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Variance in Antioxidant Potentials and Neuroprotective Effect of Black Tea due to Seasonal Effect

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Fresh tea leaves; Secondary metabolites; Seasonal variations; Antioxidant; Neuroprotective effect.

ABSTRACT: This article reports the effect of seasonal variations on antioxidant and neuroprotective effect of black tea. Fresh tea leaves plucked during spring monsoon and autumn and black tea prepared from them showed significant variances in phenolic content, antioxidant effect as studied by *in vitro* DPPH radical scavenging, ABTS and FRAP assays and neuroprotective effect studied by *in vitro* AChE inhibitory assay and *in vivo* studies of the ameliorative effect on brain neurotransmitters viz. norepinephrine, dopamine, serotonin in colchicine induced Alzheimer rat models. Tea leaves plucked during first flush in spring time and made tea produced thereof showed the highest yield of polyphenols, antioxidant and neuroprotective effect followed by monsoon and autumn i.e. second and third flush. The effect of seasonal variations on the yield of secondary metabolites and relevant pharmacologic effect have been made evidence based.

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INTRODUCTION

Black tea is a popular beverage especially in the Indian context. Recently the multidimensional and multi functional health potentials of the pharmacologically active compounds of black tea are being largely explored from different research domains. The pharmacological effect of any plant is because of the effect of the active phytomolecules and several other secondary metabolites [1]. Tea is manufactured from the tender leaves and buds of *Camellia sinensis* and tea plant being perennial in nature leaves are harvested almost all the year round [2]. Climatic change has a great worldwide impact on the agro-eco systems affecting not only the yield but also the crop quality [3]. Seasonal variations greatly affects the concentrations of the secondary metabolites of plants and hence tea. Variance in phenolic content, amounts of methy xanthenes etc. affects not only the quality of tea but also its pharmacologic profile. This research article aims to study the variance in antioxidant potentials and neuroprotective effect of fresh tea leaves obtained from three different flushes at mid Feburary, mid July and mid November and also of the made black tea manufactured from fresh leaves harvested at these time periods. Tea polyphenolic content has a correlation with its antioxidant potentials that also attributes to neuroprotection [4]. The research work focuses to study the effect of seasonal variation on the antioxidant and neuroprotective effect of back tea in an evidenced based manner.

MATERIALS & METHODS

Plant material

Fresh tea leaves were procured in different flushes at mid February, mid July and mid November and made tea prepared from them was used as the research material.

Reagents and Chemicals

All chemicals and reagents used for the experimentation were all of analytical grade and were purchased either from Merck (India) and Sigma Aldrich.

Instruments

Electric grinder (Bajaj GX 11); Centrifuge (Remi, R-8C Lab Centrifuge); UV spectrophotometer (Thermo Scientific); Fluorescence Spectrometers (Perkin Elmer)

Maintenance and care of animals

After obtaining permission from the animal ethical committee (Registration No: 1722/RO/ERe/S/13/CPCSEA, Approval No: ARTI/CPCSEA/2015/ARTI 09); animals were purchased from local vendors and healthy, adult male wistar rats weighing 200–320 g were used for the study. Animals were kept in poly carbonated cages with bedding husk and maintained in lab feed and water *ad libitum*, as per CPCSEA guidelines.

Preparation of fresh tea leaf and made tea extract

Fresh tea leaves were collected, washed, pasted with water using electric grinder (Bajaj GX 11) and subjected to aqueous extractions, filtered, concentrated (rota evaporation) and dried in hot air oven (50 $^{\circ}$C). Dried extracts were scrapped and stored at –20 $^{\circ}$C for future estimations.

For preparing made tea extracts, 2 g of black tea was added to 100 mL of boiling water and steeped for 15 min [ISO TC 34/SC 8; http://www.rsc.org]. Extracts were filtered by Whatmann No.1 filter paper for removal of residues. Filtrates were centrifuged (Remi, R-8C Lab Centrifuge) at 5000 rpm for 20 min and the supernatants were collected, concentrated (rota evaporation) and dried in hot air oven (50 $^{\circ}$C). Later extracts were scrapped and stored at –20 $^{\circ}$C for future estimations [4].

