

Turmeric black tea as a multimodal theronostic dietary adjuvant aiding neuroprotection and ameliorating hypertension

Abstract

This study reports the multifunctional potentiality of turmeric black tea (TBT) in alleviating hypertension in salt induced hypertensive animal model and aiding neuroprotection in colchicines induced Alzheimer rat models. TBT prepared basing on the desirability function of central composite design with 3.11g of black tea and 1.46g of ground turmeric exhibited optimal pharmacologic response and organoleptic acceptability. No signs of mortality were observed till 10g/kg b.w. or any hepato-renal adversities with 5000mg/kg doses. LCMS analysis of TBT showed the presence of several tea catechins, theaflavins, gallic acids and curcuminoids. Incorporation of turmeric in black tea aided in value addition as evidenced by *in vitro* and *in vivo* experimental results and has not affected the chemoprofile of black tea studied by FTIR. The multipotency of TBT is attributed due to presence of the variant pharmacologically active molecules.

Keywords: turmeric black tea, hypertension, neuroprotection, desirability function, multifunctional, organoleptic acceptability

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Baishakhi De,¹ Koushik Bhandari,² Prakash Katakam,¹ Gargi Saha,³ Tridib Kumar Goswami⁴

¹SSS Indira college of Pharmacy, India

²Advanced Technology Development Centre, Indian Institute of Technology, India

³National Tea Research Foundation, India

⁴Department of Agriculture and Food Engineering, Indian Institute of Technology, India

Correspondence: Tridib Kumar Goswami, Department of Agriculture and Food Engineering, IIT Kharagpur- 721302, west Bengal, India, Email tkg@agfe.iitkgp.ernet.in

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Abbreviations: ACE, angiotensin converting enzyme; CCBs, calcium channel blockers; AD, Alzheimer's Disease; TBT, turmeric black tea; RSM, Response Surface methodology; ANOV, analysis of variance; BT, black tea decoction; ESI, electrospray ionization; OECD, Organization for Economic Cooperation and Development; ALT, alanine Aminotransferase; AST, aspartate Aminotransferase; ALP, alkaline phosphatase; ROS, Reactive oxygen species; HHL, hippuryl L, histidyl, L, leucine

Introduction

The growing complexities of the diseases is a real challenge to the medical fraternity; thence the treatment strategies have also undergone a radical change and promotes multi-target therapeutic entities, poly therapy, nutraceuticals and food combinatorics, herbo-synthetic combinations as adjuvant therapy to achieve better therapeutic outcomes.¹⁻³ Hypertension, is a big concern worldwide affecting different levels of socio-economic classes and is the root cause of stroke, cardiovascular disorders, diabetes etc. Excessive stress, sedentary lifestyle, food habits, physical inactivity are other contributing factors. Though several beta blockers, angiotensin converting enzyme (ACE) inhibitors, calcium channel blockers (CCBs) are available in the market but are associated with side effects.^{4,5} Alzheimer's Disease (AD), is a progressive worrisome neurodegenerative disorder with devastating complications and till yet with available therapeutic options total curative outcomes has not yet been achieved.^{6,7} Epidemiological research evidences has shown that hypertension and dementia are interlinked; sustained hypertension worsens AD conditions by accelerating β -amyloid aggregation, oxidative stress and inflammatory responses; ultimately there is extensive neuronal loss and impairment of neuronal transmission. Patients suffer from cognitive decline.⁸⁻¹⁰ Multifaceted health benefits of tea is already on limelight and black tea apart from being a very refreshing beverage specially due to its astringency, its multimodal pharmacology has shown it to be a functional beverage.¹¹ Researches

with functional beverage aim to achieve for value additive synergistic potentials and thus several tea diversification products have captured the global nutraceutical market.¹² Turmeric (*Curcuma longa*), a golden yellow color cooking spice and preservative is specially preferred in Indian subcontinent, hence also called "Indian saffron" is recognized for its versatile applications and health effects.¹³ The effectivity of any natural therapeutic entity may it be phytomedicine or phytonutraceuticals are due to the wide array of pharmacologically active molecules present in them that serve as a combinatorial library each exerting its own potential.¹⁴ This research article reports chemometrics guided optimization of TBT for optimal pharmacologic effect and organoleptic acceptability and the ameliorative effect of turmeric black tea (TBT) in hypertension and aiding neuroprotection.

Material and methods

Chemicals

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

Plant material

Fresh tea leaves (TV 25 variety, *Voucher specimen*: IITKGP/HB/2018/T1) used for producing black tea were obtained from the tea garden of IIT Kharagpur and good quality turmeric rhizomes (*Voucher specimen*: IITKGP/HB/2018/T2) from the medicinal garden of Agriculture and food engineering department of IIT Kharagpur.

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 180–200g were used for

the study. Maintenance and care of animals were done as per CPCSEA guidelines.

Chemometrics optimized preparation of turmeric tea

Response Surface methodology (RSM), is an empirical modeling technique to find out the relationship amongst set of experimental factors and observed results. RSM involves the steps of developing the polynomial equation, generation of the analysis of variance (ANOVA) table, generation of response surface plots with different combinations of input parameters, determining the optimal level from desirability analysis and confirmation test for validation.^{15,16}

Basing on the desirability function, 3.11g of black tea and 1.46g of ground turmeric powder was added to 120mL of boiling water and steeped for 15 min; the strained liquid is the turmeric black tea (TBT) to which a pinch of black pepper have been added. Under this condition, pharmacologic response (acetylcholinesterase inhibitory effect) and organoleptic acceptability were maintained at optimal.

