated exposure to Caffeine has been reported to induce tolerance, sensitization or no change in motor response induced by direct or indirect DA receptor agonist administration [22]. Non-motor features of PD invariably do not respond to dopaminergic medication and are probably the major recent challenge faced in clinical management of PD [3].

Current research on PD is largely devoted to investigating the etiology of the disease with an objective to identify and evaluate preventive rather than merely symptomatic relief treatment.

It seems of crucial importance to investigate potential of decaffeinated tea extract (DTE) as an adjacent remedy in management of PD. The investigation was done through reported screening procedures in literature.

2. MATERIALS AND METHODS

2.1 Animals

Albino mice weighing 22–25 g were housed under standard laboratory conditions in groups of five. The animals were fed *ad libitum* with standard food and water. They were deprived of food 6 h before testing but had free access to water. All the experiments were carried out between 08:00 and 14:00 h. The protocol of animal experiments was approved by Institutional Animal Ethical Committee (IAEC).

2.2 Drugs

Acetylcholine (Research lab, Mumbai), Atropine sulphate (Sigma, USA) Haloperidol (RPG Life sciences, India), Caffeine (HiMedia Laboratories Pvt. Ltd., Mumbai) and Decaffeinated tea extract (DTE). Drugs were dissolved in normal saline immediately before the use and were administered intraperitoneally in a volume of 10 ml/kg.

2.3 Preparation of the extract

Decaffeinated tea powder of TATA- *Tetly* was used for the present study. Decaffeinated Tea power 250 g was extracted using acetone- water solvent system (80:20) by standard maceration method of extraction. The extract was concentrated in rotating evaporator under reduced pressure until dry yielding 20.8 % (52 g) residue.

2.4 Effect of DTE on Ach-induced contractions on using goat tracheal chain (GTC)

Isolated goat trachea gives specific response to autonomic drugs [23]. Goat trachea was obtained from slaughter house immediately after sacrifice and kept in Krebs-Henseleit solution with optimum supply of aeration. Krebs-Henseleit solution was of the following composition (mM): NaCl, 115; KCl, 4.7; $CaCl_2$, 2; $NaHCO_3$, 25; KH_2PO_4, 1.2; $MgCl_2$, 1.2; glucose, 11.5. GTC preparation was mounted in Student's organ bath equipped with temperature and aeration control. Contractions induced by Ach (100 µg/ml) alone and in presence of DTE (0.5 ml of 25 mg/ml), Caffeine (100 µg/ ml) and Atropine sulphate (50 µg/ ml) are recorded. The contact time maintained was 60s.

2.5 Effect of DTE on Haloperidol- induced catalepsy

Catalepsy is defined as reduced ability to initiate the movements and failure to achieve the correct posture, was measured by bar test. Catalepsy of an individual mouse was measured in a stepwise manner by the scoring method as described [24]. Mice were divided into 5 groups; each group composed of 5 animals (n=5). Group- I received Vehicle + Haloperidol (1 mg/kg, i.p.) while Group- II, III, IV & V received Caffeine (3 mg/ kg i.p.), Atropine (4.1 mg/ kg) DTE (100 mg/kg, i.p.) and DTE (200 mg/kg, i.p.) respectively. The duration of catalepsy (measured in seconds) was recorded at 30, 60, 90, 120 and 180 min after Haloperidol administration, using the Bar test [25]. In brief, the forepaws of mice were placed on a horizontal wooden bar (2.54 cm diameter) elevated at 3.5 cm and the duration of catalepsy was recorded for each mouse as the time for which the forepaws remained on bar. The forepaws were placed twice on the bar and the higher value was considered for the assessment of catalepsy.

2.6 Statistical analysis

Data is presented as mean ± SEM. The data was analyzed by one-way ANOVA followed by Dennett's test or student's t-test (unpaired). $P < 0.05$ was the criteria for statistical significance.

3. RESULTS AND DISCUSSION

3.1 Effect of DTE on Ach-induced contractions on using goat tracheal chain (GTC):

Log dose - response (-Log M vs Percent contraction) curve was plotted. Ach produced dose-dependent contraction on GTC. Atropine the competitive muscarinic antagonist was used as reference antimuscarinic. DTE significantly reduced the amplitude of Ach- induced contractions. DTE *per se* had no effect on GTC. Inhibition is comparable to that of Atropine (50 µg/ ml). Caffeine did not cause significant effect on Ach- induced contractions (Fig. 1).
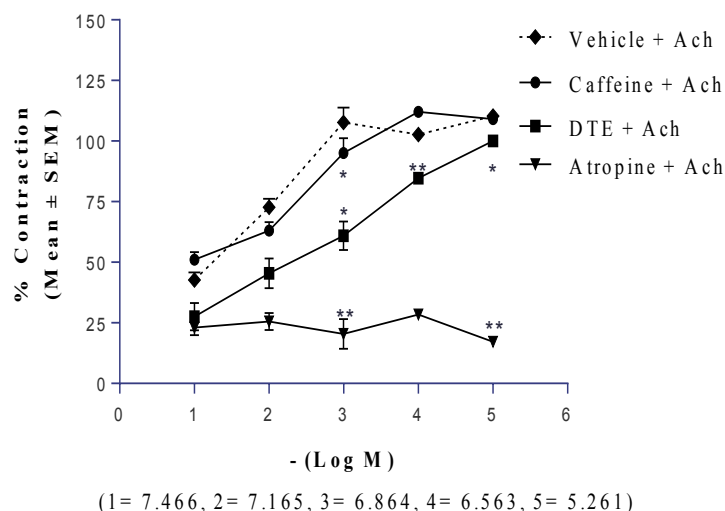
$(1= 7.466, 2= 7.165, 3= 6.864, 4= 6.563, 5= 5.261)$

