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Organic Farming Practice for Quality Improvement of Tea and Its Anti Parkinsonism Effect on Health Defense

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Abstract

Tea is an important beverage consumed worldwide. It is a source of important secondary metabolites like catechins monoterpenoids, carotenoids etc. Catechins are responsible for the beneficial health effects of tea. Over the decades conventional tea cultivation practice using synthetic fertilizer and pesticides has jeopardize the soil health particularly due to micronutrient deficiency, instability in yield and reduced product quality. Such threat has led to emergence of organic farming practice for improvement of crop yield and product quality that finally has an impact on human health defence. Field experiments conducted using organic farming practice has shown improved soil health by improving availability of micronutrients which in terms improved crop yield and quality of tea. The content of secondary phenolics compounds like total phenolics, GCG, EGCC, ECG was higher in when tea grown organically. Antioxidant property of tea extract from different farming practice was studied by DPPH method resulting higher radical scavenging capacity than tea grown conventionally. From the pharmacological study the organic tea extract has shown better control of Parkinsonism in two different animal model experiments. Organic tea extract exhibited better performance in reduction of Superoxide dismutase and catalase activity in brain in Parkinson's disease induced in test mice model than the tea extract obtained from conventional farming practice. The value of SOD and catalase activity in MPTP induced mice model given tea extract grown in control, conventional farming, vermicompost and vermicompost+vermiwash treatment are respectively 1.31 ± 0.16^{c}, 1.8 ± 0.16^{d}, 0.95 ± 0.14^{e}, 0.79 ± 0.06; and 0.98 ± 0.07^{c}, 1.10 ± 0.07^{d}, 0.78 ± 0.07^{e}, 0.69 ± 0.05^{a} for Tv 25 variety. Similar result is obtained for Tv1 variety also.The present study generated information on soil, crop performance, yield and quality of tea related to health defense, which should be regarded as valuable information for a perennial crop like tea.

Keywords: Antioxidant property; Organic farming; Phenolics; Secondary metabolites; SOD; Catalase

Introduction

In age old tea farming, tea crop has undergone a phasic change from organic to chemical production system. In recent years, chemical farming practice has brought drastic changes in soil ecology, crop productivity and quality, in tea farming system. To combat such adverse consequences and regain of lost resources, organic farming practice will be an option.

Initiation of organic cultivation practice is involved with consideration of certification standards, adaptation of organic inputs like fertilizer and pesticide, cultural practices and harvest and postharvest processes of tea. Research finding reveals that any organically produced food including tea possess high quality than conventional one [1,2]. This concern arises because conventional agricultural practices utilize levels of pesticides and fertilizers that can result in a disruption of the natural production of secondary metabolites in the plant [3]. There are evidences indicating that organic crops mostly contain higher levels of phenolic metabolites than conventionally grown crops, and very few studies have directly addressed this issue [4], particularly for tea crops. In organic tea, the quality is generally considered with respect to minimum or zero chemical residues (heavy metal and pesticides) in final product, high level of secondary metabolites including polyphenols. Evaluation of these parameters in marketed organic tea is important.

The important chemical constituents of tea include polyphenols (20-30%) flavonols and flavonol glycosides (3-4%) caffeine (3-4%) theobromine (0.2%), theophylline (0.5%), amino acids (4% to 5%), cellulose and hemicellulose (4% to 7%), chlorophylls and other pigments (0.5% to 0.6%), and volatiles (0.01% to 0.02%). The major beneficial physiological effect of tea consumption is associated with the presence of eight naturally occurring tea catechins: (+)-catechin, (-)-epicatechin, (-) gallocatechin, (-)-epigallocatechin, (-)-catechin gallate, (-)-gallocatechin gallate, (-)-epicatechin gallate, and (-)-epigallocatechin gallate. Tea catechins are effective scavengers of free radicals [5], with more effective cate-chins having a galloyl moiety at C3 [6] and a trihydroxy structure in the B ring [7]. These natural products also have numerous potentially beneficial medicinal properties, including inhibition of carcinogenesis, tumorigenesis, and muta-genesis. Additionally, they have antibacterial, antiviral, antiarteriosclerotic, hypocholesterolaemic, antidiarrhoeal, and antiallergic properties and have been demonstrated to induce apoptosis and inhibit platelate aggregation [8].

