Comparative assessment of organic and inorganic tea leaf extract feeding on anxiety behaviour status of colchicine-induced rat model of Alzheimer’s disease

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Abstract
Tea (Camellia sinensis), having anti-inflammatory, antioxidant, and free radical scavenging properties, may be beneficial to prevent the symptoms of neurodegenerative disorders like Alzheimer’s disease (AD). In this present study, field experiments using the productive tea clone (TV25) with four nutrient management treatments were conducted during 2015 to 2017 in the research farm of Agricultural and Food Engineering Department, Indian Institute of Technology Kharagpur. The four nutrient management treatments were no application of fertilizer (control), organic fertilizer (OF), inorganic fertilizer (IF), and integration of OF and IF (IF + OF). The contents of different catechins of tea leaves grown under these treatments were measured using High Performance Liquid Chromatography. Tea leaf samples of these treatments were fed to the intracerebroventricular (ICV) colchicine administered rats. The animal study was double-blinded and randomized. Assessment of anxiety status was done for the rat model in an elevated open field with a novel object in two intervals (14-day and 21-day study). Anxiolytic behaviour with the lower corticosterone (CORT) level (82 ng/ml) was observed in ICV colchicine administered rat models of AD. After feeding of organically and inorganically grown tea extract (10, 20, and 30 mg/kg) for 14 days and 21 days, it was found that the anxiolytic behaviour decreased with the increased concentration of serum CORT. However, organic tea showed greater increase in CORT level (216.1 ng/ml) as compared to inorganic tea (214 ng/ml). Thus, this study showed organic tea may act as a favourable agent or adjuvant in the improvement of the anxiolytic behaviour in rat model of AD.

Keywords Tea polyphenols · Alzheimer’s disease · Anxiolytic behaviour · Corticosterone level

Introduction
Alzheimer’s disease (AD) is a neurodegenerative disorder. It is mainly distinguished by memory loss, language disability, poor learning, reasoning ability and dysfunction in cognition in elderly people. AD is specified by gradual neuronal loss and generation of amyloid beta plaques (Aβ), hyperphosphorylated tau proteins in brain (Glenner 1983; Masters et al. 1985; Huang and Jiang 2009). Studies showed, generation of amyloid beta plaques, neurofibrillary tangles, tau protein, generation of reactive oxygen species and neuronal loss are the primary causes of AD (Bonda et al. 2010; Galasko and Montine 2010; Schrag et al. 2013). Change in anxiety status is one of the most common syndromes of AD (Vloeberghs et al. 2007). Studies showed that plant food rich in phenolics and flavonoids can resist AD in many ways because of their antioxidant property (Muthaiyah et al. 2014). It has also been found that extract of tea, which is naturally rich in polyphenol and flavonoid content can reduce oxidative stress by inhibiting reactive oxygen species in cultured neurons associated with AD (Mishra et al. 2011). The polyphenol content and free radical scavenging properties of tea varies depending upon the field level nutrient management practices. It was found that organically grown tea is enriched with much better polyphenol content than tea grown inorganically (Bagchi et al. 2020).

For many years, different types of animal models are considered to be important implements for developing many therapeutic models for human diseases (Benedikz et al. 2009). Gradual memory loss, dysfunction in cognition
leading to poor learning and reasoning ability are the most commonly found characteristics of AD. Studies showed, varying anxiety status in rat model of AD depends on the preparatory process. For an example, Raghavendra et al. (2008) showed anxiogenic behaviour in AD rats in an elevated plus maze (EPM), whereas Sil and Ghosh (2015) has reported anxiolytic behaviour of AD rats in an open field space which is elevated with a novel object in the centre. It was found that intracerebroventricular (ICV) administration of colchicine to rats assists to a progressive neurodegeneration, cognitive impairment, neuronal loss and memory impairment. Therefore, this type of model should be considered as a significant model of AD (Kumar et al. 2006, 2007, 2008, 2010). Studies have shown that tea leaf extract contains several polyphenols and has antioxidant, anti-inflammatory properties (Khan and Mukhtar 2018). Besides, several studies related to the therapeutic effect of tea leaf extract on different diseases, very few studies have delineated on the Anti-Alzheimer’s property of tea. Based on available literature, which showed that tea has antioxidant properties, it can be hypothesized that tea leaf extract should be effective to treat AD like pathology. In view of this, the present study mostly covered the anti-anxiolytic property of tea leaves grown under different field level nutrient management practices on rat models of AD. So far, the study demonstrating the anti-anxiolytic effect of organically and inorganically grown tea leaf extract on AD animal model is very sparse. Therefore, the present study was conducted to evaluate the anti-anxiolytic effect of organically and inorganically grown tea to ICV colchicine-injected AD rats.

