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Research Article

Incorporation of Cinnamon Showed Evidence Based Value Addition in Black Tea

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Abstract

This study reports value additive synergistic hypoglycemic and hypolipidemic effects on addition of Cinnamon Black Tea (CBT) in comparison to black tea alone. Basing on desirability function of chemometrics and practical experimentation, 1.09 g of cinnamon was added to 4.27 g of black tea in 120 ml water and boiled for 15 min. The content of cinnamon and black tea in CBT was adjusted to keep the sensory attributes and pharmacological response optimal. Addition of cinnamon has not affected the chemoprofile of black tea as evidenced by FTIR studies. CBT has shown a good safety profile and the presence of wide range of molecules viz. catechins, theaflavins, cinnamaldehyde, procyanidins, coumarins etc., by LCMS analysis. Results of in vitro assays and in vivo experimentation in terms of antioxidant, hypoglycemic and hypolipidemic potentials exhibited greater potency of CBT in comparison to black tea.

Keywords: Cinnamon black tea; Value additive; Synergistic; Desirability function; Hypoglycemic; Hypolipidemic

Abbreviations

CBT: Cinnamon Black Tea; TLC: Therapeutic Lifestyle Changes; RSM: Response Surface Methodology; CCD: Central Composite Design; BT: Black Tea Decoction; DMSO: Dimethyl Sulfoxide; OECD: Organization for Economic Cooperation and Development; ALT: Alanine Aminotransferase; AST: Aspartate Aminotransferase; ALP: Alkaline Phosphatase; SOD: Superoxide Dismutase; CAT: Catalase; GSH: Reduced Glutathione; STZ: Streptozotocin; FBG: Fasting Blood Glucose; OGTT: Oral Glucose Tolerance Test; HbA1c: Glycosylated Hemoglobin; ITT: Insulin Tolerance Test; FI: Fasting Insulin; GLUT-4: Glucose Transporter-4; PPAR: Peroxisome Proliferator Activated Receptor; GLP-1: Glucagon-Like-Peptide-1

Introduction

Teas (*Camellia sinensis*), is not only a worldwide popular beverage but has multiple health benefits [1]. In the domain of phytomedicine or dietary supplements and nutraceuticals often a combination of bioactive principles are applied so as to achieve synergistic health benefits. Thus polytherapy gets a priority over monotherapy [2,3]. The main purpose of developing different tea diversification products, personalized tailored foods, rationalized food combinatorics, multi food actives concentrates is "value addition" [4]. Therapeutic Lifestyle Changes (TLC) and dietary interventions are recommended as adjunct to pharmacotherapy with synthetic drugs especially in chronic ailments like Type 2 diabetes, dyslipidemia, obesity [5]. Cinnamon (*Cinnamomum zeylanicum*) is a well known spice and its wide spread use is not only because of its special flavor due to cinnamaldehyde but its polymerized polyphenols the procyanidins have different pharmacological effects of hypoglycemic, antioxidant, etc., [6]. This research article aims to develop chemometrically optimized Cinnamon added Black Tea (CBT) with optimal pharmacologic response and organoleptic acceptance and study its synergistic value additive hypoglycemic and hypolipidemic effect in comparison to black tea alone.

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Materials and Methods

Reagents and chemicals

Good quality fresh tea leaves were procured from the tea garden of IIT Kharagpur and was used for producing black tea in Tea Engineering center of IIT Kharagpur. Cinnamon bark was collected from the medicinal garden of Agricultural and food engineering department of IIT Kharagpur. All chemicals and reagents used for the experimentation were all of analytical grade and were purchased either from Merck (India) and Sigma Aldrich.

Maintenance and care of animals

Due permission from the animal ethical committee was obtained (Registration No: 1722/RO/ERe/S/13/CPCSEA, Approval No: ARTI/CPCSEA/2015/ARTI 09). Adult male wistar rats (175 g ± 5 g) were used for the study. Care of animals was done as per the guidelines issued by Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA).

Chemometrics optimized preparation of cinnamon incorporated black tea

Response Surface Methodology (RSM) using desirability function helps to design an experimental methodology that helps to save experimentation time by reducing trials, expenditure in terms of material and personnel cost. It also offers the advantage of statistical analysis of experimental process parameters [2]. In this research work, Central Composite Design (CCD) of RSM [2] have been applied to optimize the amounts of black tea and cinnamon to prepare the Cinnamon Black Tea (CBT). The aim was to keep pharmacologic response (here antioxidant potentials were considered) and organoleptic acceptability optimal. Basing on the desirability function and practical experimentation, 4.27 g of black tea and 1.09 g of

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cinnamon powder was added to 120 mL of water preheated to boiling and kept for 15 min; the strained liquid is the Cinnamon Black Tea (CBT). The Black Tea decoction (BT) was prepared by adding 5.36 g of black tea to 120 mL 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 cinnamon powder mentioned above in CBT preparation.

Organoleptic assessment of finalized Cinnamon Black Tea (CBT)

Basing on the desirability function, the optimized CBT thus prepared was reevaluated for organoleptic acceptance on the basis of 9-point hedonic scale. Basing on this scale, evaluation of CBT was done in terms of flavor, taste, color and overall acceptability by trained taste panelists [7,8].

Compatibility studies of Cinnamon Black Tea (CBT)

Cinnamon was added to black tea for the purpose of value addition. However authors aimed that potentiation of health effects of black tea by adding cinnamon powder should not compromise with the sensory attributes of black tea and the chemical quality of black tea is retained. Thus chemical compatibility of cinnamon with black tea has been studied by FTIR. Individual spectrum of aqueous extracts of black tea, cinnamon and cinnamon incorporated black tea were recorded in FTIR studies. The basic purpose was to observe any changes in the spectral pattern of the black tea due to incorporation of cinnamon and thus identify the chances of any chemical interactions.