The present investigation was carried out to assess the organic tea quality based on catechins and other biochemical compounds and to determine their radical scavenging capacity and anti-Parkinsonism property. The pharmacological actions of green tea extract and its polyphenols, have ability to penetrate the brain, fulfilled the requirements for a potential neuroprotective action. Neuroprotective effects of green tea extract and its isolated polyphenol (Epigallocatechin

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gallate-EGCG) can be proved by their effect on striatal DA depletion and neuronal loss in substantia nigra of mice induced by N-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) [8,9]. The present investigation was carried out to assess the organic tea quality based on catechins and other biochemical compounds and to determine their radical scavenging capacity and anti-Parkinsonism property.

Material s and Methods

Animals

Inbred adult male Albino mice (30-35 g) from the Institute colony were used in this study. The animals were kept in a 12 h light/dark cycle, at 22°C and 60% humidity, with food and water *ad libitum*. The experimental protocols met with the National Guidelines on the *Proper Care and Use of Animals in Laboratory Research* (Indian National Science Academy, New Delhi, 2000) and were approved by the Animal Ethics Committee of the Institute (Approval No. 164/08-2002)

Chemicals

MPTP, Thiobarbituric acid (TBA), reduced glutathione, 3,5-dithio-bis- nitrobenzoic acid (DTNB), Ethanol, HPLC-grade methanol, perchloric acid, anthrone reagent, petroleum ether, sulfuric acid, sodium hydroxide, sodium carbonate, trifluroacetic acid, ferrous ammonium sulfate, sodium chloride, Folin-Ciocalteu's phenol reagent, gallic acid monohy-drate, standard (-)-epigallocatechin gallate [(-)-EGCG], (-)-gallocatechin gallate[(-)-GCG], (-)-epicatechin gallate [(-)-ECG], 2,2-diphenyl-1-picrylhydrazyl (DPPH) were obtained from Sigma chemical Co. (St. Louis, MO. USA)

Agricultural condition and soil characteristics

A field experiment was conducted on fallow land that had not been cultivated for more than 50 years. The soil at the experimental site was acid laterite (type Haplustalf, pH 5.4), sandy-loam in texture, which is low in organic carbon (2.8 g/kg), and available N (73 mg/kg), P (4 mg/kg) and K (16mg/kg). Two organic fertilizations, i.e., vermicompost (V) and vermicompost+vermiwash (V+Vw), one inorganic fertilization (IF), and one control treatment were used for two cultivars of tea (TV1 and TV25) in the experimentation. The experiment was laid in a split-plot design with four repli-cations, where cultivar was allocated to the main plot and fertilizer source to the subplot of the design. The treatments receiving organic and inorganic sources of fertilization were added yearly with nutrients as shown in Table 1.

Determination of tea quality parameter

The quality parameters such as catechin content of the tea leaves were analyzed.

Determination of Catechin

Fresh tea leaves (0.5 g) were crushed and extracted with 100 ml of 70% methanol in a Soxhlet apparatus for 45 min. The residue was redissolved in 1 ml of 70% methanol. Catechin standards selected for this study were (-)-epigallocatechingallate [(-)-EGCG], (-)-gallocatechin gallate [(-)-GCG], and (-)-epicatechin gallate [(-)-ECG]. Standard solutions were prepared by dissolving 2 mg of each catechin in 2 ml of 70% methanol. Six different calibration levels were used to prepare the standard calibration plot of each catechin standard.