Materials and methods

Tea leaf sampling

The tea leaf samples as two leaves and a bud were collected in three commercial harvest time (May, July, and September) from different fertilizer management treatments in the years 2016 and 2017 from the tea garden of Agricultural and Food Engineering Department, Indian Institute of Technology, Kharagpur. Plucking of tea leaves depends on the flush of the leaves. The interval between successive plucking varied from 7 to 10 days. After plucking, the tea leaves were washed thoroughly and air dried in a drying chamber (Hot air oven) at 60 °C for 6 h (Chan et al. 2008; Palit et al. 2008). The dried samples were grounded in Wiley mill and passed through 80 mesh sieves. The dried samples were then stored in polythene packets for chemical analysis.

Measurement of total phenolics in tea extracts

The total phenolics content of tea samples at two leaves and a bud stage grown under different nutrient management practices were evaluated.

Preparation of tea leaf extract

The stored dried leaf samples were crushed thoroughly with mortar and pestle with deionized water. The extracts were centrifuged at 5000 rpm for 20 min. The supernatant after centrifugation was collected after centrifugation and stored at 20 °C for further experiments.

Evaluation of individual polyphenols in the tea leaf extract

High-performance liquid chromatography (HPLC) analysis was performed to evaluate the concentration of different polyphenols in tea leaves grown under different nutrient management practices. The concentrations of [(-)EGC], [(-)EC], [(-)EGCG], [(-)GCG], and [(+)ECG] in tea leaf extracts were measured.

Preparation of tea leaf extract

About 0.5 g of stored dried tea leaves were crushed in mortar and pestle and extracted with 100 ml of 70% methanol by Soxhlet apparatus for 45 min (Palit et al. 2008). The methanol of the extract was then removed with the help of rotary vacuum evaporator. After evaporation, the residue remained, was dissolved in 1 ml of 70% methanol.

Preparation of standard solution of tea catechins

The standard tea catechins considered for this study were [(+)EGC], [(+)EC], [(+)EGCG], [(+)GCG], and [(+)ECG]. The standards of the tea catechins were purchased from SigmaAldrich. The catechin standards were prepared by dissolving 2 mg of each catechin in 2 ml 70% of methanol.

Measurement of tea catechins

For HPLC study, HPLC grade methanol and trifluoroacetic acid were purchased from Merck (Mumbai, India). The deionized water was obtained from DiamondNanopureTM water purification system (Barnstead/ Thermolyne, Dubuque, IA, USA). HPLC was performed on a BREEZETM HPLC system (Waters, Milford, USA). A C18 reversephase HPLC column (SynergiTM HydoRP) was used for this study. The internal particle of the column was 5 μm. The internal diameter of the column was 4.6 nm and the length was 250 nm. In this process, a linear isocratic solvent system was used. An aqueous trifluoroacetic acid (1 mM)/ metha-
nol (17:8) solution was used as the solvent system to elute the tea catechins. The identification of the phenolic compound was confirmed by comparing with the retention time of the external standard.

**Animal study**

**Experimental animals**

Healthy albino rats (Charles Foster strain) were selected for this experiment. Each of the experimental rat weighed 200–250 g (6–8 weeks of age). Total animals used were two hundred and seventy. Rats were kept individually in polypropylene animal cages. For the experimental animals the proper ethical guidelines were followed (Jaykaran and Kantharia 2011). The standard protocol used was accepted by the institutional animal ethics committee of Institute of Reproductive medicine IRM Kolkata, India (473/01/a/CPCSEA dated 5/9/2013). The rats were assigned in 5 groups with 54 rats in each group in a random and blind folded manner. All animal model experiments were performed in Razabazar Science College, University of Calcutta (India) and in School of Medical Science and Technology, Indian Institute of Technology, Kharagpur (India).

**Preparation of Alzheimer’s disease rat models**

For preparation of experimental rat models of AD 2.5 μL of artificial cerebrospinal fluid (ACSF) was taken. In 2.5 μL ACSF 7.5 μg colchicine was dissolved and was administered slowly for 5 min in the lateral ventricle of the experimental rats, to be treated as AD model. The lateral ventricle of both sides of the anaesthetized brain of the rats was approached stereotaxically (Kumar et al. 2006, 2007, 2010) through a steel cannula attached to a Hamilton syringe.

Group 3 were further subdivided into 3 subgroups with 18 rats in each subgroup. Each subgroup was further divided into three sub-sub groups with six rats in each sub-sub group. In each subgroup, different doses of tea extract (10, 20, and 30 mg/kg IP.) treatment of different nutrient management practices was given (Rezai-Zadeh et al. 2005).