In vitro studies of antioxidant, hypoglycemic and hypolipidemic effects

As per literature methodologies, *in vitro* antioxidant potentials of CBT and BT was determined by 2,2-diphenyl-1-picrylhydrazyl or DPPH radical scavenging [9-11], 2, 2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid or ABTS [11-13] and Ferric ion reducing antioxidant power or FRAP assay [13,14]. The comparative *in vitro* hypoglycemic and *in vitro* hypolipidemic effect of CBT and BT were studied by *in vitro* alpha amylase and alpha glucosidase inhibitory assays [15,16] and *in vitro* lipase inhibitory assay [17-19].

LC-MS of CBT

Chemo profiling of CBT was done by LC-MS, following the chromatographic and mass spectroscopic conditions as per literature [20,21].

Safety profile of CBT

Safety profile of CBT was studied in rodent model (wistar rats) as per the guidelines of Organization for Economic Cooperation and Development (OECD) guideline 425. The study protocol for both acute and sub-acute toxicity studies was followed as per literature [22,23].

Administration of BT and CBT to experimental animals

Black tea decoction or BT prepared as mentioned earlier gave a percent yield of 20.5 and the solid content was calculated to be 4.58 mg. 0.5 mL of BT was orally administered to experimental rats twice a day (9.16 mg/rat/day) via gastric gavage needle.

Considering the case of CBT, the percent yield was found to be 11.6 and the solid content was calculated to be 4.17 mg; each rat were orally administered twice with 0.5 mL of CBT via gastric gavage needle and thus each rat received (8.34 mg/rat/day) solid content.

In vivo studies of oxidative stress, hypoglycemic and hypolipidemic effects

The *in vivo* antioxidant potentials of CBT and BT were studied by measuring their effect on Superoxide Dismutase (SOD), Catalase (CAT) and reduced Glutathione (GSH) [24-27].

The *in vivo* hypoglycemic and hypolipidemic effects of CBT and BT have been studied in adult wistar rats (170 g to 185 g) as per literature methods [28,29]. The relevant animal model for studying the hypoglycemic and hypolipidemic activity of the above test substances (CBT and BT) was developed by intravenous injection of streptozotocin in tail vein in a dose of 45 mg/kg (Sigma, USA) with simultaneous high glucose and fat diet. After 3 days of such treatment the fasting blood glucose was measured in Glucometer. Fasting blood glucose >250 mg/dl confirmed the diabetic state of the animal model.

Amongst six groups, with each group having six animals, the normal control (Group I) was treated with distilled water (5 ml/kg); the negative control (Group II) was treated with vehicle; the STZ treated diabetic controls were considered as Group III; Group IV was treated with Black Tea (BT, 1 ml at 150 mg/kg doses, twice daily) group who received; Group V was administered with CBT (1 mL, 150 mg/kg, twice daily), the positive control group (Group VI) was treated with metformin (150 mg/kg). Oral administration of test substances to the experimental animals was done with the help of gavage needle. Fasting Blood glucose (FBG) level and Oral Glucose Tolerance Test (OGTT) where glucose content was analyzed at 60 min and 120 min, after a fasting of 12 hrs to 14 hrs was determined with the help of glucometer [30]. Glycosylated hemoglobin (HbA1c) was analyzed as per literature [2]. Blood lipid profile (Total Cholesterol (TC), HDL-cholesterol, Triglycerides (TG), LDL cholesterol and VLDL cholesterol) was estimated spectrophotometrically using commercial kits [2] by withdrawing blood from heart of animals sacrificed under deep anesthesia. After statistical analysis of experimental results data were presented as mean ± SD and analyzed by Student's t-test (paired or unpaired, as desired) and $P < 0.05$ was considered significant.

Serum insulin and insulin sensitivity

Insulin Tolerance Test (ITT) was done where serum insulin level was estimated quantitatively by ELISA so as to assess the peripheral insulin resistance. HOMA-IR was calculated by using Fasting Blood Glucose (FBG) and Fasting Insulin (FI) level [30,31-33]. FBG and FI levels were used for the determination of hepatic insulin resistance. The insulin sensitivity level was calculated using the following formula: HOMA-IR=FI (μU/mL) × FBG (mg/dL)/22.5.

Stability studies of CBT

The stability studies of CBT was done by low temperature Differential scanning calorimetry within the temperature range of -20°C to 200°C [34].

Results and Discussion

Response surface methodology

Considering organoleptic score as 1st response and antioxidant potential by DPPH radical scavenging as 2nd response, the RSM was done. The process order fitted to quadratic design model. The result details on the adequacy of models (Tables 1 and 2), model summary statistics (Tables 3 and 4) and analysis of variance (Tables 5 and 6) was justifiable. The focus of model summary statistics was in maximizing the "Adjusted R-squared" and the "predicted R-squared". A good fitness between the actual and that obtained from the response model

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Table 1: Model adequacy considering organoleptic score as first response.

Source	Sum of squares	df	Mean square	F value	p-value Prob>F	
Mean *vs* total	456.08	1	456.08			
Linear *vs* mean	7.58	2	3.79	0.92	0.4311	
2FI *vs* Linear	1	1	1	0.22	0.6479	
Quadratic *vs* 2FI	**37.3**	**2**	**18.65**	**42.85**	**0.0001**	**Suggested**
Cubic *vs* quadratic	1.92	2	0.96	4.27	0.0829	Aliased
Residual	1.13	5	0.23			
Total	505	13	38.85			

Table 2: Model adequacy considering antioxidant potential (DPPH radical scavenging) as second response.

Source	Sum of squares	df	Mean square	F value	p-value Prob>F	
Mean *vs* total	19695.08	1	19695.08			
Linear *vs* mean	696.63	2	348.31	30.48	<0.0001	
2FI *vs* Linear	6.25	1	6.25	0.52	0.4889	
Quadratic *vs* 2FI	**97.17**	**2**	**48.59**	**31.28**	**0.0003**	**Suggested**
Cubic *vs* quadratic	4.37	2	2.19	1.68	0.2764	Aliased
Residual	6.5	5	1.3			
Total	20506	13	1577.38			

Table 3: Statistics summary of the model with organoleptic score as first response.