The black tea decoction (BT) was prepared by adding 4.57g of black tea to 120mL of boiled water and steeped for 15 min (ISO TC 34/SC 8; <http://www.rsc.org>). Here the weight of black tea is the cumulative weight of black tea and turmeric powder mentioned above in TBT.

Organoleptic evaluation of the finalized turmeric black tea (TBT)

Organoleptic acceptability of TBT that have been optimized as per desirability solutions and selected for further experimentations was reevaluated on the basis of 9-point hedonic scale by trained taste panelists.^{17,18}

Compatibility studies of turmeric black tea

The purpose of preparing TBT is to achieve value additive synergistic pharmacologic response; however achievement of the same should not compromise with the original quality of black tea and for that purpose the chemical compatibility of turmeric with black tea have been studied by FTIR. Individual spectrum of aqueous extracts of black tea, turmeric and turmeric black tea were recorded in FTIR so as to observe any changes in the spectrum pattern of the black tea due to incorporation of turmeric and thus identify the chances of any chemical interactions.

LC-MS analysis of TBT

Chemo profiling of TBT was carried out by LC-MS analysis using Zorbax SB-C18 column, acetonitrile and 0.4% acetic acid as the mobile phase, injection volume of 20 μ L and flow rate of 1mL/min, a run time of 30min and detector wavelength was kept at 430nm. Mass spectrometer with electrospray ionization (ESI) and operating between the positive and negative polarity conditions were used for detecting the catechins in ESI negative modes and the ESI positive mode was used for detecting the theaflavins. The capillary voltage was maintained at 3KV, cone voltage at 30V and extractor voltage at 4V for both positive and negative mode to achieve the best sensitivity for the analytes. The source temperature and desolvation temperature were maintained at 150°C and 625°C respectively. The cone gas flow rate was kept at 50L/h and that of salvation gas flow rate at 1100L/h.^{19,20}

Safety profile of TBT

Acute toxicity studies were carried out in male wistar rats as per Organization for Economic Cooperation and Development (OECD)

guideline 425 and sub-acute toxicity studies for a period of 28 days. In acute toxicity studies signs of mortality, behavioral and physical abnormalities were observed from the cage side. In sub chronic toxicity studies the effects of TBT on body weight of experimental animals, hepato-renal effect by observing the effects of TBT on hepatic enzymes viz., alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), and urea-creatinine for renal effect were studied.^{21,22}

Administration of BT and TBT to experimental animals

Black tea decoction or BT (TV 25 variety, tea garden of IIT Kharagpur, India) prepared as mentioned earlier gave a percent yield of 20.5 and the solid content was calculated to be 3.91 mg. 0.5mL of BT was orally administered to experimental rats twice a day (7.81mg/rat/day) via gastric gavage needle.

Considering the case of TBT as prepared earlier, the percent yield found to be 12.1 and the solid content was calculated to be 3.39mg; each rat were orally administered twice with 0.5mL of TBT via gastric gavage needle and thus each rat received (6.79mg/rat/day) solid content.

Development of colchicine induced Alzheimer rat models

Colchicine induced AD rat models were developed as per the literature methodologies.^{7,23-25}

In vitro and *in vivo* evaluation of AChE inhibitory effect of turmeric black tea

In vitro AChE inhibitory assay

Since acetylcholinesterase (AChE) involves breakdown of acetylcholine, inhibition of AChE is considered as one of the treatment strategies against several neurological disorders including Alzheimer.²⁶ 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. AChE activity was measured using 1-naphthyl acetate as a substrate. Production of 1-naphthol was monitored spectrophotometrically by measuring the absorbance at 320nm.²⁶

In vivo estimation of AChE level by Ellman method

The AChE activity in the hippocampus was assessed by Ellman method.²⁷ The change in absorbance was measured for 15minutes at 1minute interval at 412nm in spectrophotometer. Results were analyzed as micromoles of acetylthiocholine iodide hydrolyzed per minute per mg protein and expressed as percent of control.

Estimation of Reactive oxygen species (ROS) and nitrite level

ROS was estimated in hippocampal homogenate by spectrofluorometry²⁸ and the same hippocampal homogenate was used for Nitrite level estimation as per literature methodologies.²⁹

In vitro estimation of ACE inhibitor effect of BT and TBT

Angiotensin converting enzyme (ACE) activates angiotensin-I into a potent vasoconstrictor called angiotensin-II that influences aldosterone release which increases blood pressure. So ACE

inhibitors are preferred anti hypertensives. The assay method is based on the liberation of hippuric acid from hippuryl-L-histidyl-L-leucine (HHL) catalyzed by the ACE. The assay procedure was carried as per literature.^{2,30-32}

Development of salt induced hypertensive rat models

Salt induced hypertensive rat models were developed as per literature.⁴ Animals were divided into five groups with six animals in each group. Group I are the normotensive rats administered with water (10mL/kg/day), Group II are the salt induced hypertensive rats who were administered with 18%w/v NaCl solution (10mL/kg/day), Group III is the BT treated group, Group IV is treated with TBT and the Group V served as positive control treated with captopril (20mg/kg/day). Oral administration was done to experimental animals by oral gavage needle. Blood pressure measurement: Systolic and diastolic arterial blood pressure of experimental animals was measured by tail-cuff method.^{4,5}