Seasonal variation of Total poly phenol and Total flavonoid content

Total poly-phenol content (TPC) was determined with Folin-Ciocalteu (FC) reagent according to the literatures methods [5,6]. The concentrations of the total polyphenols were determined in terms of Gallic equivalents (GAE) per gram of the extract.

Total Flavonoid Content (TFC) of the extracts was determined basing on the formation of a complex of flavonoid-aluminium and the concentration of the flavonoids is expressed in terms of quercetin equivalent (QE) per gram of the extract [7].

Seasonal variation of antioxidant potentials

DPPH assay

DPPH radical scavenging was done according to Shakila Banu S et al., 2010 [8]. Briefly, 0.2mM DPPH solution was prepared by dissolving 0.08g of DPPH in methanol in a 100ml standard flask and volume made up to mark with methanol. Next 1.5ml of 0.2mM DPPH solution and 1.5ml of sample solutions in different concentrations were mixed. In another series 1.5ml of different concentrations of sample solutions were mixed with 1.5ml of methanol. All solutions were kept for 30 min at room temperature and allowed to react. Absorbance was read at 517nm.

ABTS assay

The assay was carried out according to Shalaby et al., 2013 [9]. The main principle is based on the ability of test samples to scavenge 2, 2'-azino-bis (ethylbenzthiazoline-6-sulphonic acid or $ABTS^{+}$) radical cation. The anti-oxidative activities of the tested samples were calculated by determining the decrease in absorbance at different concentrations by using the equation:

$$E= [(A_c – A_t)/A_c] \text{ X } 100$$

where at and A_c are the respective absorbance of tested samples and $ABTS^{\cdot +}$ expressed as µmol.

FRAP assay

Total antioxidant activity was determined by the FRAP assay as per Patel et al., 2014[10]. The procedure is based on the reduction of ferric to ferrous form in the presence of antioxidants in the tea samples. Tea samples (200 µL) were allowed to react with FRAP solution (2900-3000 µL) for 30 min in the dark. Absorbance of the colored product formed (ferrous tri-pyridyl triazine complex) was recorded at 595 nm. Results were expressed in µM equivalent to $FeSO_4$ by extrapolation from the calibration curve.

Dot blot assay

Rapid screening of antioxidant properties by dot blot assay with DPPH staining was carried out as per the literature [11]. An aliquot of each dilution of different tea extracts were loaded on a 10 × 20 cm silica gel (60) TLC plate (Merck) and allowed to dry. Drops of each sample were loaded in order of decreasing concentrations along the column. The staining of silica plate was done by dipping the plate in 0.4 mM DPPH solution in methanol for 2–3 sec. The intensity of the yellow

color depends upon the amount and nature of radical scavenger present in the sample.

Seasonal variation in neuroprotective effect

AChE inhibitory assay

Inhibition of AChE, the key enzyme in the breakdown of acetylcholine, is considered as one of the treatment strategies against several neurological disorders including Alzheimer [12]. AChE inhibitory activity of the tea samples was measured using 1-naphthyl acetate as a substrate. The test relies on the cleavage by AChE of 1-naphthyl acetate to form α-naphthol, which in turn reacts with fast blue B salt to give a purple colored diazonium dye. Production of 1-naphthol was monitored spectrophotometrically, absorbance was measured at 320 nm for 5 min at 0.5 min intervals as absorption maxima of the product α-naphthol is 320 nm [13].

Spectrofluorometric assessment on effect of brain catecholamines

The effect of seasonal variations on the neuroprotective effect of black tea was also studied on other neurotransmitters viz. nor-epinephrine (NE), dopamine (DA) and serotonin (5-HT) in colchicines induced Alzheimer rat models [4, 14]. Biochemical estimations of NE, DA and 5-HT was carried out using fluorescence spectrophotometer as per the available literature methodologies [15]. Alzheimer induced animals were sacrificed by cervical dislocation, brain tissues were dissected out, washed in ice cold saline (4ºC) subjected to homogenization in 10 ml of acidified butanol. 4ml of the homogenate was mixed with 10 ml of heptane (10%) and 5 ml of 0.001N HCl, shaken for 5 min and was centrifuged at 200g for 10 min. The acid layer was mixed with alumina and 1ml of 2M sodium acetate, shaken for 5 min and centrifuged at 200g for 10 min. The supernatant was used for estimating 5-HT and the precipitate for estimating DA and NE. Estimation is done based on a fluorometric assay where the fluorescence product in case of DA and NE are formed by reaction with a mixture of alkaline sulfite and iodine solution and in case of 5-HT by reaction with ortho- phthalaldehyde. Fluorescence for DA was read at excitation 320 nm and emission 375 nm, for NE excitation at 380 nm and emission at 480 nm and for 5-HT excitation at 355 nm and emission at 470 nm. The results were expressed as µg/g wet tissue [4, 15].