Source	Std. Dev.	R-squared	Adjusted R-squared	Predicted R-squared	PRESS	
Linear	2.03	0.1549	-0.0141	-0.4097	68.97	
2FI	2.12	0.1753	-0.0995	-0.6664	81.52	
Quadratic	**0.66**	**0.9377**	**0.8932**	**0.5572**	**21.66**	**Suggested**
Cubic	0.47	0.977	0.9448	-0.4717	72	Aliased

Table 4: Statistics summary of the model with antioxidant potential (DPPH radical scavenging) as second response.

Source	Std. Dev.	R-squared	Adjusted R-squared	Predicted R-squared	PRESS	
Linear	3.38	0.8591	0.8309	0.7518	201.29	
2FI	3.46	0.8668	0.8224	0.7367	213.48	
Quadratic	**1.25**	**0.9866**	**0.977**	**0.9184**	**66.21**	**Suggested**
Cubic	1.14	0.992	0.9808	0.641	291.13	Aliased

Table 5: Analysis of Variance (ANOVA) table with organoleptic score as first response.

Source	Sum of squares	df	Mean square	F value	p-value Prob>F	
Model	45.88	5	9.18	21.08	0.0004	Significant
A-BT	0.25	1	0.25	0.57	0.4732	
B-CT	7.33	1	7.33	16.84	0.0046	
AB	1	1	1	2.3	0.1733	
A2	29.59	1	29.59	67.99	<0.0001	
B2	11.98	1	11.98	27.53	0.0012	
Residual	3.05	7	0.44			
Lack of fit	3.05	3	1.02			
Pure error	0	4	0			
Cor Total	48.92	12				

Table 6: Analysis of Variance (ANOVA) table with antioxidant potential (DPPH radical scavenging) as second response.

Source	Sum of squares	df	Mean square	F value	p-value Prob>F	
Model	800.05	5	160.01	103.03	<0.0001	Significant
A-BT	5.54	1	5.54	3.57	0.1009	
B-CT	7.33	1	7.33	16.84	0.0046	
AB	1	1	1	2.3	0.1733	
A2	29.59	1	29.59	67.99	<0.0001	
B2	11.98	1	11.98	27.53	0.0012	
Residual	3.05	7	0.44			
Lack of fit	3.05	3	1.02			
Pure error	0	4	0			
Cor Total	48.92	12				

was observed where the R-squared values of 0.9377 (Table 3) and 0.9866 (Table 4) were found close to unity. May be due to block effect, as per normal expectation, the "Pred R-Squared" value of 0.5572 is not as close to the "Adj R-Squared" of 0.8932 (Table 3) and thus model reduction or response transformation is needed to be considered. "Adeq Precision" that measures the signal to noise ratio and a desirable value of greater than 4 is expected; however the ratio of 10.387 (for response 1) indicates an adequate signal and the model can be used to navigate the design space. Considering the 2nd response, the values of "Pred R-Squared" and "Adj R-Squared" of 0.9184 and 0.9770 (Table 4) are in reasonable agreement. For 2nd response, the "Adeq Precision" of 31.051 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.

The significance of the models were confirmed by the p-values

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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. In case of 1st response, the F-value of 21.08 implies the significance of the model. 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 0.0500 indicate that the model terms are significant. B, A^2, B^2 are significant model terms (Table 5) considering the 1st response. Considering the 2nd response, the model F-value of 103.03 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. The significant model terms in this case are B, A^2, B^2 (Table 6). The "lack of fit F-value" of 5.91 is not significant relative to the pure error. Internally studentized residuals helps to measure the number of standard deviations separating the actual and predicted values. In this case, the very less scattering of points and fitting almost to a straight line (Figure 1) satisfies the assumption of normality. The software generated final equations in terms of coded and actual factors for the responses have been presented in Table 7.

The values for desirability of the responses are shown in Figure 2. The optimal level of the parameter is indicated by the dot on each ramp function graph. Value of desirability function ranges from zero, outside of the limits to one at the goal. The main aim behind is to maximize the function that begins at a random starting point and proceeds up the steepest slope to a maximum. Basing on the desirability function, both pharmacologic response (antioxidant potential) and organoleptic acceptance were achieved at optimal with 4.27 g of black tea and 1.09 g of cinnamon powder.

The organoleptic acceptability of the finalized CBT used for further experimentations that was evaluated on the basis of 9-point hedonic scale gave a preference rating score of 8.8 for taste, 8.7 for texture, 8.9 for flavor and a overall preference rating value of 8.06 that indicated "like very much".

Compatibility studies

The FTIR spectra for compatibility studies are provided in Figure 3. From the peaks of IR spectrum of black tea (red spectrum) (Figure 3), peak at 3600 cm^{-1} to 3350 cm^{-1} corresponds to Str vib of OH gr; 1695 cm^{-1} to 1558 cm^{-1} (Caffeine & theobromine bands), 1650 cm^{-1} to 1400 cm^{-1} (Phenolic acids), 1600 cm^{-1} to 800 cm^{-1} (catechins), gallocatechins showed peaks at 1373 cm^{-1} to1280 cm^{-1}, epicatechin gallate at 1238 cm^{-1}, epicatechins at 820 cm^{-1}, and peaks at 833 cm^{-1} 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 cinnamon (green spectrum) (Figure 3) the IR peaks at 1727 cm^{-1} correspond to the aldehyde of saturated fat and peaks at