In vivo estimation of concentration of Renin and ACE

Rats were anesthetized and blood samples were collected from the inferior venacava, kept for 15-20min at room temperature and then centrifuged at 3000 rpm; the separated serum stored at -80°C and rennin and ACE concentrations measured at ng/mL using ELISA kits.^{4,5}

Aortic ring assay

Rats were sacrificed by decapitation, the thoracic aorta was isolated, cleaned of fat and connective tissue, and all aortas were denuded of endothelium film by gentle mechanical procedure and, finally, cut into rings of about 4-5 mm of width. The rings were tied to stainless steel hooks with silk thread and immersed into 10mL organ baths of Krebs solution at 37°C and oxygenated (O₂/CO₂, 95 : 5). A basal tension of 2g was established for all tissues. The temperature was maintained

at 37°C throughout the experiment. The isometric tension generated by the coronary artery was measured using a force-displacement transducer. The contraction of each prepared aorta was maximized by administration of KCl (120mM) and was recorded. Further tissues were pre-incubated with test samples (BT and TBT). Relaxation was expressed as a percentage change from KCl contracted levels i.e. by comparison between maximum vascular contraction before and after addition of test samples (BT and TBT).^{4,5,33}

Results

Response surface methodology

Considering RSM, the process order fits to quadratic design model, the adequacy of models (Table 1 & 2) and model summary statistics (Table 3 & 4) justified by the analysis of variance (Table 5 & 6) considering organoleptic score as response 1 and inhibition of AChE as response 2. The model summary statistics focus on the model maximizing the “Adjusted R-squared” and the “predicted R-squared”. The R-squared values of 0.9397 (Table 3) and 0.9817 (Table 4) having closeness to unity showed a good fitness between the actual and that obtained from the response model. The “Pred R-Squared” value of 0.5735 is not as close to the “Adj R-Squared” of 0.8966 (Table 3) as normally expected and the reason may be due to block effect and to consider model reduction or response transformation. However, “Adeq Precision” that measures the signal to noise ratio, a ratio greater than 4 is desirable. The ratio of 11.106 (for response 1) indicates an adequate signal and the model can be used to navigate the design space. Considering response 2, the “Pred R-Squared” value of 0.8736 is in reasonable agreement with the “Adj R-Squared” of 0.9687 (Table 4) and the “Adeq Precision” of 22.062 (for response 2) indicates an adequate signal and the model can be used to navigate the design space. The average leverage was 0.4615 and VIF close to 1.0 was obtained.

Table 1 Software generated adequacy of model considering organoleptic score as Response 1

Source	Sum of squares	df	Mean square	F value	p-value	Prob>F
Mean vs total	418.96	1	418.96			
Linear vs mean	2.5	2	1.25	0.21	0.8108	
2FI vs Linear	0.49	1	0.49	0.076	0.7888	
Quadratic vs 2FI	54.26	2	27.13	51.66	<0.0001	Suggested
Cubic vs quadratic	0.27	2	0.13	0.2	0.0877	Aliased
Residual	3.41	5	0.68			
Total	479.88	13	36.91			

Table 2 Software generated adequacy of model considering inhibition of AChE as Response 2

Source	Sum of squares	df	Mean square	F value	p-value	Prob > F
Mean vs. total	65277.65	1	65277.65			
Linear vs. mean	1812.33	2	906.16	5.18	0.0286	
2FI vs. Linear	4.41	1	4.41	0.023	0.8834	
Quadratic vs. 2FI	1679.16	2	839.58	90.26	<0.0001	Suggested
Cubic vs. quadratic	40.32	2	20.16	4.07	0.0895	Aliased
Residual	24.79	5	4.96			
Total	68838.66	13	5295.28			

The significance of the models were confirmed by the p-values of 0.0004 (Table 5) and 0.0001 (Table 6). The model F-value is the ratio of mean square for the individual term to the mean square for the residual. The model F-value of 21.08 implies the model is significant (response 1). There is only a 0.04% chance that a “Model F-value” this large could occur due to noise. The Prob>F value is the probability of F-statistics value and is used to test the null hypothesis. Values of “Prob > F” less than 0.05 indicates that the model terms are significant. For response 1, A², B² are significant model terms (Table 5). The “lack of fit F-value” of 173.70 implies the lack of fit is significant. There is only a 0.01% chance that a “Lack of fit F value” this large could occur due to noise. For response 2, the model F-value of 75.17 implies that the model is significant and there is only a 0.01% chance that a model

F-value this large could occur due to noise. Values of “Prob > F” less than 0.0500 indicate model terms are significant. The significant model terms in this case are A, B, A², B² (Table 6). The “lack of fit F-value” of 35.64 implies the “lack of fit” is significant. There is only a 0.24% chance that a “Lack of fit F value” this large could occur due to noise. Internally studentized residuals helps to measure the number of standard deviations separating the actual and predicted values; here the normality assumption was satisfied with very less scattering of points and fitting almost to a straight line (Figure 1).

The software generated final equation in terms of coded factors and actual factors for organoleptic score (response 1) and inhibition of AChE (response 2) is presented in Table 7.