Calibration standards in the concentration range of 10–300 gm/ml were prepared by diluting the stock solution of the respective catechin with aqueous methanol (50:50, v/v). Each calibration solution was injected into HPLC in triplicate. The calibration curve was drawn by plotting the peak area against the concentration of the compound. The calibration curves, characterized by slope and intercept, were used to determine the concentration of respective catechins in the sample. Chromatography was performed on a Waters HPLC system (Waters Corp., Milford, Mass.), which consists of a 1500 series HPLC pump, 2487 dual-wavelength absorbance detector, and Breeze software (ver. 3.2) for instrument control and data processing. A C18 reverse-phase HPLC column (Synergi Hydro-RP, Phenomenex, and Torrance, Cal.) was used for the study. The column has an internal particle size of 5 µm with 250 mm length and 4.6 mm internal diameter. A linear isocratic solvent system consisting of aqueous trifluoroacetic acid (1 mM) and methanol (17:8) at a flow rate of 1 ml/min for 20 min at room temperature was used to elute the tea catechins. The identification of each phenolic compound was confirmed by comparing retention time and UV spectra with external standards procured from Sigma Aldrich.

Antioxidant activity

Antioxidant activity of tea leaves was determined as follows: a) 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical assay: The antioxidant activity of tea leaf extracts was measured in terms of hydrogen donating or radical scavenging ability using the s Table radical DPPH [10,11]. Stock solutions of the tea leaf extracts obtained from different treatments were prepared by dissolving 1 mg of lyophilized extract in 1 ml of 60% methanol. A fresh solution of DPPH in methanol (6 × 10-5M) was prepared before the measurements. A methanolic solution (0.1 ml) of sample of various concentrations (50, 100, 150, 200, and 300 µgm/ml prepared from the stock solution) was placed in a cuvette, and 2.9 ml of DPPH was added. The mixture was shaken vigorously, and absorbance measurements were recorded immediately. The decrease in absorbance at 515 nm was observed continuously at 5 min intervals until the reaction reached a plateau. Methanol was used as blank. Radical scavenging activity was calculated as shown in equation 2 [12]:

$$\text{Percent inhibition} = [(AB-AA)/AB] \times 100 \quad (2)$$

Where AB=absorption of blank sample (t=0)

AA=absorption of tested extract solution (t=T).

b) Experimental Animals: The experiments were carried out with male albino rats (Sprague Dawley strain) each weighing 150-200 g and male ICR mice (25-30 g) bred in the institute's animal house. The animals were housed under conditions of 22°C ± 2°C, 50 ± 10% humidity and 12 h light/dark cycle. During maintenance, the animals received a diet of food pellets (fortified with minerals and vitamins), water *ad libitum*. Although food uptake was not fully moni-tored, no major change was observed in body weight between treated and

Agricultural practice	Fertilizer	Description	Rate	Timing
conventional	Standard commercial chemical fertilizers: urea, single super phosphate, and muriate of potash	N_2O, P_2O_5 and K_2O	200:60:120 kg/ha	4 splits in a year
organic	Vermicompost		11.1/ha	4 splits in a year
	vermiwash		650 L/ha	4 splits in a year

Table 1: Fertilizer usage records for tea crop.

untreated animals. Before drug administration the animals were subjected to fasting overnight (18 h).

Preparation of hot water extract of tea

An amount of 10 g of processed tea obtained from tea plants subjected to different to different fertilizer treatments and sampled at three different growing seasons i.e., April, June, and November was soaked separately in 100 ml of boiling water for 2 min and filtered. The filtrate was designed as 'green tea extract (GTE)" and accordingly 24 different GTEs were prepared for the experiment. The GTE was fed intragastrically by mouth (1ml/100 g) to animals at a temperature of 37 ± 1°C. It was observed that 0.28-0.3 g of dried material was present in 1% solution of GTE The mice were divided into five groups of six animals each. The first group of mice was kept as control. The mice of the second group received injections of MPTP (i.p., 20 mg/kg body weight), one each on 4 consecutive days. The GTE was not given to the control group. The mice from the third group were injected with MPTP (i.p., 20 mg/kg body weight), one each on 4 consecutive days, followed by intragastrically administration of tea grown conventionally [13] for 28 days. The fourth group of mice injected with MPTP (i.p., 20 mg/kg body weight), one each on 4 consecutive days, followed by intragastrically administration of tea grown in vermicompost for 28 days. The mice of the fifth group injected with MPTP (i.p., 20 mg/kg body weight), one each on 4 consecutive days, followed by intragastrically administration of tea grown in vermicompost+vermiwash for 28 days. At the end of the experiment (28 days after the first MPTP injection), mice were killed by cervical dislocation on the following day, and their brains were dissected via the method described by Glowinski and Iversen [14] to procure the midbrains for the analyses of SOD [15], CAT [16].