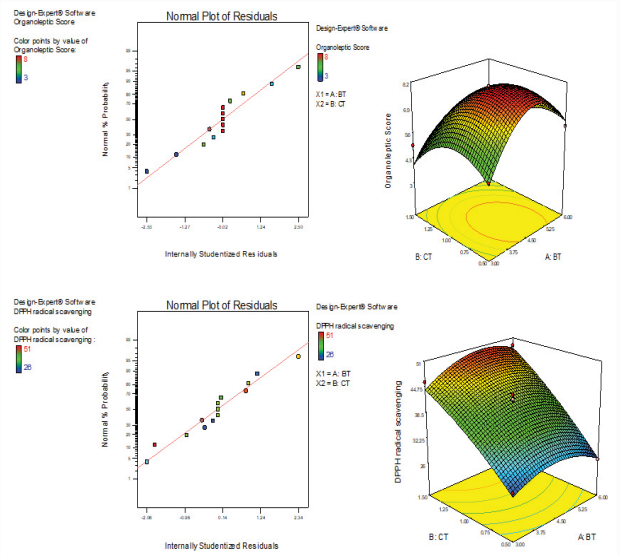

Figure 1: Internally studentized normal plot of residuals and 3D surface plots.

1678 cm^{-1} correspond to the stretching vibration of an aldehyde of carbonyl group C-O; presence of broad band's at 3500 cm^{-1} and 3200 cm^{-1} indicates O-H stretch, bands at 3000 cm^{-1} and 2850 cm^{-1} indicate H-bonding of alcohol and phenols, band at 1666.90 cm^{-1} revealed the presence of C=O of aldehyde, wide aldehyde peaks are due to conjugation and aromatic ring, bands between 1680 cm^{-1} to 1600 cm^{-1} are due to C=C stretches of alkenes; the strong absorption bands between 900 cm^{-1} and 675 cm^{-1} indicates the presence of aromatic C=C.

Considering the FTIR spectral pattern of black tea (red), cinnamon (green), cinnamon tea (pink) (Figure 3) each of the components are maintaining their individual identity and on intermixing no significant changes have been observed in chemical fingerprint. So it can be considered that addition of cinnamon has not affected the chemical identity of black tea.

In vitro assays

In vitro antioxidant DPPH radical scavenging activities of BT and CBT studied in the concentration range 50 µg/mL, 100 µg/mL, 200 µg/mL, 300 µg/mL were determined to be 20.74 ± 2.19, 37.92 ± 3.16, 62.24 ± 2.14 and 64.26 ± 2.14 respectively for BT and the corresponding values of CBT were 39.16 ± 2.05, 56.56 ± 2.16, 74.82 ± 3.29 and 82.96 ± 3.16 respectively. The results of ABTS and FRAP assays have shown greater antioxidant potentials of CBT in comparison to BT. The IC50

Table 7: Coded and actual factors for the final equations of the two responses.

Variables	Coded factors of the final equation	Actual factors of the final equation
Response 1: Organoleptic scores	8	-17.42862
	+0.18*A	+9.03452*BT
	-0.96*B	+11.58579*CT
	-0.50*A*B	-0.66667*BT*CT
	-2.06*A^2	-0.91667*BT2
	-1.31*B^2	-5.25000*CT2
Response 2: DPPH radical scavenging	42	-9.71016
	+0.83*A	+13.38807*BT
	+9.29*B	+22.08883*CT
	+1.25*A*B	+1.66667*BT*CT
	-3.63*A^2	-1.61111*BT^2
	-1.37*B^2	-5.50000*CT^2

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values of *in vitro* alpha amylase and alpha glucosidase inhibitory effects for BT were 244 µg/mL ± 1.46 µg/mL and 55 µg/mL ± 0.25 µg/mL respectively and that of CBT were 164 µg/mL ± 0.67 µg/mL and 12.8 µg/mL ± 0.78 µg/mL respectively. Considering the *in vitro* pancreatic lipase inhibitory effect, the IC50 value for BT was 14.4 µg/mL ± 0.64 µg/mL and that of CBT was 13 µg/mL ± 0.52 µg/mL. The results of *in vitro* assays have shown the greater effectivity of CBT in comparison to BT.

LC-MS of CBT

The LC-MS chromatogram of CBT (Figure 4) showed the presence of several compounds. Those with good abundances were detected within the retention time range of 0.91 min to 21.7 min. The presence of compounds were confirmed both by chromatographic and MS data. Comparison was done with retention times, UV-vis spectra and maximum absorption wavelength. Since in CBT both cinnamon and black tea are present, so bioactive compounds of both the constituents were detected in CBT. In the negative mode of Electron Spray Ionization (ESI), the tea catechins were detected. The a flavins are powerful black tea antioxidants; they are detected in the positive mode of ESI. In the mass range of 100 m/z to1000 m/z several tea compounds were detected. Gallic acid and caffeine were detected at m/z 169 and m/z 195 respectively. Major tea catechins were detected in the mentioned mass ranges i.e. m/z 289 (catechin and epicatechin), m/z 441 (catechin gallate and epicatechin gallate), m/z 457 (gallocatechin gallate and epigallocatechin gallate), gallocatechins and epigallocatechin (m/z 305). Quercetin and kaempferol -3-O-glucoside were also detected. Cinnamaldehyde and coumarins are important compounds of cinnamon detected at m/z 133 and m/z 147 respectively. Different polymerized polyphenols, the procyanidins were also detected e.g. Type A trimer proanthocyanin (m/z 865), Type B trimers (m/z 867.2134), and tetramers (m/z 1153.2629, 1155.2782), pentamers (m/z 1441.3259) were detected. The research results are supported with our other corroborative research studies [20,21].

