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RADICAL SCAVENGING ABILITY OF BLACK TEA INFUSIONS WITH OR WITHOUT MILK IN COMBINATION WITH *OCIMUM GRATISSIMUM*

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Abstract

Tea is consumed in most parts of world for its aroma, flavour, taste and health promoting potential. Black tea can be consumed with/without the addition of milk, that can alter its antioxidant capacity. Moreover, in India black tea is commonly supplemented with medicinal herbs along with milk. One of the herbs used for supplementation is tulsi (*Ocimum. Gratissimum (O. gratissimum)*). This is for the first-time combined effect of tulsi (*O. gratissimum*) and milk on the antioxidant capability of black tea is undertaken. Black tea alone showed more antioxidant potential than *O. gratissimum*. The binary combination of black tea and *O. gratissimum* displayed additive effect, the antioxidant capacity enhanced for DPPH, LPO and ABTS as compared to black tea. The addition of milk to black tea, *O. gratissimum* or their combination lead to decrease in DPPH scavenging potential, but in case of LPO and ABTS test the activity was increased. Overall, ternary combination of black tea, *O.gratissimum* and milk demonstrated highest combined antioxidant capability in ABTS and LPO assay.
***Keywords*:** Black tea, Tulsi, Antioxidant, Milk, DPPH, ABTS,

Introduction

Tea is one of the popular beverages in the world (Awasom, 2011; Kumar *et al.*, 2018) that is consumed for its flavor, aroma, taste and health benefits. All tea come from *Camellia sinensis* plant. The major portion of tea phytochemicals belongs to polyphenols which possess unique antioxidant properties to fight against free radicals and promote anti-ageing effects (Balentinen, 1997; Nankar *et al.*, 2017). The free radicals involved in various disorders are hydroxyl radical, superoxide radicals, alkoxyl radical, hydroperoxyl radical, peroxyl radical and nitric oxide radical (Hou *et al.,* 2003; Kaur *et al.*, 2016; Sharma, 2016). These free radicals are associated with lipid peroxidation, DNA damage, carcinogenesis, cardiovascular and neuro degenerative diseases (Kahkonen *et al.,* 1999; Sudhakar *et al.*, 2015). These harmful actions can be controlled by antioxidant substances, which detoxify the organism by scavenging free radicals (Sharma *et al.*, 2019; Vyas, 2019).

Tea is rich in phenolic compounds and are important source of antioxidants (Al-Rejaie *et al.,* 2009; Prasher *et al.*, 2018). The processing of tea may alter the property of tea type. There are mainly three types of teas, black, oolong and green (Singh *et al*., 2011; Gul *et al*., 2016; Upadhyay *et al.*, 2019). Black tea is obtained by maximum-oxidation, oolong tea by semi-oxidation and green by least-oxidation. Tea products vary in their biochemical profiles; oolong and green tea contain high amount of catechins while black tea is rich in theaflavins (TFs) and thearubigins (TRs) (Hollman *et al.,* 1997). The total production of different types of tea throughout the world is 78% (black tea), 20% (green tea) and <2% (oolong tea) (Kuroda and Hara, 1999). Tea is associated with various health promoting effects such as anticarcinogenic (Dufresne and Farnworth, 2000; Kumar *et al.*, 2018), along with reduction of cardiovascular diseases (Sano *et al.,* 2004; Kuriyama *et al.,* 2006) and antioxidative action (Benzie and Szeto, 1999). Major constituents important for these health benefits are polyphenols that account for 30% of the leaf weight and comprises the largest group of chemical composition in black tea followed by protein and carbohydrates (Leenen *et al.,* 2000; Jain *et al.*, 2018).

Ocimum gratissimum is an aromatic, perennial herb, it attains 1-3 m height; stem is dark brown in color bearing leaves from top to bottom, woody at the base, often with epidermis peeling in strips (Paton, 1992; Saxena *et al.*, 2016; Farooq and Sehgal, 2019a, 2019b). *O. gratissimum* (Tulsi) bears essential oils in its stem and leaves (Sulistiarini, 1999). It belongs to family Labiateae, and widely distributed in tropical Africa and South-East Asia, largely in India and Hawaii. Tulsi is also used for the treatment of anti-convulsive, anti-malaria, paralysis, high fever, epilepsy, diarrhea, influenza, cough, sunstroke, gonorrhea and mental illness (Sulistiarini, 1999; Kar *et al.*, 2018; Sharma *et al.*, 2017).