Statistical analysis

All the data was expressed as the means ± SD of a number of experiments (n=6). The statistical significance was evalu-ated by the one-way analysis of variance (ANOVA) using SPSS version 10.0, and individual comparisons were ob-tained using Duncan's Multiple Range Test (DMRT). Values were considered statistically significant if not sharing a common superscript letter or symbol. All the results proved to be statistically significant at $p<0.05$.

Results

Polyphenols are biochemical constituents that affect tea quality. The effect of organic and inorganic sources of fertilizers on synthesis of phenolic antioxidants in tea plants is shown in Table 2. Significantly lower total phenolics content was observed in leaves of tea plant grown with the IF treatment (197.45 mg/g in GAE) as compared to the organic treatments, i.e., V (279.05 mg/g in GAE), V+Vw (288.26 mg/g in GAE), and C (251.65 mg/g in GAE). A similar result was also reported for other crops like peach and pear, where organically grown crops had higher levels of total phenol-ics than those grown under conventional agricultural practices [17]. It was observed that the total contents of [(-)-EGCG], [(-)-GCG], and [(-)-ECG] were higher in V and V+Vw than in IF. Increase in polyphenol content of organically grown field crops has also been confirmed in previous studies [18-20]. Tea catechins are potent antioxidants, which modulate key biological pathways in vivo in mammals [21,22]. In order to determine the radical scavenging/ antioxidant activity of tea extracts DPPH assay [23] was used. The assay is based on the measurement of the scavenging ability of antioxidants of a free radical, 2,2-diphenyl-1-picrylhydrazyl (DPPH), transforming it to the corresponding hydrazine when it reacts with hydrogen donors [24]. From a methodological point of view, the DPPH method is recommended as easy and accurate with regard to measuring the antioxidant activity of fruit, vege Table juices, and plant extracts [25]. The results are highly reproducible and comparable to other free radical scavenging methods [26]. The radical scavenging ability of lyophilized tea extracts at various concentrations (50, 100, 200, and 300 gm/ml) is presented in Table 3. A higher radical scavenging activity in tea leaf extracts of the V+Vw

Variety	Treatment(a)	Concentration of catachin (mg g- tea leaves)			Total content of catachin (mg g- tea leaves)	Percentage of Increase/Decrease of catachin Content over control treatment
		EGCG	GCG	ECG		
TV 25	C	7.65	3.83	3.31	14.79	--
	IF	2.41	3.30	1.61	7.32	50.5(-)
	V	9304	4.26	3.07	16.37	10.7(+)
	V+VW	8.20	6.94	3.55	18.69	26.4(+)
TV1	C	6.35	3.13	3.13	12.61	--
	IF	1.76	2.15	1.03	4.94	60.82(-)
	V	7.96	4.37	3.61	15.94	26.4(+)
	V+VW	8.55	4.71	3.22	16.48	30.7(+)

C: Control; IF: Chemical Fertilizer; V: Vermicompost; V+VW: Vermicompost+Vermiwash

Table 2: Catechin content (mg/gm) in tea leaf as influenced by fertiliser source.

	Concentration of tea extract (µg/ml)			
	50	100	200	300
Variety				
TV 25	22.3	36.3	58.6	58.4
TV 1	23.7	36.9	60.7	60.5
LSD (5%)	NS	NS	NS	NS
Fertilizer				
C	22.1	34.6	57.4	57.5
IF	22.2	31.5	47.6	47.7
V	23.7	38.4	67.1	67.0
V+VW	25.9	42.1	74.1	74.4
LSD (5%)	2.1	2.5	4.1	4.3

[a] C: Control; IF: Chemical Fertilizer; V: Vermicompost; V+VW: Vermicompost+Vermiwash; LSD: Least Significant Difference; NS: Not Significant

Table 3: Scavenging effect for DPPH radical (percent inhibition) of tea leaf extracts as influenced by fertilizer sources.