RESULTS

The details of the effect of seasonal variation of polyphenolic and flavonoid content of fresh tea leaves and made tea are presented in **Table 1**. The variances in antioxidant potentials due to seasonal effect are provided in **Table 2** and **Figures 1-5**. The changes in neuroprotective effect of black tea as observed in different seasons are presented in **Table 3** and **Figures 6-8**.

Table1: UV estimations of Poly phenol and Flavonoid of fresh and made tea extracts in different seasons.

Tea in different seasons	UV estimations of Polyphenol and Flavonoid of fresh and made tea extracts			
	Total Polyphenol (TPC)		Total Flavonoid (TFC)	
	Fresh Leaves Water	Made Tea Water	Fresh Leaves Water	Made Tea Water
Spring	23.24±2.76	6.75±1.65	19.64±2.34	2.8±0.31
Monsoon	20.16±2.56	4.51±1.11	16.28±1.79	2.41±1.15
Autumn	20.13±2.78	3.74±1.31	15.97±1.32	1.94±1.56

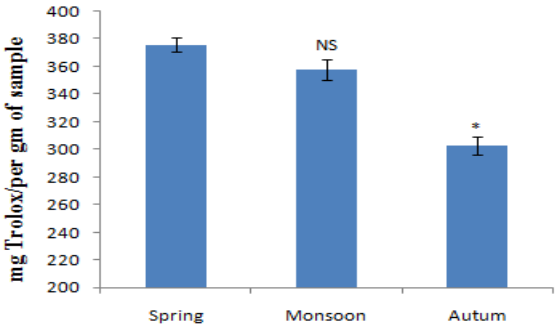

Figure 1: Comparative histogram of ABTS assay (expressed in mg Trolox per gm of sample) for fresh tea leaves collected in different seasons. Results are expressed as mean ± SD, (n = 10), *means significant at p<0.05 level, NS= Not Significant

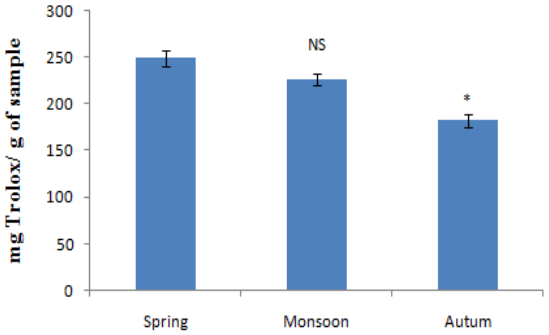

Figure 2: Comparative histogram of ABTS assay (expressed in mg Trolox per gm of sample) for made tea obtained at seasons. Results are expressed as mean ± SD, (n = 10), *means significant at p<0.05 level, NS= Not Significant

Table 2: DPPH radical scavenging of different fresh tea leaves and made tea (CTC) of TV varieties

Tea of different season	Aqueous Extract Concentration (µg/ml)							
	Scavenging Activity							
	50 µg/ml		100 µg/ml		200 µg/ml		300 µg/ml	
	Fresh Leaves	Made Tea	Fresh Leaves	Made Tea	Fresh Leaves	Made Tea	Fresh Leaves	Made Tea
Spring	25.43±1.78	15.43±1.28	42.78±2.06	31.78±1.16	74.75±2.14	65.75±1.14	75.76±1.16	65.16±1.06
Monsoon	28.27±1.97	16.27±1.25	41.16±2.56	32.26±1.26	77.5±1.13	64.5±2.19	78±2.75	67.23±1.55
Autumn	25.14±1.74	16.14±1.14	45.21±2.78	34.87±2.92	72.0±1.11	63.0±1.23	73±1.41	66.21±1.11

Table 3. Comparative evaluation of AChE inhibitory potentials of black tea due to seasonal effect