**Treatment schedule of drugs**

Standard fresh solutions of colchicine (St. Louis, MO, USA) were made at the start of each experiment. For ICV administration, a 15 mg dose of colchicine dissolved in ACSF should be given in a 5 mL injection volume. For therapeutic purpose, tea leaf extract (2 g of fresh tea leaves macerated in 100 ml of drinking water) was administered intraperitoneally (IP) at dosages of 10, 20, and 30 mg/kg, respectively, to Group 3 for 21 days. The tea extract treatment was started from 4 days prior to the ICV administration of colchicine (Halawany et al. 2017). In group 5 rats (positive control), celecoxib (10 mg/kg) was given through IP injection for 21 days starting from 4 days before the intracerebroventricular administration of colchicine (Halawany et al. 2017).

**Anti-anxiolytic property of tea extract**

The anxiety behaviour was assessed in an elevated open field space with an object in the centre (Ennaceur et al. 2006). The anxiety status was evaluated on 14th and 21st day after ICV administration of colchicine to the AD rats. In each session of the test, all the experimental animals were used for 10 min of anxiety test. Rats were kept for 10 min to traverse the area of the open space. Different parameters of this behavioural analysis like the delay and frequency of entry to the inner area, amount of time spent in inner and outer area, delay and frequency of approach to the object area were assessed in each trial of the experiment. Experimental rats in elevated open field experiment are shown in Fig. 1.

**Serum corticosterone**

About 1.5 ml blood was taken from the heart of each anesthetized rat by a syringe for collection of the serum. With the blood serum, the corticosterone (CORT) level was determined in each group of rats by using a commercially available kit.

**Results**

**HPLC determination of catechins**

The individual polyphenol content like the concentration of [( ) EGC], [( ) EC], [( ) EGCG], [( ) GCG], and [( ) ECG]) in tea leaves sampled in the months of May, July, and September in 2016 and 2017 are presented in Figs. 2, 3, 4, 5, and 6. In general, the contents of these polyphenols in tea leaves were significantly higher in July sampling than May and September in both years. The highest content of these individual polyphenols like [( ) EGC], [( ) EC],[( ) EGCG],[( ) GCG], and [( ) ECG]) in tea leaves were found in OF treatment and these were significantly higher than control and IF treatments (Figs. 2, 3, 4, 5, 6). The values of [( ) EGC],[( ) EC],[( ) EGCG], [( ) GCG], and [( ) ECG]) in tea leaves were found in OF treatment were 19 ± 0.7 mg/g, 16 ± 0.6 mg/g, 42 ± 1.9 mg/g, 18.8 ± 1.2 mg/g, and 18 ± 0.88 mg/g, respectively, in the year 2016 and 22 ± 0.78 mg/g, 18.2 ± 0.77 mg/g, 43.8 ± 1.8 mg/g, 20.2 ± 0.75 mg/g, and 19.3 ± 0.82 mg/g, respectively, in the year 2017. The IF treatment gave the lowest value of all individual polyphenol content in tea leaf extracts, except GCG.
In general, the total content of these individual polyphenols was higher in second year than first year of sampling.

**Anti-anxioytic property of tea leaves grown organically and inorganically**

Anxiety behaviour in rat model was assessed as per the protocol described in Ennaceur et al. 2006. Different anxiety parameters as described in the subsection 2.3.4 were evaluated in each trial of the experiment.

**Delay/latency of entry of the rats to the inner area**

The data in Table 1 stated that the delay or latency of entry to the inner area were significantly higher in untreated AD rats (Group 4) as compared to the normal and sham rats (Groups 1 and 2) on both 14th and 21st day. Chronic treatment with tea extract (10,20, and 30 mg/kg IP) of different nutrient management practices showed a significant decline of this parameter in AD rats (Group 3) as compared to untreated AD rats (Group 4). Among the subgroups of Group 3 rats, the subgroup, treated with organic tea extract (OF) showed maximum reduction in latency as compared to inorganic tea extract (IF) in both days. Moreover, the different dosages of tea extract
showed a gradual decrease to this parameter on both 14th and 21st day. However, the celecoxib-treated AD rats (Group 5) had significantly lower latency than the tea extracts treated group (Group 3).

Frequency of entry to the inner area

Frequency of entry to the inner area was lowered in untreated AD rats (Group 4) as compared to that of respective normal and sham rats (Groups 1 and 2) on 14th and 21st day after ICV administration (Fig. 7). Treatment with tea extract (10, 20, and 30 mg/kg IP.) of different nutrient management practices were almost comparable and resulted a significant increase to the frequency as compared to untreated AD rats (Group 4) on both 14th and 21st day. However, in group 3 rats, sometimes, the OF treatment showed better result as compared to IF and IF+OF. Also, the different dosages of tea extract showed a gradual increase to this parameter on both 14th and 21st day. Thus, this result is establishing the fact that treatment with tea extracts of different nutrient management practices is effective to ameliorate the anxiety status in AD rats.