Fig. 1: Effect of DTE on Ach- induced contractions on isolated GTC

Values in parentheses indicate the dose. Data was expressed as Mean ± SEM.   Statistical significance was determined by student's t- test (unpaired),

*: $P<0.05$, **: $P<0.01$ as compared to Vehicle + Ach (n=5).

3.2 Effect of DTE on Haloperidol- induced catalepsy:

In this study, mice were pretreated with Vehicle + Haloperidol caused progressive increase of descend time measured in the bar test. Haloperidol exhibited time- dependent changes in the duration of catalepsy, and maximum catalepsy (183.2 ± 6.9 s) was observed 90 min after haloperidol (1 mg/kg, i.p.) administration. Pretreatment with Caffeine (3 mg/ kg, i.p.), Atropine (4.1 mg/kg, i.p.) and DTE (100 mg/kg & 200 mg/kg, i.p.) significantly inhibited catalepsy. Inhibition is more in case of DTE (200 mg/kg, i.p.) (Fig.2).
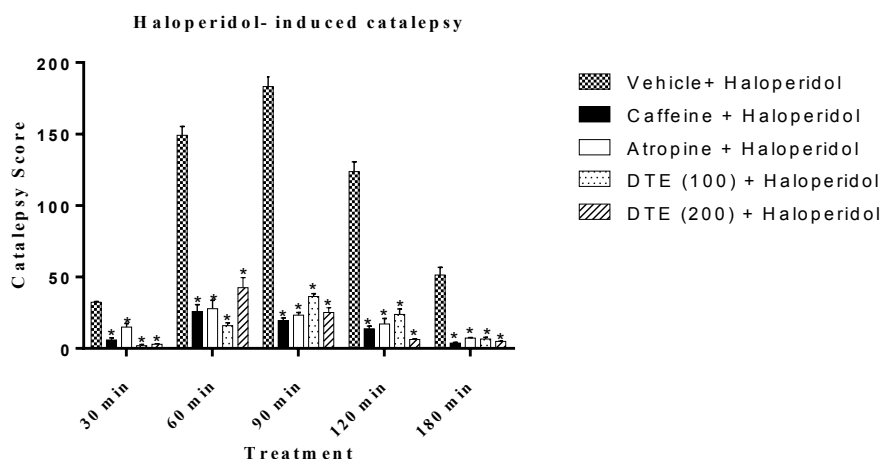

Fig. 2: Effect of DTE (100 & 200 mg/kg, i.p.) on Haloperidol-induced catalepsy.

Data is expressed as mean ± SEM, analyzed by two way ANOVA followed by Dunnett's test.*: $P<0.05$ compared to control (n=5).