Concentration (µg/ml)	IC_{50}= 33.83µg/ml	IC_{50}= 36.93µg/ml	IC_{50}= 39.97µg/ml
	% of AChE Inhibition (Spring)	% of AChE Inhibition (Monsoon)	% of AChE Inhibition BT (Autumn)
50.1	75.2	71.6	66.1
40.2	54.9	50.2	47.1
30.1	43.7	39.3	34.9
20.1	34.7	27.4	25.8
10.2	19.9	16.3	15.3
5.0	11.2	9.6	8.2

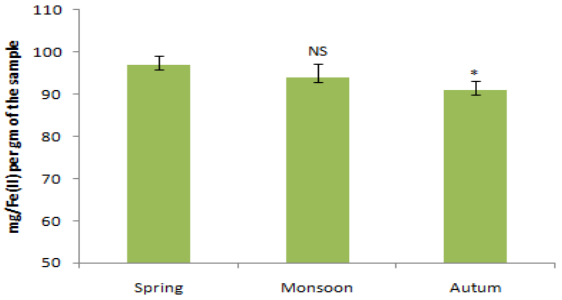

Figure 3: Comparative histogram of FRAP assay for fresh tea leaves collected in different seasons. Results are expressed as mean ± SD, (n = 10), *means significant at p<0.05 level, NS= Not Significant

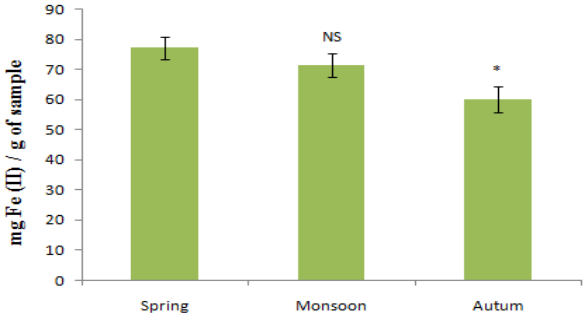

Figure 4: Comparative histogram of FRAP assay of made tea obtained at different seasons Results are expressed as mean ± SD, (n = 10), *means significant at p<0.05 level, NS= Not Significant

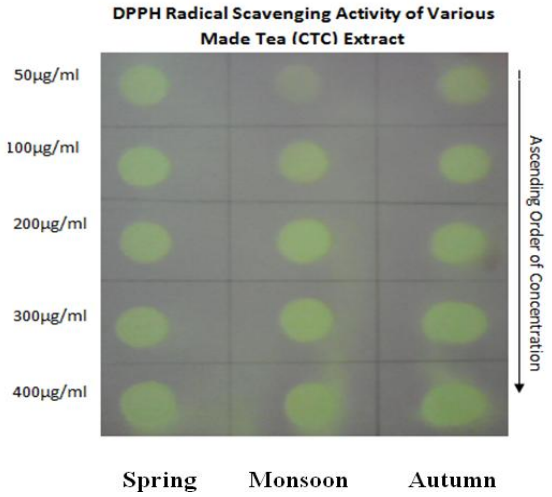

Figure 5: TLC plate for Dot blot assay of made tea

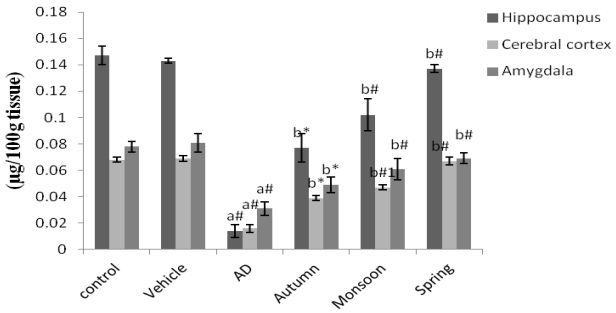

Figure 6: Effect of seasonal variation of black tea on Norepinephrine (NE) levels (µg/100g of tissue) in colchicine induced AD rats. Experimental animals in

different groups were given black tea procured in different seasons. Results are expressed as mean ± standard deviation of three independent experiments with eight animals in each group. 'a' means compared to control (Gr. 1), 'b' means compared to AD (Gr. 3) * means significant at p<0.05; # means significant at p<0.005