Amount of time spent in the inner area

Figure 8 stated that the total amount of time spent in the inner area by the untreated AD rats (Group 4) were 3.16 ± 0.21 min on 14th day and 3.64 ± 0.22 min on 21st day, which were significantly lower than rest groups of rats. Feeding tea extract (10, 20, and 30 mg/kg IP.) of different nutrient management practices in Group 3 showed a noteworthy increment in this parameter as compared to the untreated AD rats (Group 4). The tea extract treatments of different nutrients management did not differ significantly in increasing the amount of time spent in group 3 rats. However, the different dosages of tea extract in group 3 showed a gradual increase to this parameter on both 14th and 21st day. Moreover, this parameter in AD rats + Tea (30 mg/kg IP.) (group 3) was comparable with celecoxib-treated AD rats (Group 5) on the day 14th, though the latter group of rats spent significantly higher time than the former on 21st day.

Amount of time spent in the outer area

Figure 9 states that the amount of time spent in the outer area was remarkably higher in untreated AD rats (Group 4) on both 14th and 21st day as compared to the other experimental group of rats. Feeding of tea extract (10, 20, and 30 mg/kg IP) of different nutrient management in group 3 rats significantly decreased this parameter as compared to
The delay/latency of first approach to the object area

Table 1. Effect of tea extract and celecoxib on latency of first entry to the inner area (min) of different experimental groups of rats on 14th and 21st day after ICV administration.

| Groups            | Tea extract | Day 14th | Day 21th |
|------------------|-------------|----------|----------|
| Normal rats      | IF          | 0.11 ± 0.013<sup>a</sup> | 0.07 ± 0.016<sup>a</sup> |
|                  | OF          | 0.09 ± 0.013<sup>a</sup> | 0.05 ± 0.014<sup>a</sup> |
|                  | IF + OF     | 0.096 ± 0.007<sup>b</sup> | 0.08 ± 0.017<sup>b</sup> |
| Normal rats + ACSF | IF          | 0.12 ± 0.016<sup>a</sup> | 0.1 ± 0.03<sup>a</sup> |
|                  | OF          | 0.07 ± 0.01<sup>a</sup> | 0.09 ± 0.02<sup>a</sup> |
|                  | IF + OF     | 0.09 ± 0.006<sup>b</sup> | 0.07 ± 0.01<sup>a</sup> |
| AD rats + Tea (10) | IF          | 1.5 ± 0.19<sup>d</sup> | 1.19 ± 0.2<sup>d</sup> |
|                  | OF          | 1.25 ± 0.1<sup>c</sup> | 0.92 ± 0.076<sup>c</sup> |
|                  | IF + OF     | 1.53 ± 0.133<sup>d</sup> | 1.11 ± 0.16<sup>d</sup> |
| AD rats + Tea (20) | IF          | 1.18 ± 0.13<sup>d</sup> | 1.17 ± 0.15<sup>d</sup> |
|                  | OF          | 0.88 ± 0.2<sup>d</sup> | 0.79 ± 0.2<sup>d</sup> |
|                  | IF + OF     | 1.15 ± 0.18<sup>d</sup> | 1.13 ± 0.13<sup>d</sup> |
| AD rats + Tea (30) | IF          | 1.13 ± 0.11<sup>d</sup> | 0.9 ± 0.13<sup>d</sup> |
|                  | OF          | 0.71 ± 0.08<sup>d</sup> | 0.69 ± 0.1<sup>d</sup> |
|                  | IF + OF     | 1.0 ± 0.14<sup>d</sup> | 0.99 ± 0.09<sup>d</sup> |
| AD rats          |             | 5.9 ± 0.3<sup>e</sup> | 7.8 ± 0.34<sup>e</sup> |
| AD rats + Celecoxib (10) |         | 0.9 ± 0.07<sup>b</sup> | 0.44 ± 0.04<sup>b</sup> |

Each superscript present statistically significant values at *p* < 0.05. The groups are: Normal (Group 1): Tea extract was not fed and no ICV administration; Normal + ACSF (Group 2): Only ICV artificial cerebrospinal fluid (ACSF) was administered; AD rats + Tea (Group 3): tea extract was fed to ICV colchicine administered rats; Untreated AD rats (negative control): ICV colchicine administered rats without feeding of tea extracts; AD rats + Celecoxib (positive control): the NSAID celecoxib was given to ICV colchicine administered rats. OF: organic fertilizer, IF: inorganic fertilizer, IF + OF: integrated fertilizer.