Table 3 Software generated model summary statistics considering organoleptic score as Response 1

Source	Std. Dev.	R-squared	Adjusted R-squared	Predicted R-squared	PRESS	
Linear	2.42	0.0411	-0.1507	-0.6098	98.08	
2FI	2.54	0.0491	-0.2678	-1.651	161.51	
Quadratic	0.72	0.9397	0.8966	0.5735	25.98	Suggested
Cubic	0.83	0.9441	0.8657	-2.5514	216.36	Aliased

Table 4 Software generated model summary statistics considering inhibition of AChE as Response 2

Source	Std. Dev.	R-squared	Adjusted R-squared	Predicted R-squared	PRESS	
Linear	13.22	0.5089	0.4107	0.1939	2870.56	
2FI	13.92	0.5102	0.3469	-0.2261	4366.26	
Quadratic	3.05	0.9817	0.9687	0.8736	450	Suggested
Cubic	2.23	0.993	0.9833	0.5956	1440.15	Aliased

Table 5 Software generated ANOVA table considering organoleptic score as response 1

Source	Sum of squares	df	Mean square	F value	p-value	Prob>F	
Model	57.25	5	11.45	21.8	0.0004		Significant
A-Black tea	0.35	1	0.35	0.67	0.4387		
B-turmeric powder	2.15	1	2.15	4.09	0.0828		
AB	0.49	1	0.49	0.93	0.3662		
A2	46.49	1	46.49	88.52	<0.0001		
B2	13.34	1	13.34	25.41	0.0015		
Residual	3.68	7	0.53				
Lack of fit	3.65	3	1.22	173.7	0.0001		Significant
Pure error	0.028	4	7.00E-03				
Cor Total	60.92	12					

Table 6 Software generated ANOVA table considering inhibition of AChE as response 2

Source	Sum of squares	df	Mean square	F value	p-value	Prob>F	
Model	3495.9	5	699.18	75.17	<0.0001		Significant
A-black tea	1240.19	1	1240.19	133.33	<0.0001		
B-turmeric powder	572.13	1	572.13	61.51	0.0001		
AB	4.41	1	4.41	0.47	0.5133		
A2	1373.72	1	1373.72	147.68	<0.0001		
B2	491.14	1	491.14	52.8	0.0002		
Residual	65.11	7	9.3				
Lack of fit	62.77	3	20.92	35.64	0.0024		Significant
Pure error	2.35	4	0.59				
Cor Total	3561.01	12					

Table 7 Software generated final equations in terms of coded and actual factors for the responses

Variables	Final equation in terms of coded factors	Final equation in terms of actual factors
Response 1: Organoleptic scores	8.12	-6.02966
	+0.21*A	+7.38357*Black tea
	-0.52*B	+4.33680*Turmeric powder
	-0.35*A*B	-0.23333*Black tea *Turmeric powder
	0	-1.14889*Black Tea2
	0	-1.38500*Turmeric powder2
Response 2: Inhibition of AChE	84.68	-31.17253
	+12.45*A	+46.82392*Black Tea
	+8.46*B	+35.76426*Turmeric powder
	-1.05*A*B	-0.70000*Black Tea *Turmeric powder
	0	-6.24556*Black Tea2
	0	-8.40250*Turmeric powder2