Black tea can be consumed with/without the addition of milk. The addition of milk can give various effects, and these can be categorized into negative, positive, neutral and dual effects (Graham, 1992; Gupta *et al.,* 2002; Katiyar and Mukhtar 1996). The incorporation of milk into tea can either decrease or completely inhibit tea antioxidant properties. Milk components, caseins after interacting with polyphenolic catechins from tea, decreases its antioxidant property (Rashid *et al.,* 2015; Singh *et al.*, 2016).

In India black tea is commonly supplemented with medicinal herbs to enhance its flavor and health benefits. This is for first time the combined effect of tulsi and milk on the antioxidant capability of black tea is being evaluated.

Material and methods

Plant material

The Leaves of *Ocimum gratissimum* (OG) was collected from herbal garden, Lovely Professional University, Phagwara (Punjab) in month of January 2017. Further the leaves were shade-dried at room temperature for six days. The commercially available black tea bags were purchased from the market (Brooke Bond, Taj Mahal, Hindustan Unilever limited).

Preparation of aqueous extracts

The aqueous infusion of black tea (1%) or tulsi (1%) is prepared individually or in combination by brewing in 10ml of distilled water with or without milk at 90-95^0 c for 5 minutes. The obtained extracts are filtered and kept at 4^{0}c for further studies (Gupta *et al.*, 2013; Jyoti *et al.*, 2018).

Determination of antioxidant activity

The antioxidant activity of individual infusion or combination was measured using DPPH (1, 1-diphenyl-2-picrylhydrazyl) assay (Mensor *et al.*, *2001*). For measuring the antioxidant activity of different infusion, another test assay ABTS was used (Chouhan *et al.*, 2017; Vyas, 2017). The antilipid peroxidation of various samples was determined at 532nm (Ohkawa *et al.*, *1979*). Total phenolic content was calculated using Folin-ciocalteau assay and gallic acid as standard (Singleton *et al.,* 1999; Sivakumar *et al.*, 2011, Mishra *et al.*, 2018).

Combination index (CI)

The interactions between binary mixture of black tea with *Ocimum gratissium* (additive or antognostic or synergistic) at a ratio of 1:1 was tested using combination index (CI) (Chou, 2006). If CI < 1 (synergistic), CI > 1 (antagonistic), and CI = 1 (additive effect) (Prabhakar *et al.*, 2013, 2014).

Statistical analysis:

The results obtained were presented as mean ± standard deviation (SD). Each sample is run in triplicates. To test inter group differences the obtained data is scrutinized by post-hoc test (Tukey′s honestly significant difference test) using SPSS software (version 18). p-value ≤ 0.05 was regarded as statistically significant.

Results and Discussion

Antioxidant activity was determined using standard chemical methods such as DPPH and ABTS. Moreover, ex-vivo antioxidant activity was also evaluated by employing chick liver lipid peroxidation test. In all the performed antioxidant assays, EC_{50} value of infusions was calculated. The lower the EC_{50} value the higher antioxidant potential of the infusion. The total phenolic content of different infusions was also determined using gallic acid.

EC_{50} of infusions for DPPH, ABTS and lipid peroxidation assays

The findings revealed that various individual aqueous infusions may act differently against different radicals. Among the individual infusions black tea exhibited the lowest EC_{50} (that is highest antioxidant potential) in all the assays performed (DPPH, ABTS and LPO). In DPPH assay, EC_{50} of black tea (BT), *Ocimum gratissimum* (OG), black tea and milk (BTM), *Ocimum gratissimum* and milk (OGM), black tea and *Ocimum gratissimum* (BTOG), and black tea in combination with *Ocimum gratissimum* and milk (BTOGM) were 58.48µg/ml, 75.13µg/ml, 67.01µg/ml, 86.55µg/ml, 63.50 µg/ml and 81.07µg/ml, respectively. Among all samples, BT showed least EC_{50} and highest antioxidant activity whereas BTOGM showed highest EC_{50} and lowest antioxidant activity as depicted in Table 1.

Table 1: DPPH free radical scavenging activity compared with black tea.

Drug/Combo	EC_{50}	$1/EC_{50}$	% Change in DPPH scavenging activity
BT	58.48	0.017	-
OG	75.13	0.013	22.16 (-)
BTM	67.01	0.014	12.73 (-)
OGM	86.55	0.011	32.43 (-)
BTOG	63.50	0.015	7.911 (-)
BTOGM	81.07	0.012	27.87 (-)

BT, OG, BTM, OGM, BTOG and BTOGM represent Black Tea, *O. gratissimum*, Black tea with Milk, *O. gratissimum* with Milk, Black tea with *O. gratissimum*, Black tea in combination with *O. gratissimum* and Milk. EC_{50} (Effective concentration causing 50% scavenging activity).