The untreated AD rats (group 4). On 14th day, this parameter was comparable between tea extract fed AD rats (Group 3) and celecoxib-treated AD rats (Group 5). Moreover, the dosage of 30 mg/kg IP. showed better improvement in total serum CORT level as compared to the other two dosages (10 and 20 mg/kg IP.) in group 3 rats.

The delay/latency of first approach to the object area was highest (8.48 ± 0.19 min on day 14th and 9.68 ± 0.17 min on day 21st) in untreated AD rats (Group 4), which was significantly higher than rest groups on 14th as well as on 21st day after ICV administration of colchicine (Fig. 10). The latencies were (4.75 ± 0.19 min, 4.6 ± 0.13 min, 4.67 ± 0.12 min) and 2.9 ± 0.17 min, respectively, on 14th and (4.05 ± 0.11 min, 3.9 ± 0.1 min, 4.05 ± 0.13 min) and 2.32 ± 0.2 min, respectively, on 21st day in tea extract (10 mg/kg IP.) treated AD rats (Group 3) and celecoxib-treated AD rats (Group 5), which were significantly higher than normal and normal + ACSF rats (Group 1 and 2). Feeding of tea extracts (10, 20, and 30 mg/kg IP.) of different nutrient management did not bring any significant variation in the latency value among the Group 3 rats though the dosage of 20 mg/kg and 30 mg/kg showed better improvement in ameliorating this parameter as compared to dosage of 10 mg/kg. However, the celecoxib-treated AD rats (Group 5) showed a significant reduction of this parameter over tea extract-treated AD rats (Group 3) on both days (day 14th and day 21st).

Frequency of approach to the object area

Figure 11 reveals that the frequency of approach to the object area was lowest (3.42 ± 0.19 on day 14th and 2.82 ± 0.15 on day 21st) in untreated AD rats (Group 4) and this was remarkably lower than rest of the rat groups on both days. Feeding tea extracts (10, 20, and 30 mg/kg IP) of different nutrient management practices showed a significant increase in the frequencies in group 3 rats as compared to the untreated AD rats (Group 4) on both 14th and 21st day, though the values were significantly lower than normal rats (Group 1 and 2). The tea extracts of different nutrient management were comparable in increasing the frequency in group 3 rats on 14th day. However, dosages of 20 mg/kg and 30 mg/kg IP. in group 3 showed a significant improvement in alleviating this parameter as compared to the dose of 10 mg/kg IP. on day 21st. The Celecoxib-treated rats (Group 5) showed a significant increase in this parameter as compared to the tea extract-treated AD rats (Group 3) on both 14th and 21st day and this was also comparable to normal rats on 14th day.

Serum corticosterone level

The serum corticosterone (CORT) level in untreated AD rats (Group 4) were 82 ± 2.06 ng/ml on 14th day and 79.6 ± 3.19 ng/ml on 21st day, which were significantly lower than rest group of rats (Fig. 12). The tea extracts (10, 20, and 30 mg/kg IP) treatment of different nutrient management practices in group 3 rats showed a significant improvement in total serum CORT level as compared to the untreated AD group (Group 4). Among the different nutrient management, OF and IF + OF were better effective on 14th day and all (OF, IF + OF and IF) were comparable on 21st day in increasing this parameter in Group 3 rats. Moreover, the dosage of 30 mg/kg IP. showed better result in improving this parameter as compared to the other two dosages (10 and 20 mg/kg) in group 3 rats on both of
the days. The Celecoxib-treated AD rats (Group 5) had significantly higher serum CORT level as compared to the tea extract-treated AD rats (Group 3) on 21st day, but they were comparable on 14th day.

**Discussion**

Colchicine is a plant-derived alkaloid which acts as a...
A microtubule disrupting agent. It irreversibly binds with the microtubules leading to the depolymerization of the microtubules, thereby inhibiting their association. This will eventually lead to disruption of apoptosis of cerebellar cells and cholinergic neurons, dysfunction of cytoskeletal and axonal transport (Kumar et al. 2009). Colchicine is responsible for free radical generated neuroinflammation which eventually leads to cognitive impairment in rodents (Kumar et al. 2009). Moreover, it was reported by several studies that colchicine-mediated neurodegeneration is specifically mediated by cyclooxygenase (COX) induced neuroinflammation (Sil and Ghosh 2015). There are mainly two varieties of COX, COX-1 and COX-2 which are responsible for neuroinflammation particularly in the hippocampus. Also, it has been reported that excessive amount of prostaglandin (PG) is known to cause neuroinflammation (Lima et al. 2012).