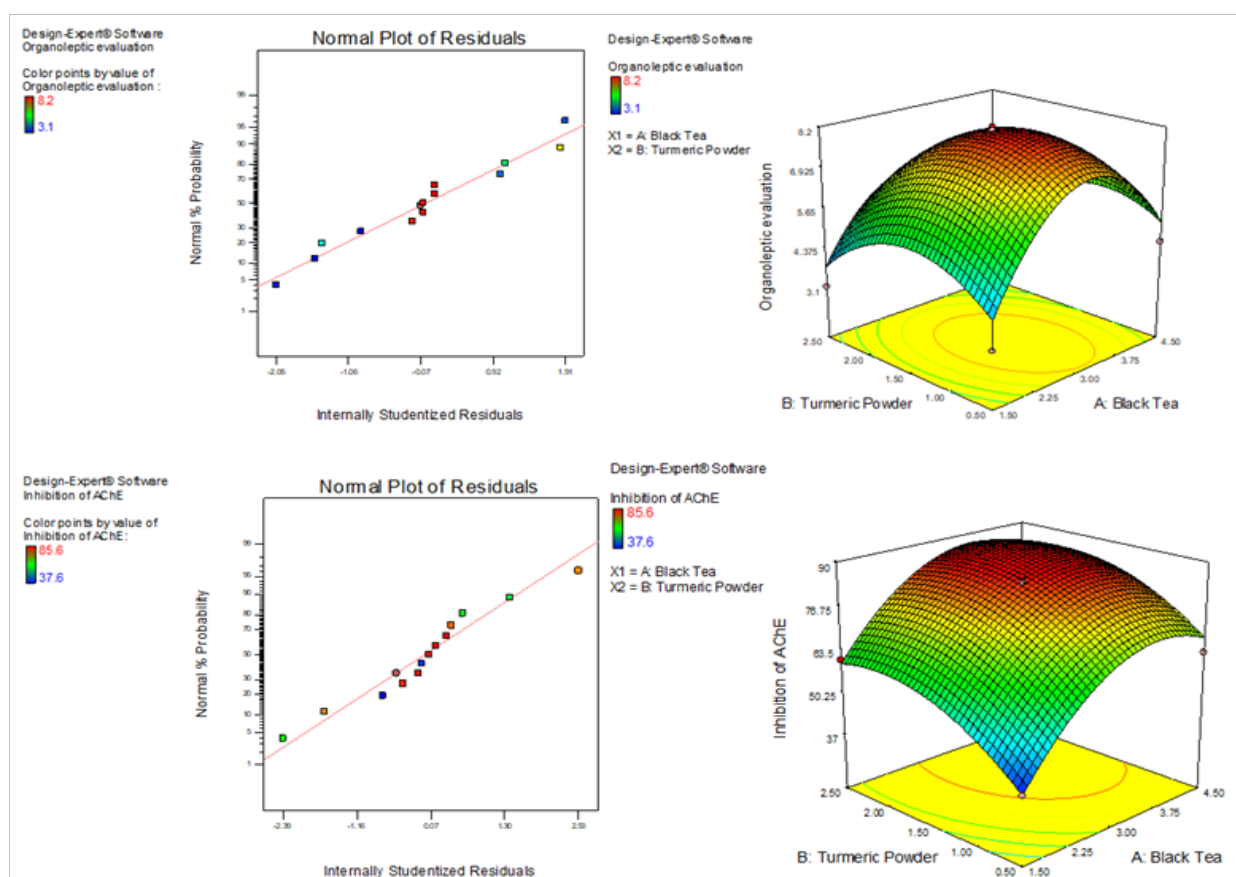


Figure 1 Internally studentized normal plot of residuals and 3D surface plots.

The desirability values for responses are shown in (Figure 2). The dot on each ramp function graph indicates the optimal level of the parameter. Desirability function value ranges from zero, outside of the limits to one at the goal, the purpose of the program is to maximize the function; seeking of goal begins at a random starting

point and proceeds up the steepest slope to a maximum. Basing on the desirability function, both pharmacologic response (inhibition of AChE) and organoleptic acceptance were achieved at optimal with 3.11g of black tea and 1.46g of turmeric powder.

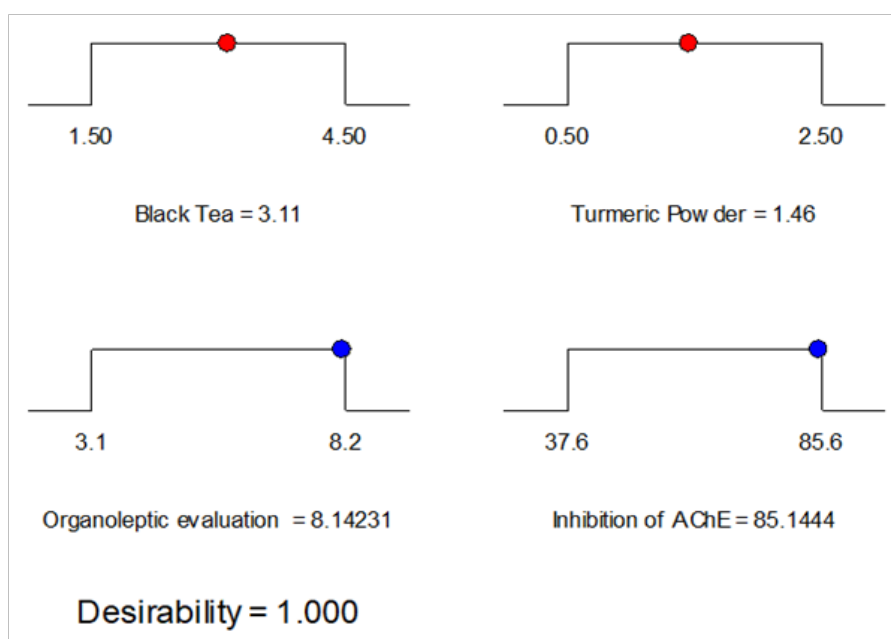


Figure 2 Desirability ramp function graph.

The organoleptic acceptability of the finalized TBT used for further experimentations assessed on 9-point hedonic scale gave a preference rating value for taste as 7.1, for flavor as 6.9 and an overall assessment score of 7 meaning moderate likeliness.

Compatibility studies

The FTIR spectra for compatibility studies are provided in (Figure 3). From the peaks of IR spectrum of black tea (purple spectrum) Figure 3, peak at $3600\text{-}3350\text{ cm}^{-1}$ corresponds to Str vib of OH gr; $1695\text{-}1558\text{ cm}^{-1}$ (Caffeine & theobromine bands), $1650\text{-}1400\text{ cm}^{-1}$ (Phenolic acids), $1600\text{-}800\text{ cm}^{-1}$ (catechins), gallocatechins showed peaks at $1373\text{-}1280\text{ cm}^{-1}$, epicatechin gallate at 1238 cm^{-1} , epicatechins at 820 cm^{-1} , and peaks at 833 and 518 cm^{-1} corresponds to C-H, C-C out of plane bending vibration, associated with 1,4-disubstituted benzene molecules. Considering the FTIR spectrum of turmeric (blue spectrum), the strong IR band at 3393 cm^{-1} are due to O-H stretching vibrations; bands at 2924 cm^{-1} is due to asymmetric stretching vibrations of the CH_2 group; the strong band at 1630 cm^{-1} and 1514 cm^{-1} shows the mixed character of C=C and C=O stretching vibration and also C-H bending at 1514 cm^{-1} ; C-CH deformation vibrations can be assigned to the presence of medium strong bands at 1451 cm^{-1} and 1432 cm^{-1} ; the band at 1378 cm^{-1} are due to C-OH bending vibrations; C=CH bending vibrations are assigned to the IR band at 1281 cm^{-1} . Considering the FTIR spectral pattern of black tea (purple spectrum), turmeric (blue spectrum) and turmeric black tea (red spectrum) each of the components are maintaining their individual identity and on intermixing no significant changes have been observed in their chemical fingerprint. So it can be considered that addition of turmeric has not affected the chemical identity of black tea.