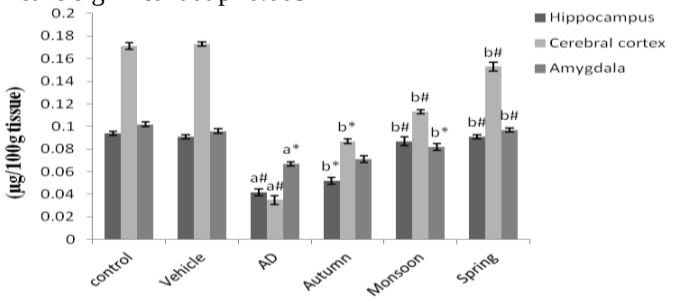

Figure 7: **Effect of seasonal variation of black tea on Dopamine (DA) levels (µg/100g of tissue) in colchicine induced AD rats**. Experimental animals in different groups were given BTE, drug and BTE+ drug. Results are expressed as mean ± standard deviation of three independent experiments with eight animals in each group. 'a' means compared to control (Gr. 1), 'b' means compared to AD (Gr. 3) * means significant at p<0.05; # means significant at p<0.005

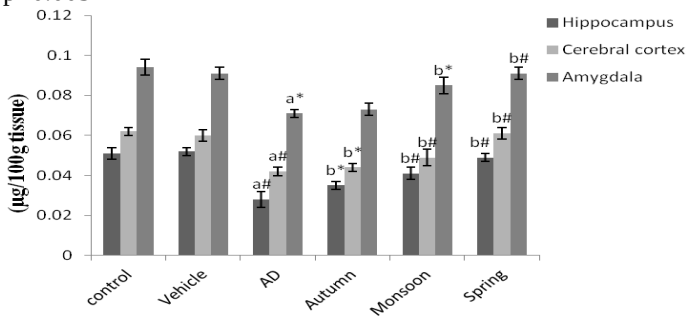

Figure 8: **Effect of seasonal variation of black tea on serotonin (5-HT) levels (µg/100g of tissue) in colchicine induced AD rats**. Experimental animals in different groups were given BTE, drug and BTE+ drug. Results are expressed as mean ± standard deviation of three independent experiments with eight animals in each group. 'a' means compared to control (Gr. 1), 'b' means compared to AD (Gr. 3) * means significant at p<0.05; # means significant at p<0.005

DISCUSSION

Both fresh tea leaves and made tea showed significant yield of polyphenol (**Table 1**). Leaves obtained after first flush in the month of mid February (spring season) showed maximum polyphenol content in comparison to tea leaves obtained during monsoon or autumn i.e. second or third flush. The same fact was witnessed regarding flavonoid content that also varied with the seasonal variations and the maximum flavonoid content was found amongst the leaves of first flush (spring time) in comparison to leaves plucked during monsoon or autumn (**Table 1**). The screening of antioxidant properties by different *in vitro* assays, have shown that both the fresh tea leaves & made tea showed that highest antioxidant activity during first flush plucking at the spring time (**Table 2 and Fig. 1-5**). A correlation thus exists between the polyphenolic content and antioxidant potentials of tea which is also supported by corroborative literature evidences [13]. Antioxidant potentials of black tea also contribute to its varied pharmacological actions including neuroprotection since oxidative stress contributes significantly to the pathogenicity of several diseases including neurodegenerative disorders. With seasonal variations, changes were observed in polyphenolic and flavonoid content that was reflected in variations in antioxidant potentials and also in the neuroprotective effect (**Table 3 and Fig. 6-8**). Effect of tea leaves obtained from first flushing in inhibiting AChE and recovering the depleted neurotransmitters viz. norepinephrine, dopamine, serotonin were significantly more in comparison to the effect of leaves plucked in monsoon or autumn (**Table 3 and Fig. 6-8**).

CONCLUSION

Climatic influence and seasonal variations greatly influences the chemo-pharmacological effects of plant secondary metabolites. Black tea is a popular beverage with multifaceted health benefits. The findings in this research article have shown the variances in polyphenolic content in tea leaves obtained from different flushes and this variance had significant effect on the antioxidant and neuroprotective effect of tea. Tea leaves plucked during spring and made tea produced thereof from them showed the highest yield of polyphenols and thus antioxidant and neuroprotective effect followed by monsoon and autumn i.e. second and third flush.

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