**Fig. 9** Impact of tea extract of different field level nutrient management and celecoxib on amount of time spent in the outer area in different experimental groups of rats. IF inorganic fertilizer, OF organic fertilizer, IF + OF integrated fertilizer. Each bar represents mean ± SE value, and different letters showed statistical significance at $p < 0.05$. The groups are: Normal (Group 1): Tea extract was not fed and no ICV administration; Normal + ACSF (Group 2): Only ICV artificial cerebrospinal fluid (ACSF) was administered; AD rats + Tea (Group 3): tea extract was fed to ICV colchicine administered rats; Untreated AD rats (negative control): ICV colchicine administered rats without feeding of tea extracts; AD rats + Celecoxib (positive control): the NSAID celecoxib was given to ICV colchicine administered rats.

**Fig. 10** Impact of tea extract of different field level nutrient management and celecoxib on latency of first approach to the object area in different experimental groups of rats. IF inorganic fertilizer, OF organic fertilizer, IF + OF integrated fertilizer. Each bar represents mean ± SE value, and different letters in the bars showed statistical significance at $p < 0.05$. The groups are: Normal (Group 1): Tea extract was not fed and no ICV administration; Normal + ACSF (Group 2): Only ICV artificial cerebrospinal fluid (ACSF) was administered; AD rats + Tea (Group 3): tea extract was fed to ICV colchicine administered rats; Untreated AD rats (negative control): ICV colchicine administered rats without feeding of tea extracts; AD rats + Celecoxib (positive control): the NSAID celecoxib was given to ICV colchicine administered rats.
PG such as PG1 and PG 2 are responsible for neurotoxicity and can stimulate the release of glutamate from astrocyte. In case of AD, the concentration of COX increases (Hoozemans et al. 2008) and COX is a rate limiting enzyme in the synthesis of prostanoids from arachidonic acid (Brock et al. 1999). COX 1 and COX 2 are the two rate limiting enzymes in prostaglandin biosynthesis. COX 1 is responsible for maintaining the normal cellular biosynthesis and it
serves as housekeeping enzyme. On the other hand, COX 2 has inducible isoforms and overexpression of COX 2 is rapidly induced by many growth factors, tumour promoters, and carcinogens (Hussain et al. 2003). In AD, COX 2 is generated from Aβ peptides and acts as proinflammatory cytokine which is responsible for neuroinflammation. In this present study, ICV colchicine administered rat models of AD produced amyloid β plaques in different areas of brain leading to neurodegeneration showing resemblance with AD pathology (Sil and Ghosh 2015). Several studies showed that memory dysfunction in ICV colchicine administered rat models of AD exhibited comparability with AD patients (Kumar et al. 2002; Kumar et al. 2006, 2007). Thus, ICV colchicine administered animal model is contemplated as a potent model of AD by many researchers and has been taken as a model for the discovery of new therapeutic agents for AD (Kumar et al. 2006, 2007; Pitchaimani et al. 2012). Therefore, rat models of AD prepared from ICV colchicine administration is relevant for studying different behavioural and pathological characteristics of AD. Neuroinflammation and oxidative stress due to free radical damage are most common causes of AD. Tea catechins can enhance the resistance of the cells to oxidative stress by its free radical scavenging and iron chelating property (Salah et al. 1995; Lin et al. 1998). Thus, special interest should be given to the therapeutic effects of nutritional antioxidants and free radical scavengers in preventing neurodegenerative diseases (Gotz et al. 1990; Halliwell 1992; Mandel et al. 2004; Weinreb et al. 2004). AD patients have been reported with anxiety along with dementia for a long period of time. Though most of the animal models of AD showed memory impairment, inconsistency with anxiety behaviour can be seen to some extent. This inconsistency in anxiety behaviour is affected not only by different animal models of AD but also by the method of study (Ennaceur et al. 2006). The results of the present study exhibited different parameters of anxiety status in rat models of AD. This result demonstrates anxiolytic behaviour in group 4 rats (untreated AD rats). But the anxiolytic behaviour was reversed after feeding with tea extract for 14 and 21 days. Thus, the result of the present study shows improvement in the anxiolytic behaviour in AD rat models after treatment with organic and inorganic tea extract. It was also found that organic tea extract treatment showed better improvement in anxiolytic behaviour in rat models of AD than inorganic tea extract treatment. The anxiolytic behaviour in the untreated AD rats (group 4) may be the cumulative effect of the damaged function of neuronal areas in brain which were contrived by colchicine administration. The recovery from neurodegeneration of these brain areas after chronic feeding of tea extract may be the reason behind the improvement of anxiolytic behaviour presented in this study. The major catechins of tea leaves can traverse the blood brain barrier (Weinreb et al. 2004) and can protect colchicine-induced neurodegeneration. The decreased serum corticosterone level was seen in the untreated AD rats and the feeding of 10, 20, and 30 mg/kg body weight of organic and inorganic tea leaf extract stemmed in almost complete recovery of corticosterone on 14th and 21st days of study. This convalescence of corticosterone in tea extract-treated AD rats can be supported by the recovery from anxiolytic behaviour. The hypothalamo-hypophysial-adrenal (HPA) axis which is mainly required in the regulation of CORT secretion, may be non-functional in untreated AD rats due to the colchicine produced damage of the neuronal cells and free radical generated oxidative stress (Herman et al. 2016). Hindrance from this neuronal and free radical damage by tea polyphenols may be helpful to recuperate the normal activity of the HPA axis which is corroborated by this study. Earlier studies demonstrated that organic tea has higher amount of polyphenol content and better antioxidant property as compared to tea leaves grown under inorganic nutrient management practice (Bagchi et al. 2020). In this present study, it was found that organic tea can be more effective in ameliorating the anxiolytic behaviour in rat model of AD as compared to tea leaves grown under inorganic fertilizer management. Thus, it can be stated that this study is the first of its category which revealed the comparison of the anxiety status in rat models of AD, treated with tea leaf extracts grown under organic and inorganic nutrient management practices.