LC-MS of TBT

Chemo profiling of TBT by LC-MS Figure 4 showed the presence of several compounds, however those with good abundances were detected within the retention time range of 0.9-30.9min. The presence of compounds were confirmed both by chromatographic and MS data and also comparison with retention times as well as UV-vis spectra

and maximum absorption wavelength. Tea catechins were detected in the ESI negative mode and the powerful black tea antioxidants, the theaflavins were detected in the ESI positive mode; considering the retention time and comparing with the UV absorption maxima curcuminoids were detected in the wavelength range of 254-500 nm; amongst other curcuminoids, curcumin was detected between 423-425nm, demethoxycurcumin was detected at 420-421nm, bisdemethoxycurcumin was detected between 413-418nm.

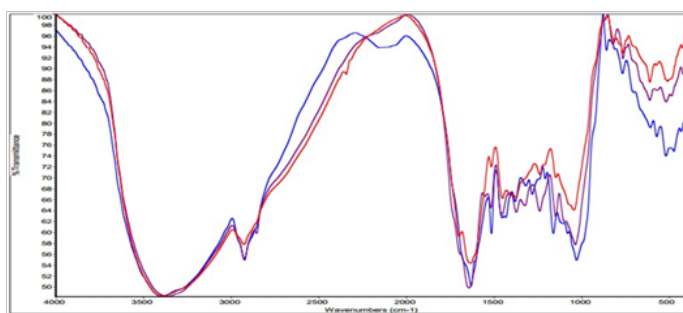


Figure 3 Overlapped FTIR spectra of black tea (purple), turmeric (blue) and turmeric black tea (red).

Safety profile of TBT

In acute toxicity studies with TBT, till 10g/kg body weight (b.w.) no signs of mortality or any physical or behavioral abnormalities were found as observed from cage side. The first death was observed at 15g/kg b.w. and the LD50 was determined to be 30g/kg b.w. Sub chronic toxicity studies for 28 days studied with doses 100, 500, 1000, 2500, 5000 mg/kg b.w. didn't exhibit any significant changes on body weight in the doses range of 100-1000 mg/kg b.w., however with doses of 2500 and 5000 mg/kg b.w. significant reductions in weight was observed from the 21st day. The studied dosage range didn't exhibit any adverse hepato-renal effect and thus no alterations were observed in the hepatic enzyme levels viz. AST, ALT and ALP or the urea, creatinine, Na and K thus supporting the safety profile of TBT.

In vitro and in vivo estimations of AChE

The %inhibition of AChE *in vitro* Figure 5 and also *in vivo* Figure

6 exhibited greater potency of TBT (IC_{50} 32.51 μ g/ml) in comparison to BT (IC_{50} 39.59 μ g/ml) thus substantiating the neuroprotective potentiality of TBT.

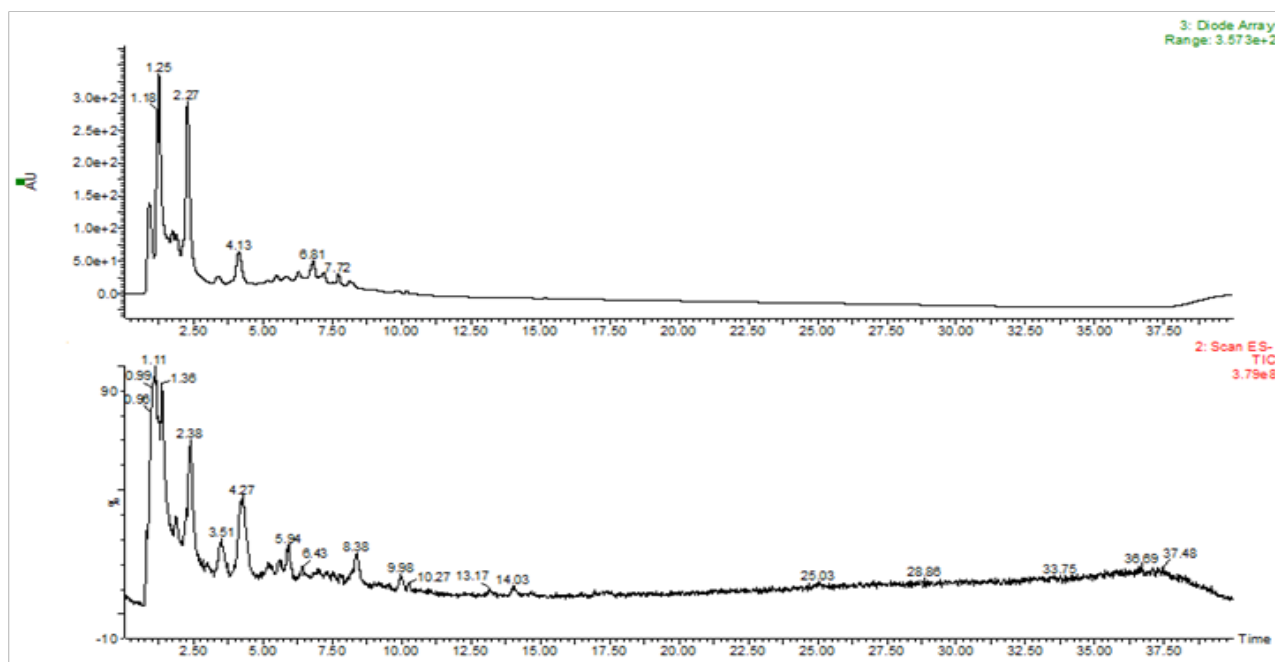


Figure 4 LCMS analysis of TBT.