Safety profile of CBT

In acute toxicity studies with CBT, till 7.5 g/kg 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 10 g/kg b.w. and the LD50 was determined to be 25 g/kg b.w. In sub chronic toxicity studies for 28 days, the effect of doses (100, 500, 1000, 2500, 5000 mg/kg b.w.) on body weight didn't show any significant changes till 21st day, however on 28th day significant lowering of body weight was observed with 100 mg/kg, doses 500 mg/kg and 1000 mg/kg exhibited body weight reduction from 21st day, with the increment in doses reduction in body weight was observed from the 14th day. No significant change in hepatic enzyme level (AST, ALT and ALP) was observed up to doses of 2500 mg/kg, however significant changes were observed at dose of 5000 mg/kg. The urea, creatinine, Na and K level didn't exhibit any significant change up to a dose of 5000 mg/kg. Considering the results of acute and sub chronic toxicity studies, the safety margin of CBT was found to be significantly high.

In vivo animal studies

CBT exhibited comparatively greater potency in recovering the endogenous antioxidant levels than BT (Figures 5-7) and the greater hypoglycemic potentials of CBT was further justified by its effect on FBG, OGTT (Figure 8), glycosylated hemoglobin, plasma insulin and HOMA and the *in vivo* hypolipidemic effects (Table 8) in comparison to BT alone. There was a significant elevation in FBG level ($p< 0.005$) amongst the diabetic group that was significantly lowered in BT ($p< 0.05$) and CBT treated group ($p< 0.005$); the hypoglycemic potentiality of CBT was found to be more in comparison to BT and the effects of CBT were comparable with the drug treated group ($p< 0.005$). The same hypoglycemic potentiality of CBT was observed in OGTT (Figure 8). Significant elevation in glycated hemoglobin level ($p< 0.005$) was observed in the diabetic group that was significantly reduced ($p< 0.05$) on treatment with BT, but reduction was more on treating with CBT ($p< 0.005$) and the effect of CBT was comparable to drug treated group ($p< 0.005$). The plasma insulin level that was significantly reduced in the diabetic group ($p< 0.05$) was found to improve significantly ($p< 0.05$) on treatment with BT, CBT and drug. The HOMA-IR values that was significantly ($p< 0.05$) increased in diabetic group (2.89) was found to reduce significantly in BT (2.15, $p< 0.05$), CBT (1.96, $p< 0.005$) and drug treated groups (1.88, $p< 0.005$).

Considering the comparative hypolipidemic effect of BT and CBT, the values of blood lipid parameters viz. TC, TG, HDL, LDL, VLDL was found to be significantly ($p< 0.05$) increased in disease induced group III (169.5 ± 10.5, 244.5 ± 12.3, 17.2 ± 5.5, 116.1 ± 9.4, 41.9 ± 9.5 respectively) in comparison to group I normal control (71.9 ± 8.9, 91.2 ± 11.4, 45.2 ± 6.6, 69.8 ± 9.6, 16.5 ± 7.8 respectively). The values of blood lipid parameters (TC, TG, HDL, LDL, VLDL) of vehicle treated (group II) are 72.5 ± 9.5, 89.1 ± 14.8, 44.2 ± 6.2, 68.2 ± 10.1, 15.9 ± 8.9 respectively. On treatment with BT (150 mg/kg), in group IV, the blood lipid parameters were significantly ($p< 0.05$) reduced in comparison to group III (diabetic control) and the blood lipid values being 156.4 ± 10.7, 200.2 ± 12.4, 25.9 ± 10.2, 99.6 ± 9.5, and 32.6 ± 10.5 respectively. However further significant ($p< 0.05$) reduction in the escalated blood lipid values was found in group V (treated with CBT, 150 mg/kg) in comparison to the diabetic control group (group III) and the values were found to be 110. 5 ± 8.5, 122.4 ± 10.4, 39.8 ± 9.4, 82.6 ± 9.8, 23.5 ± 11.2 respectively. In the Metformin (150 mg/kg) treated positive control group VI the significant reduction ($p<0.005$) in lipid profile values (99. 8 ± 9.5, 120.9 ± 8.4, 41.5 ± 5.4, 80.2 ± 11.8, 20.5 ± 10.6 respectively) in comparison to group III was obtained and the values are found to be comparable with the lipid lowering potency of CBT (Group V). Further the experimental values suggest the synergistic lipid lowering effect of CBT (Group V) in comparison to the BT treated group IV.

The thermal stability of CBT as studied by DSC, exhibited a glass transition temperature (Tg) of 114.51°C and a ΔCp value of 1.719 J/g*°C, for black tea the Tg value is 113.66°C and a ΔCp value of 0.951 J/g*°C, and for cinnamon the Tg value is 111.62°C and a ΔCp value of 1.042 J/g*°C. As regards stability aspects, the higher glass transition temperature values exhibits the thermal stability of cinnamon black tea and also black tea and cinnamon; moreover the closeness of Tg values of CBT with black tea and cinnamon shows that black tea and cinnamon have retained their individuality and incorporation of cinnamon aided in value addition of black tea without altering the original chemical qualities of black tea.

Hyphenation of conventional therapy with the evidence based complementary therapies is the underlying concept of integrative medicine [35]. Type 2 diabetes is multi-etiological and are influenced by poor glycaemic control leading to hyperglycemia, insulin resistance, oxidative stress leading to peroxidation of membrane lipids and protein glycation, excessive flux of fatty acids etc [36,37]. Black tea is not only a popular beverage but is reported to be of versatile pharmacology. Black tea can mimic insulin action and improve insulin

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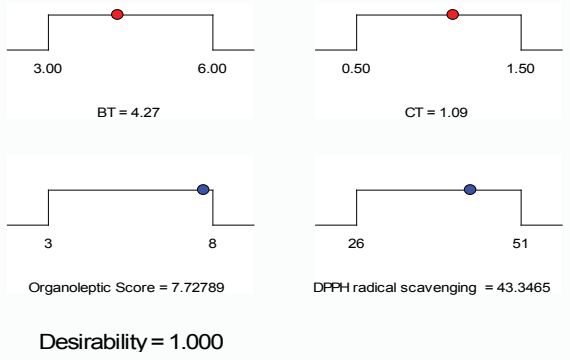

Figure 2: Desirability ramp function graph.