Conclusions

Tea (Camellia sinensis) grown under organic nutrient management resulted in improved quality as total phenolics content increased by approximately 30% as compared to inorganic nutrient management in subtropical climate. The July harvesting under high humidity and sufficient water availability could give better tea quality as compared to May and September harvest. Accumulation of the secondary plant metabolites i.e., EGC, EC, ECGC, and ECG in tea leaves were increased with organic nutrients, but decreased with inorganic nutrients management as compared to no fertilizer application. In animal model experiment, feeding rats with organic tea leaf extract acted as a favourable adjuvant or pharmacological agent to alleviate the anxiolytic symptoms of AD in the rats. Moreover, the effectiveness of the organic tea extract was comparable with the traditional nonsteroidal anti-inflammatory drug ‘celecoxib’ for improvement in the anxiolytic behaviour of the AD rats.

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Data availability The datasets generated during the current study are available.

Declarations

Conflict of interest The authors declare no conflict of interest among themselves.

Ethical approval For animal experiments, guidelines of CPCSEA, were followed. The ethical standard for animal experiments was approved by the animal ethical committee of the Institute of Reproductive Medicine (IRM) Kolkata, India (473/01/a/CPCSEA dated 5/9/2013).

References

Bagchi A, Swain DK, Mitra A (2020) Neuroprotective effect of organic and inorganically grown tea on oxidative damage in rat model of Alzheimer’s disease. Adv Tradit Med 20:439–450. https://doi.org/10.1007/s13596-020-00428-8

Benedikz E, Kloskowska E, Winblad B (2009) The rat as an animal model of Alzheimer’s disease. J Cell Mol Med 13:1034–1042. https://doi.org/10.1111/j.1582-4934.2009.00781.x

Bonda DJ, Wang X, Perry G, Nunomura A, Tabaton M, Zhu X, Smith MA (2010) Oxidative stress in Alzheimer disease: a possibility for prevention. Neuropharmacology 59:290–294. https://doi.org/10.1016/j.neuropharm.2010.04.005

Brock TG, McNish RW, PetersGolden M (1999) Arachidonic acid is preferentially metabolized by cyclooxygenase 2 to prostacyclin and prostaglandin e2. J Biol Chem 74:11660–11666

Chan EWC, Lim YY, Wong LF et al (2008) Antioxidant and tyrosinase inhibition properties of leaves and rhizomes of ginger species. Food Chem 109:477–483. https://doi.org/10.1016/j.foodchem.2008.02.016

Ennaceur A, Michalikova S, Chazot PL (2006) Models of anxiety: responses of rats to novelty in an open space and an enclosed space. Behav Brain Res 171:26–49. https://doi.org/10.1016/j.bbr.2006.03.016

Galasko D, Montine TJ (2010) Biomarkers of oxidative damage and inflammation in Alzheimer’s disease. Biomark Med 4:27–36. https://doi.org/10.2217/bmm.09.89

Glennon GG (1983) Alzheimer’s disease. The commonest form of amyloidosisis. Arch Pathol Lab Med 107:281–282

Hoozemans JJ, Rozemuller JM, Van Haastert ES, Veerhuis R, Eikelenboom P (2008) Cyclooxygenase1 and 2 in the different stages of Alzheimer’s disease pathology. Curr Pharm Des 14:1419–1427

Kumar A, Segal N, Padi SS, Naidu PS (2007) Effect of curcumin on intracerebroventricular colchicine-induced cognitive impairment and oxidative stress in rats. J Med Food 10:486–494. https://doi.org/10.1089/jmf.2006.076

Kumar A, Segal N, Padi SS, Naidu PS (2008) Protective effect of quercetin against ICV colchicine induced cognitive dysfunctions and oxidative damage in rats. Phytother Res 22:1563–1569. https://doi.org/10.1002/ptr.2454