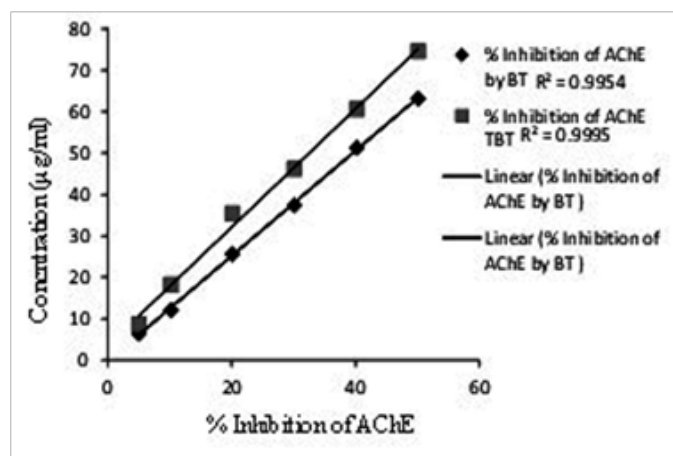


Figure 5 Percent inhibition of AChE *in vitro* by TBT and BT.

ROS and nitrite level

The effect of TBT and BT on ROS and nitrite level is presented in (Table 8). The AD induced experimental animals showed significant increase in the ROS level as observed in hippocampal tissues and serum Table 8 in comparison to control and sham operated group; on vehicle treatment no significant alterations were observed in ROS level (hippocampal tissues and serum) in the three groups; BT treatment didn't exhibit any significant effect on the ROS level in the control and sham group (hippocampus and serum) but the ROS level was significantly lowered in AD induced group, the same fact was observed on treatment with drug both in the hippocampus and serum

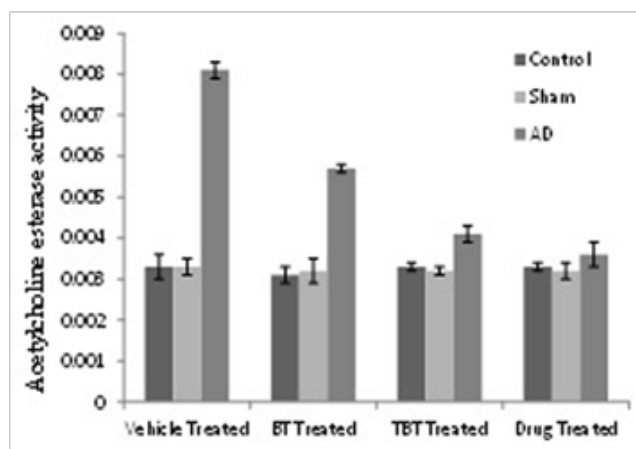


Figure 6 *In vivo* AChE inhibitory effect of TBT and BT in hippocampal region.

(Table 8) however the quenching of ROS on TBT treatment was much greater in AD groups (hippocampus and serum) in comparison to BT treatment. The AD induced animals showed significant increase in nitrite levels (hippocampus and serum) in comparison to control and sham group. On vehicle treatment no significant alterations were observed amongst the three groups; on treatment with BT significant reduction in nitrite levels was observed in AD groups (hippocampus and serum), significant reductions in nitrite level amongst AD groups was also observed on drug treatment; however TBT treatment exhibited greater reduction in nitrite levels in both hippocampus and serum in comparison to BT treatment (Table 8).

Table 8 Effect of TBT and BT on ROS and nitrite levels

ROS level (% of control)			Nitrite level (% of control)			
Hippocampus			Hippocampus			
In Tissue			In Tissue			
Group	Control	Sham	AD	Control	Sham	AD
Vehicle treated	98.6±4.2	99.2±3.6	190.6±3.9	101.2±4.6	100.5±3.9	202.9±5.1
BT treated	97.7±2.6	99.1±1.7	112.6±4.7	96.7±2.1	96.7±4.2	118.6±4.6
TBT treated	90.2±2.5	95.7±3.6	94±3.4	92.6±2.2	93.4±3.7	99.7±2.8
Drug treated	91.2±2.9	93.2±2.4	91.6±1.9	90.2±3.7	91.5±3.8	92.4±2.9
In Serum			In Serum			
Group	Control	Sham	AD	Control	Sham	AD
Vehicle treated	99.1±4.6	105.6±3.4	134.9±2.1	102.5±2.6	103.1±3.3	136.6±4.7
BT treated	99.2±4.2	105.2±2.4	105.6±3.9	101.2±3.8	104.3±3.6	112.2±5.1
TBT treated	95.2±3.1	104.1±3.9	94.8±3.5	102.4±4.5	102.6±3.4	96.5±4.7
Drug treated	95.7±3.7	104.5±1.9	81.6±2.2	101.4±2.9	103.4±3.5	84.1±3.4

In vitro ACE estimations

The antihypertensive effect of BT in *in vitro* ACE inhibitor assay expressed in terms of IC50 values was found to be 7.01µg/mL for BT and with TBT the IC50 value was found to be 0.38µg/mL showing the greater antihypertensive potency of TBT in comparison to BT.