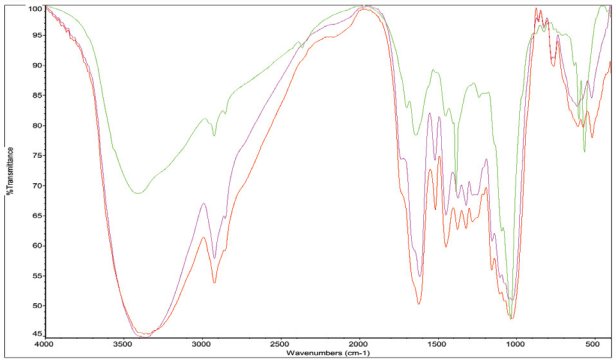

Figure 3: Overlapped FTIR spectra for black tea (red), cinnamon (green) and cinnamon black tea (green).

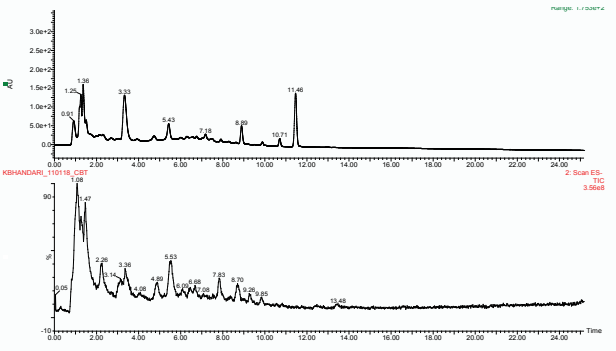

Figure 4: LCMS chromatogram of CBT.

signaling [1]. As per literature evidence, enhancing phosphorylation of the insulin receptor, activation of Glucose Transporter-4 (GLUT-4) to increase entry of glucose into the cells, increasing the expression of Peroxisome Proliferator Activated Receptor (PPAR) and thus enhancing insulin sensitivity, increasing the levels of Glucagon-Like-Peptide-1(GLP-1), stimulation of glycogen synthesis, inhibition of gluconeogenesis are some of the associated mechanisms for the glucose lowering effect of cinnamon [35,37]. Our research results showed inhibition of pancreatic amylase and intestinal glucosidases by Cinnamon Black Tea (CBT) is responsible for its hypoglycemic activity. The mechanism is also supported by corroborative researches in the hypoglycemic potency of cinnamon [35,37]. Oxidative stress increases lipid peroxidation leading to hypercholesterolemia. Cinnamon exerts hypolipidemic effect by inhibiting the activity of

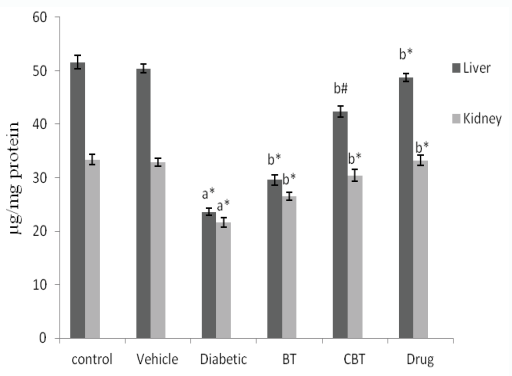

Figure 5: The comparative effect of BT and CBT on endogenous antioxidant level GSH. All results are expressed in ± SD after performing experiments in triplicate. Comparison with normal control group is presented as "a" and comparison to diabetic control group means "b"; significance at p<0.05 is marked as * and at p<0.005 is marked as #.

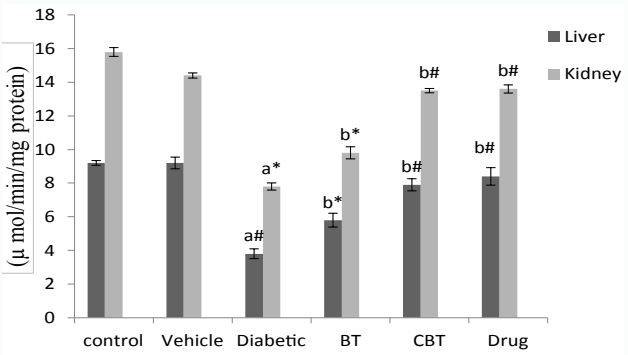

Figure 6: The comparative effect of BT and CBT on endogenous antioxidant level SOD. All results are expressed in ± SD after performing experiments in triplicate. Comparison with normal control group is presented as "a" and comparison to diabetic control group means "b"; significance at p<0.05 is marked as * and at p<0.005 is marked as #.

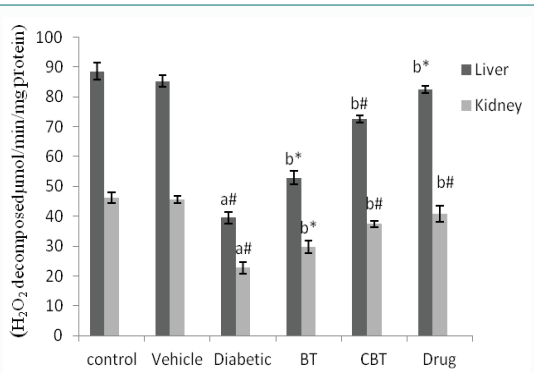

Figure 7: The comparative effect of BT and CBT on endogenous antioxidant level SOD. All results are expressed in ± SD after performing experiments in triplicate. Comparison with normal control group is presented as "a" and comparison to diabetic control group means "b"; significance at p<0.05 is marked as * and at p<0.005 is marked as #.

hepatic HMG-CoA reductase [38,39]. Basing on desirability function, CBT have been developed with chemometrically optimized weight ratios of black tea and cinnamon, maintaining the sensory attributes of black tea and antioxidant potentials at optimal level (Figure 2). Our research results have shown that CBT with the synergistic benefits