Kumar A, Dogra S, Prakash A (2009) Neuroprotective effects of Curcilla asiatica against intracerebroventricular colchicine-induced cognitive impairment and oxidative stress. Int J Alzheimers Dis 2009:972178

Kumar A, Dogra S, Prakash A (2010) Protective effect of naringin, a citrus flavonoid, against colchicine induced cognitive dysfunction and oxidative damage in rats. J Med Food 13:976–984. https://doi.org/10.1089/jmf.2009.1251

Lima IV, Bastos LFS, Limborch M, Fiebich BL, Pinheiro AC (2012) Role of prostaglandins in neuro inflammatory and neurodegenerative diseases. Mediat Inflamm. 2012:946813

Lin AM, Chyi BY, Wu LY, Hwang LS, Ho LT (1998) The antioxidative property of green tea against iron-induced oxidative stress in rat brain. Chin J Physiol 41:189–194

Manuel S, Weinreb O, Amit T, Youdim MB (2004) Cell signalling pathways in the neuroprotective actions of the green tea polyphenol (−)epigallocatechin-3-gallate: implications for neurodegenerative diseases. J Neurochem 88:1555–1569. https://doi.org/10.1046/j.1471-4159.2003.02291.x

Masters CL, Simms G, Weinman NA, Mulhaug G, McDonald BL, Beyreuther K (1985) Amyloid plaque core protein in Alzheimer disease and Down syndrome. Proc Natl Acad Sci U S A 82:4245–4249. https://doi.org/10.1073/pnas.82.12.4245

Mishra S, Mishra M, Seth P, Sharma SK (2011) Tetrahydro curcumin confers protection against amyloid beta-induced toxicity. NeuroReport 22:23–27. https://doi.org/10.1097/WNR.0b013e3283e1414

Muthiah B, Essa MM, Lee M, Chauhan V, Kaur K, Chauhan A (2014) Dietary supplementation of walnuts improves memory deficits and learning skills in transgenic mouse model of Alzheimer’s disease. J Alzheimer’s Dis 42:1397–1405. https://doi.org/10.3233/JAD-140675

Palit S, Ghosh BC, Dutta Gupta S, Swain DK (2008) Studies on tea quality grown through conventional and organic management practices: its impact on antioxidant and anti diarrhoeal activity. Trans ASABE 51:2227–2238. https://doi.org/10.10301/2013.25376

Pitchaimani V, Arumugam S, Thandavarayan RA (2012) Nootropic activity of acetaminophen against colchicine induced cognitive
Impairment in rats. J Clin Biochem Nutr 50(3):241–244. https://doi.org/10.3164/jcbn.11-73

Raghavendra M, Maiti R, Kumar S, Acharya SB (2008) Role of Ocimum sanctum in the experimental model of Alzheimer’s disease in rats. Int J Green Pharm 3(6–15):3. https://doi.org/10.4103/0973-8258.49368

Rezai-Zadeh K, Shytle D, Sun N et al (2005) Green tea epigallocatechin-3gallate (EGCG) modulates amyloid precursor protein cleavage and reduces cerebral amyloidosis in Alzheimer transgenic mice. J Neurosci 25:8807–8814. https://doi.org/10.1523/JNEUROSCI.1521-05.2005

Salah N, Miller NJ, Paganga G, Tijburg L, Bolwell GP, Rice-Evans C (1995) Polyphenolic flavanols as scavengers of aqueous phase radicals and as chain-breaking antioxidants. Arch Biochem Biophys 322:339–346. https://doi.org/10.1006/abbi.1995.1473

Schrag M, Mueller C, Zabel M, Crofton A, Kirsch WM, Ghribi O, Squitti R, Perry G (2013) Oxidative stress in blood in Alzheimer’s disease and mild cognitive impairment: a meta-analysis. Neurobiol Dis 59:100–110. https://doi.org/10.1016/j.nbd.2013.07.005

Sil S, Ghosh TK (2015) Amelioration of anxiolytic behavior in intracerebroventricular colchicine injected rats by naproxen. Asian J Pharm Clin Res 8(5):189–196. https://innovareacademics.in/journals/index.php/ajpcr/article/view/7230

Vloeberghs E, Van Dam D, Franck F, Staufenbiel M, De Deyn PP (2007) Mood and male sexual behaviour in the APP23 model of Alzheimer’s disease. Behav Brain Res 180:146–151. https://doi.org/10.1016/j.bbr.2007.03.002

Weinreb O, Mandel S, Amit T, Youdim MB (2004) Neurological mechanisms of green tea polyphenols in Alzheimer’s and Parkinson’s diseases. J Nutr Biochem 15:506–516. https://doi.org/10.1016/j.jnutbio.2004.05.002

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