In vivo studies in hypertensive rats: There was elevation in both systolic and diastolic BP in experimentally induced hypertensive rats in comparison to the normotensive rats; lowering of both systolic and diastolic BP was observed in both BT treated as well as in drug treated groups, however the antihypertensive effect of TBT was found to be more with respect to BT treated groups (Table 9). The renin level that was decreased in hypertensive rats in comparison to the normotensive rats was increased on treatment with BT as well as in drug treatment however the comparative increase with respect to BT is found to be more on treatment with TBT. The elevation in ACE level in hypertensive rats w.r.t. to the normotensive animals was decreased on treatment with BT as well as drug, however TBT exhibited greater effectivity in reducing elevated ACE levels in comparison to BT. In aortic ring assay, the maximally contracted aortic rings in hypertensive rats showed percent relaxation of 62.82±6.19 on treatment with BT, 78.54±5.54 on treatment with TBT and 92.64±3.4 on drug treatment. The experimental results exhibited greater anti-hypertensive potency of TBT in comparison to BT.

Discussion and Conclusion

TBT, prepared as per the chemometrically optimized desirability function (Figure 2) gave optimal pharmacologic response and organoleptic acceptability. Tea being a source of several potent molecules exhibited versatile pharmacology,¹¹ however to achieve synergistic add-on now tea diversification products have occupied a significant place in global nutraceutical market.¹² Therapeutic life style changes, dietary interventions are becoming prominent in the

changing treatment statement¹ and also the incorporation of integrative medicine that hyphenates the evidence based complementary therapy with the conventional approaches.³⁰ Basing on the concept of integrative medicine, in house remedial measures with preventative and curative potentials so as to halt at early stages of a disease onset and restrict from developing further complications is a safe, acceptable naturistic therapy.^{2,3,7,22} TBT, is found to contain several potent molecules, the flavonoids, catechins, benzotropolone compounds the theaflavins, the curcuminoids viz. curcumin, demethoxycurcumin, bisdemethoxycurcumin (Figure 4) and has exhibited significant antihypertensive Table 9, neuroprotective (Figure 5 & 6) and abilities to quench ROS and nitrite level (Table 8). Our research results have shown higher antihypertensive and neuroprotective potentiality of TBT in comparison to black tea alone (Figure 5 & 6) and (Table 8 & 9). Epidemiological research evidences have shown that regular consumption of black tea is found to lower elevated blood pressure.³¹⁻³³ Black tea also exhibited neuroprotective effect in colchicine induced alzheimer models by its significant antioxidant and acetylcholinesterase inhibitory effect and elevating the effect of depleted neurotransmitters viz. norepinephrine, dopamine, serotonin.^{7,22} Consumption of black tea is found to improve neurocognitive performance of patients with early onset of Alzheimer.³⁴ Methylxanthines in black tea viz. caffeine, theobromine, etc. are found to have a BP lowering effect and caffeine can affect the heart rate.^{35,36} Theanine, a novel amino acid found only in tea can influence heart rate, has antianxiety effect and improves sleep quality expectedly via attenuation of sympathetic nervous activation.^{35,37-39} L-theanine is a neuroprotective and in combination with caffeine can act as brain booster; moreover it can cross the blood brain barrier in 30min and exert its neuroprotective effect, supported by corroborative studies.^{7,11} TBT showed the presence of curcuminoids (Figure 4); curcumin is found to show a dose dependent effect on the hemodynamic variables of heart⁴⁰ and down regulation of the AT₁ receptors in vascular smooth muscle cells is responsible for its

antihypertensive effects.⁴¹ Curcumin aids neuroprotection by reducing brain inflammations, decreasing β amyloid formation, by its lipophilic action it acts intracellularly and improves cognitive function in Alzheimer patients; has anti-proliferative actions on microglia, helps the macrophages to clear amyloid plaques.⁴²⁻⁴⁵ However TBT acting as a combinatorial library of several potent pharmacophores and being a cocktail of multipotent antioxidants, polyphenols, curcuminoids exhibited multidimensional pharmacology and enhanced effects due to synergistic cumulative effect of the pharmacophores in the common matrix, also supported by corroborative research evidences.¹⁴

Black tea is not only a popular beverage owing to its flavor and astringency but the multitude of pharmacologically active molecules in black tea owes to its versatile pharmacology. Inclusion of proper compatible additives in definitive proportions is found to synergize the existing health effects of black tea. TBT, is a value added black tea diversification product with theronostic dietary adjuvant potentiality in combating hypertension and aiding neuroprotection and also a multitarget therapeutic agent whose further applications warrant rigorous human trials.

Table 9 Effect of TBT and BT on spontaneously induced hypertensive rats

Groups	NT (water 10ml/kg/day)	SIH (HS 10ml/kg/day)	BT treated	TBT treated	Drug treated
Systolic BP(mm of Hg)	123.4±9.2	201.8±12.6	161.9±8.6	142.1±9.6	120.9±8.6
Diastolic(mm of Hg)	82.4±6.5	151.5±11.6	137.2±9.4	102.4±9.2	84.3±7.6
Renin (ng/ml)	3.58±0.67	1.27±0.21	2.89±0.47	3.23±0.36	4.21±0.35
ACE (ng/ml)	16.96±1.36	22.34±2.45	19.87±1.98	18.54±1.24	17.25±1.68
Aortic ring assay	Near to 93% relaxation	Sustained contraction with 5363±541 at 120mM KCl was considered max	62.82±6.19 % relaxation	78.54±5.54 % relaxation	92.64±3.4 % relaxation

*NT-normotensive; SIH, spontaneously induced hypertensive; HS, high saline

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Conflict of interest

All authors declare that there is no conflict of interest.

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