Catalepsy occurs when more than 80% of D_2 receptors are occupied by the drug [26]. Catalepsy is mediated through excitatory adenosine and glutamatergic inputs acting on adenosine A_{2A} and N-Methyl, D- Aspartate (NMDA) receptors in striatum [27]. Catalepsy induced by antipsychotic drug Haloperidol is reduced by the administration of nootropic drugs. Catalepsy is also reduced by $α_2$ receptor antagonists ($α_{2C}$ or $α_{2A}$) [28] or 5 HT_{1A} agonists [29], as well as by metabotropic glutamate receptor-4 agonists [30]. Chronic stressful stimuli cause an increased expression and density of adenosine A_{2A} receptor in animal model of PD [31, 32]. Moreover, adenosine A_{2A} antagonists confer neuroprotection against a broad spectrum of brain injury induced by excitotoxicity and ischemia [4]. Glutamate in brain acts as protein constituent, neurotransmitter and involved in intermediary metabolism. Another side of Glutamate physiology indicates that it can be highly toxic to the neurons in terms of over activation of excitatory neurotransmitter receptor and leads to cell death through either rapid necrosis or delayed apoptosis [4]. More recently findings suggests A_{2A} receptor modulation of glial function has emerged out as an additional mechanism by which A_{2A} receptor antagonist may modulate Glutamate release and further consequences leading to cell death in CNS. Caffeine is potent Monoamine Oxidase- B (MAO-B) inhibitor as well as Adenosine A_{2A} antagonist [33]. It confers protection against rapid metabolism of DA by MAO-B. It also offers neuroprotection against the underlying dopaminergic neuron degeneration and influence the onset and progression of PD. Protective effect of Caffeine against PD is more evident in men and post menopausal women not consuming estrogen as it counteracts neuroprotective effect of Caffeine [34]. Neuroprotection may involve modulation of neuroinflammation; the glial cells (microglia and astrocytes) attribute to neuroinflammation. Activation of microglia in neuropathological conditions like PD contributes to neuronal damage through the release of proinflammatory and neurotoxic factors [35]. Oxidative stress associated is free radical generation which is crucial factor in etiology of PD. Caffeine has inhibited generation of hydroxyl radical too in striatum of Haloperidol treated rats with damaged nigrostriatal neuron [25]. Caffeine and muscarinic antagonists act in synergy to inhibit Haloperidol- induced catalepsy in rats, synergism persists after repeated administration of low doses of Caffeine (15 mg/kg, i.p.) [36]. Anticholinergics, however can also enhance striatal Ach release through the blocked of muscarinic autoreceptor in striatal cholinergic interneurons [37, 38]; presumably this increase in Ach concentration in synaptic cleft, would prevent binding of anticholinergics to muscarinic receptors located on postsynaptic striatal targets. Ultimately the competitive antagonistic ability of anticholinergics declines; this effect counteracts antiparkinsonian and anticataleptic action of anticholinergics. Anticholinergics even at therapeutic doses often produce amnesia and cognitive impairment [36]. Detrimental effects of commonly used anticholinergics are more intense in elderly Patients; extrapyramidal symptoms significantly contribute to non- compliance with medication [39]. The non- selective muscarinic antagonist like Scopolamine does not inhibit Haloperidol-induced catalepsy in mice lacking the M_4 receptor subtype [18] suggests that blockade of M_4 receptors must underlie the anticataleptic effect of anticholinergics in rodents [19]. Preferential M_4 receptor antagonist Tropicamide was about five fold more potent to inhibit the tremulous jaw movements in rat than that of non-selective muscarinic antagonist Atropine [40]. Haloperidol does not produce catalepsy in mice lacking M_4 receptor. Previous hypotheses can be refined with another possible mechanism that

blockade of M_4 receptors in neurons of the striatonigral pathway plays a major role in the synergism between anticholinergics and drugs that block A_{2A} receptors [41]. Safer and specific anticholinergic (M_4 receptor antagonist) without/ less pronounced CNS side effect is yet to be discovered.

Effectiveness of Caffeine in management of PD is well understood. However, current evidences indicate that psychomotor stimulant effect of Caffeine at doses similar to habitual human consumption is produced by antagonism of Adenosine A_{2A} receptor [36]. The subjects who are about to develop PD are intolerant to Caffeine [28], or that they have a low sensation seeking trait that makes them less predisposed to drinking caffeinated beverages [42]. Individual with pre-existing anxiety disorders are more sensitive to the effect of Caffeine [43]. In rare cases, rhabdomyosis has been reported attributable to Caffeine [44, 45]. It causes reduction in cerebral perfusion, that may sustain for 90 min of administration can thus reduce effectiveness of several drugs and can cause exacerbation in psychiatric illnesses in patient on maintenance therapy [46, 47]. Severe reduction in cerebral circulation and cerebral ischemia causes brain damage. Some individuals experience anxiety and distress in response to Caffeine. These effects have been shown to correlate with Adenosine A_{2A} receptor gene polymorphism, suggesting that A_{2A} receptor gene may contribute towards the development of panic disorder [48, 49].

In present study, Ach- induced contractions are significantly inhibited by DTE, comparable to that of Atropine (100 μg/ ml), the competitive antagonist of muscarinic receptor. It indicates anticholinergic potential of DTE. Caffeine did not produce significant inhibition in Ach- induced contraction. DTE significantly inhibited Haloperidol- induced catalepsy; here we postulate antagonistic effect of DTE component(s) on A_{2A} receptors. Possible synergism may exist between Adenosine A_{2A} receptor blocked and anticholinergic potential of DTE.

DTE may confer improvement in non- motor symptoms of PD. Effect on Glutamate turnover and direct/ indirect action on D_2 receptor needs to be explored further.

4. CONCLUSION

Several study reports has shown that Caffeine produces discriminative stimuli and reinforcing effects similar to those produced by classical stimulants such as Cocaine and Amphetamine. DA replacement therapy also has limitations due to gradual decline in therapeutic efficacy. Therapeutic use of anticholinergics seriously limited by various systemic and CNS side effects; and susceptibility to neurodegenerative disease. Selectivity of DTE for muscarinic M_4 receptor may add to its usefulness, it may have antioxidant property offering neuroprotection to different fields in brain. Here we may conclude that decaffeinated tea could be the best dietary adjuvant in long term management of PD and objective of improvement in quality of patient's life can be partially fulfilled.

5. ACKNOWLEDGEMENT

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