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of both black tea and cinnamon exhibited enhanced antioxidant (Figures 5-7), hypoglycemic and hypolipidemic potentials and thus can serve as an adjuvant therapy in Type 2 diabetes. CBT exhibited high organoleptic acceptability and results of compatibility studies have shown that both black tea and cinnamon have retained their own individual criteria (Figure 3) and incorporation of cinnamon haven't affected the quality attributes of black tea rather aided in neurotherapeutics value addition. LCMS analysis of CBT showed the presence of several potent molecules of black tea and cinnamon viz. catechins, benzotropolone ring containing polymerized polyphenols the theaflavins, other antioxidant compounds like gallic acid, quercetin [1] and cinnamaldehyde and procyanidins, methyl hydroxyl chalcones the potent compounds of cinnamon [6]. Theaflavins being powerful antioxidants were effective in alleviating oxidative stress; catechins, epicatechin, procyanidins quench free radicals because of their acidity and ability to transfer electrons. These molecules can also trap the intermediate reactive carbonyl species thus exhibiting antiglycation activities. Cinnamaldehyde of cinnamon not only imparts the characteristic cinnamon flavor but has antidiabetic activity due to its insulinotropic effect and enhancing glucose uptake in peripheral tissues via glucose transporter 4 [40]. The synergistic hypoglycemic and hypolipidemic effect of CBT in comparison to black tea alone may be due to positive interactions amongst the potent compounds of black tea and cinnamon thus accounting to 'value addition', also supported by corroborative research studies [2].

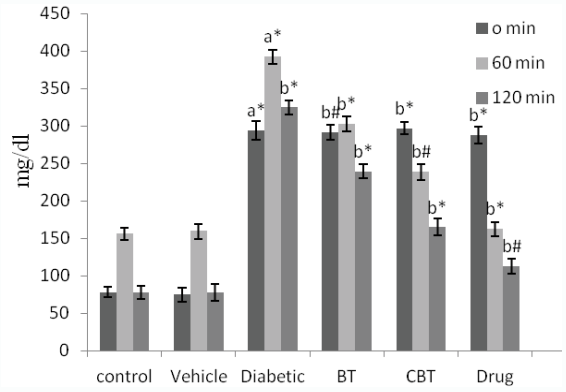

Figure 8: The comparative effect of BT and CBT on oral glucose tolerance test. All results are expressed in ± SD after performing experiments in triplicate. Comparison with normal control group is presented as "a" and comparison to diabetic control group means "b"; significance at p<0.05 is marked as * and at p<0.005 is marked as #.

Conclusion

Value addition in tea by incorporation of additives to develop tea diversification products and explore their synergistic and diversified pharmacology has opened new vistas of neurotherapeutics research. Poor glycaemic and lipidemic control is the root cause of several chronic ailments like diabetes, dyslipidemia and cardiovascular complications. Thus proper control of blood gluco-lipid profile in a naturistic manner can serve as an adjunct therapy and also form an important counterpart in integrative medicinal research. Basing on our research results CBT has enough potentiality in this regard.

References

1. Sen G, Bera B. Black tea as a part of daily diet: A boon for healthy living. Int J Tea Sci. 2013;9(2-3):51-9.
2. De B, Bhandari K, Singla RK, Katakam P, Samanta T, Kushwaha DK, et al. Chemometrics optimized extraction procedures, phytosynergistic blending and *in vitro* screening of natural enzyme inhibitors amongst leaves of Tulsi, Banyan and Jamun. Pharmacogn Mag. 2015;11(Suppl 4):S522-32.
3. De B, Bhandari K, Adhikari A, Katakam P, Adiki SK, Mitra A. Hypoglycemic hypolipidemic evaluation of a polyherbal phytocomposite and the mechanism of its synergistic anti-diabetic effect. Int J Pharm Biol Sci Arch. 2017;8(4):11-21.
4. Hajra NG, Yang Mo CW. Diversification of the tea products ---- global scenario. J Tea Sci Res. 2015;5(3):1-10.
5. Mitra A, Dewanjee D, Dey B. Mechanistic studies of lifestyle interventions in type 2 diabetes. World J Diabetes. 2012;3(12):201-7.
6. Natalia GV, Evangelopoulos A, Kollas A, Kazazis C. Hypoglycemic and hypolipidemic effects of cinnamon. Curr Top Nutraceutical Res. 2014;12 (4):127-34.
7. Adnan M, Ahmad A, Ahmed A, Khalid N, Hayat I, Ahmed I. Chemical composition and sensory evaluation of tea (*camellia sinensis*) commercialized in Pakistan. Pak J Bot. 2013;45(3):901-7.
8. Larmond E. Laboratory methods for sensory evaluation of food. Res Branch, Canada Deptt. of Agric. Canada. 1977.
9. Shalini D, Sudha G. Antioxidative activity of various tea extracts (*Camellia sinensis*). J Biol Sciences Res. 2010;1(1):271-8.
10. Banu SS, Majula AS, Latha MT, Ravi TK. De Novo drug design and synthesis of certain indole derivatives and screening for their Xanthine oxidase inhibitory activity. Int J Pharm Tech Res. 2010;2(4):2128-38.
11. Shonisani N. Effect of brewing temperature and duration on quality of black tea (*Camellia sinensis*) and equal combination of bush tea (Athrixia phylicoides DC) and black tea. Mini Dissertation, University of Limpopo. 2010.
12. Taheri M, Sairi R, Giahi M, Ghafoori H. Screening antioxidant activity of extracts from different tea samples. Pharmacologyonline. 2011;2:674-83.
13. Xu JZ, Yeung SYV, Chang Q, Huang Y, Chen ZY. Comparison of antioxidant activity and bioavailability of tea epicatechins with their epimers. Br J Nutr. 2004;91(6):873-81.
14. Patel G, Gauni B, Mehta K, Patel BN. Comparative study of antioxidant capacity of raw powder and waste black tea by FRAP assay. Res J Recent Sci. 2014;3:42-4.
15. Subramanian R, Azmawi ZM, Sadikun A. *In vitro* α-glucosidase and α-amylase enzyme inhibitory effects of Andrographis paniculata extract and andrographolide. Acta Biochim Pol. 2008;55(2):391-8.
16. Basak SS, Candan F. Chemical composition and *in vitro* anti-oxidant and anti diabetic activities of Eucalyptus camaldulensis Dehnh, Essential oil. J Iran Chem Soc. 2010;7(1):216-26.
17. Inthongkaewa P, Chatsumpunb N, Supasuteekul C, Kitisripanyac T, Putalunc W, Likhitwitayawuida K, et al. Glucosidase and pancreatic lipase inhibitory activities and glucose uptake stimulatory effect of phenolic compounds from Dendrobium formosum. Rev Bras Farmacogn. 2017;27(4):480-7.
18. Chedda U, Kaikini A, Bagle S, Madhav S, Sadhana S. *In vitro* pancreatic lipase inhibition potential of commonly used Indian spices. IOSR J Pharm. 2016;6(10):10-13.
19. Senapaty S, Yadav RP, Paul NS, Chavan R. Evaluation of *in-vitro* pancreatic lipase inhibitory activity of fenugreek seeds extracts. IJPHC. 2014;4(4):1-7.
20. Ganguly S, Kumar TG, Mantha S, Panda K. Simultaneous determination of blacktea derived catechins and theaflavins in tissues of tea consuming animals using ultra-performance liquid-chromatography tandem mass spectrometry. PLoS One. 2016;11(10):e0163498.
21. De B, Bhandari K, Singla RK, Saha G, Goswami TK. In silico molecular GRIP docking of some secondary metabolites combating diabesity. Bull Natl Res Cent. 2020;44(66):1-13.
22. De B, Bhandari K, Katakam P, Goswami TK. Development of a standardized combined plant extract containing nutraceutical formulation ameliorating metabolic