Treatment[a]	SOD [U/mg protein]	CAT [U/mg protein]
control (No MPTP)	0.76 ± 0.06[a]	0.56 ± 0.04[a]
MPTP	0.98 ± 0.15[b]	1.31 ± 0.10[b]
TV 25		
MPTP+C	1.31 ± 0.16[c]	0.98 ± 0.07[c]
MPTP+IF	1.8 ± 0.16[d]	1.10 ± 0.07[d]
MPTP+V	0.95 ± 0.14[e]	0.78 ± 0.07[e]
MPTP+V+VW	0.79 ± 0.06[a]	0.69 ± 0.05[a]
TV 1		
MPTP+C	1.38 ± 0.16[c]	1.02 ± 0.07[c]
MPTP+IF	2.0 ± 0.16[d]	1.17 ± 0.07[d]
MPTP+V	0.99 ± 0.14[e]	0.82 ± 0.07[e]
MPTP+V+VW	0.80 ± 0.06[f]	0.72 ± 0.05[f]

Table 4: Changes in the activities of SOD and CAT in the control and experimental mice.

treatment was noted, followed by V, C, and IF for both varieties. Radical scavenging capacity of the tea extracts was increased up to the concentration of 200 µgm/ml

Neuroprotective in vivo studies using N-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) have shown that green tea extract possess highly potent activities in preventing striatal dopamine depletion in mice as well as substantia nigra dopaminergic neuron loss in- duced by the parkinsonism-inducing neurotoxin. In the present invest igation, we attempted to evaluate the effect of tea extracts collected from different fertilizer treatments on anti-Parkinsonism activity using conventional mice models of parkinson's disease. Green tea extract prepared from plant grown under different fertilizer treatments exhibited differential anti Parkinsonism activity. The green tea extract appears to act by reducing the activities of SOD and catalase activities in MPTP induced mice brain. As shown in Table 4, green tea extracts from the V+Vw and V treatments have better SOD and catalase activity reducing action action than extract from the IF treatment.

Table 4 depicts the levels of activities of SOD and CAT in the midbrain of mice from the control and experimental groups for both of the varieties. The activities of SOD and CAT in the midbrain were significantly elevated in MPTP-treated animals relative to the control group. The results of the present investigation as stated above showed that organic tea has better therapeutic potential as an anti-Parkinsonism agent with higher content of total phenolics, flavanols, antiradical scavenging capacity as compared to tea grown conventionally.

Discussion

The disruption of subtantia nigra found in midbrain may result in movement disorders such as those seen in Parkinson's disease [27]. Treatment with tea might reverse the alterations in locomotor and muscle coordination in 6-hydroxy dopamine-induced parkinsonic rats via an unknown mechanism [28]. Increased levels of malondialdehyde (MDA) and lipid hydroperoxides [29,30], which are markers of oxidative damage, usually found in the midbrains of PD patients. In the brain, MPTP is metabolized to its active toxin, MPP+, by the action of monoamine oxidase (MAO). This enzymatic conversion of MPTP to MPP+ causes generation of free radicals [31]. Tea polyphenols have the free radical scavenging property. The ethanolic extract of tea increases the striatum dopamine content and attenuates the effects of 6-hydroxy dopamine-induced Parkinsonism in rats. Tea grown organically has shown improvement in quality parameters such as total polyphenol and catechins. In our study it is found that the decrease in the level of activities of SOD and CAT is more in organically cultivated tea extract treated MPTP induced mice model in comparison to inorganically cultivated tea. The value of SOD and CAT activities for tea treated with vermicompost+vermiwash are 0.79 ± 0.06[a] and 0.69 ± 0.05[a] respectively for TV 25 variety which is more closer to control group (1) where no MPTP was given. Whereas the values of SOD and CAT activities for inorganically cultivated TV 25 variety are 1.8 ± 0.06[d] and 1.10 ± 0.07[d] [32-36].

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