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syndrome components. SN Appl Sci. 2019;1(1484):1-12.

23. De B, Bhandari K, Katakam P, Mitra A. *In vivo* hypoglycemic studies of polyherbal phytoceuticals, their pharmacokinetic studies and dose extrapolation by allometric scaling. Curr Drug Discov Technol. 2017;14(4):277-92.

24. Misra HP, Fridovich I. The generation of superoxide radical during the autooxidation of hemoglobin. J Biol Chem. 1972;247(21):6960-2.

25. Cohen G, Dembiec D, Marcus J. Measurement of catalase activity in tissue extracts. Anal Biochem. 1970;34:30-8.

26. Roy C, Ghosh TK, Guha D. The anti-oxidative role of *benincasa hispida* on colchicine induced experimental rat model of alzheimer's disease. Biog Amines. 2007;21(1-2):42-55.

27. Ellman GL. Tissue sulphydryl groups. Arch Biochem Biophys. 1959;82:70-7.

28. Srinivasan K, Viswanad B, Asrat L, Kaul CL, Ramarao P. Combination of high fat diet fed and low dose streptozotocin treated rat: A model for type 2 diabetes and pharmacological screening. Pharmacol Res. 2005;52(4):313-20.

29. Zhang M, Lv XY, Li J, Xu ZG, Chen L. The characterization of high fat diet and multiple low dose streptozotocin induced type 2 diabetes rat model. Exp Diabetes Res. 2008:1-9.

30. Shamshun N, Kauser H, Rani P, Alam I. Effects of *nigella sativa* seed extract on insulin resistant non-insulin-dependent diabetic guinea pigs. Am J Ethnomed. 2015;2(1):58-67.

31. Morgan CR, Lazarow A. Immunoassay of insulin two antibody system: Plasma Insulin Levels of Normal, Subdiabetic and Diabetic Rats. Diabetes. 1963;12(2):115-26.

32. Murali B, Upadhyaya UM, Goyal RK. Effect of chronic treatment with enicostemma littorale in non-insulin- dependent diabetic (NIDDM) rats. J Ethnopharmacol. 2002;81(2):199-204.

33. Uno T, Ohsawa I, Tokudome M, Sato Y. Effect of goshajinkigan on insulin resistance in patients with type 2 diabetes. Diabetes Res Clin Pract. 2005;69(2):129-35.

34. Campelo PH, de Abreu FJ, Ferraz V, Yoshida MI, de Barros FRV, Botrel DA, et al. Hygroscopic thermal and chemical properties of cinnamon essential oil microparticles obtained by spray drying. Emir J Food Agric. 2017;29(11):884-92.

35. Medagama AB. The glycaemic outcomes of Cinnamon, a review of the experimental evidence and clinical trials. Nutr J. 2015;14:108.

36. Zhou L, Pan Y, Chonan R, Batey R, Rong X, Yamahara J, et al. Mitigation of insulin resistance by mangiferin in a rat model of fructose-induced metabolic syndrome is associated with modulation of CD36 redistribution in the skeletal muscle. J Pharmacol Exp Ther. 2016;356(1):74-84.

37. Sahib AS. Anti-diabetic and antioxidant effect of cinnamon in poorly controlled type-2 diabetic Iraqi patients: A randomized, placebo-controlled clinical trial. J Intercult Ethnopharmacol. 2016;5(2):108-13.

38. Tuzcu Z, Orhan C, Sahin N, Juturu V, Sahin K. Cinnamon polyphenol extract inhibits hyperlipidemia and inflammation by modulation of transcription factors in high-fat diet-fed rats. Oxid Med Cell Longev. 2017;2017: 1583098.

39. Rahman S, Begum H, Rahman Z, Ara F, Iqbal MJ, Yousuf AKM. Effect of cinnamon (*cinnamomum cassia*) as a lipid lowering agent on hypercholesterolemic rats. J Enam Med Coll. 2013;3(2):94-8.

40. Abrigo E, Gregnanin M. Cinnamon ally of diabetic.2012.

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