In comparison with black tea a significant decrease was found in the antioxidant activity of OGM (32.43%), BTOGM (27.87%), OG (22.16%), BTM (12.73%) and BTOG (7.91%). The addition of milk decreased the DPPH scavenging activity in the following manner in various infusions: BTM (12.73%), OGM (10.27%) and BTOGM (19.95%), respectively (Table 1). The DPPH assay is used widely to evaluate the free radical scavenging ability of various extracts; same method was adopted here to evaluate the black tea infusions. The degree of discoloration indicates the scavenging potential of extracts. The scavenging potential of black tea was more followed by BTOG and OG. After supplementation of milk, significant decrease was found in antioxidant activity of BT, OG and BTOG (Malik *et al.*, 2013; Malik *et al.*, 2016). This was because the addition of milk may mask the radical scavenging activity due to presence of proteins (Singh and Thakur, 2018; Mishra, 2019a). Earlier study also reported that radical scavenging activity of black tea increase with increase in concentration and also, the activity of BT was found to be more but when supplemented with milk less activity was found (Korir *et al.,* 2014; Mishra, 2019b) while in contradiction to other studies that evaluated that addition of milk will not influence the antioxidant potential (Kyle *et al.,* 2007; Van *et al.,* 1998; Reddy *et al.,* 2005).

Table 2: ABTS radical scavenging activity of various infusions compared with black tea.

Drug/ Combo	EC_{50}	$1/EC_{50}$	% Change in ABTS scavenging activity
BT	20.76	0.048	-
OG	42.47	0.023	51.10 (-)
BTM	18.95	0.052	9.59 (+)
OGM	38.74	0.025	46.39 (-)
BTOG	32.11	0.031	35.31 (-)
BTOGM	26.66	0.037	22.11 (-)

BT, OG, BTM, OGM, BTOG and BTOGM represent Black Tea, *O. gratissimum*, Black tea with Milk, *O. gratissimum* with Milk, Black tea with *O. gratissimum*, Black tea in combination with *O. gratissimum* and Milk. EC_{50} (Effective concentration causing 50% scavenging activity).

In ABTS assay, EC_{50} of BT, OG, BTM, OGM, BTOG and BTOGM were 20.76µg/ml, 42.47µg/ml, 18.95µg/ml, 38.74µg/ml, 32.11 µg/ml and 26.66µg/ml, respectively (Table 2). BTM showed least EC_{50} and highest antioxidant activity whereas OG showed highest EC_{50} and lowest antioxidant activity as depicted in Table 2. In comparison with black tea a significant decrease was found in the antioxidant activity of OG (51.10%), OGM (46.39%), BTOG (35.31%) and BTOGM (22.11%). In case of BTM an increase of (9.59%) was observed. The ABTS quenching

ability of BT was observed to be highest followed by BTOG and OG. After supplementing of milk in black tea, slightly increase in activity of BT, OG and BTOG was observed (Singh *et al.*, 2019; Prabhakar *et al*., 2020). This was because ABTS is used both for hydrophobic and hydrophilic substances. The hydrophobic substances which cannot perform in DPPH are performing here. Previous finding observed opposite result that milk decrease the antioxidant activity because of the presence of pure a, b, and j-caseins, the main proteins in milk, masked to different extents the ABTS-scavenging capacities black tea extracts and of some pure flavonoids typically found in teas (Bourassa *et al.,* 2013; Kaur *et al.*, 2020).

Table 3: Anti-lipid peroxidation activity of various infusions compared with black tea.

Drug/Combo	EC_{50}	$1/EC_{50}$	% Change in anti LPO scavenging activity
BT	38.46	0.025	-
OG	67.56	0.014	43.06 (-)
BTM	38.43	0.026	0.087 (+)
OGM	64.66	0.015	40.50 (-)
BTOG	46.61	0.021	17.46 (-)
BTOGM	44.23	0.022	13.04 (-)

BT, OG, BTM, OGM, BTOG and BTOGM represent Black Tea, *O. gratissimum*, Black tea with Milk, *O. gratissimum* with Milk, Black tea with *O. gratissimum*, Black tea in combination with *O. gratissimum* and Milk. EC_{50} (Effective concentration causing 50% scavenging activity).

Showed least EC_{50} and highest antioxidant activity whereas OG showed highest EC_{50} and lowest antioxidant activity (Table 3). The addition of milk increased the ABTS scavenging activity in the following manner in various infusions: BTM (9.59%), OGM (4.71%) and BTOGM (13.20%), respectively. The addition of milk had almost no effect on the antilipid peroxidation activity of BTM (0.087%) whereas slight increase was observed in OGM (2.56%) and BTOGM (4.42%), respectively. The anti-lipid peroxidation activity of black tea represented minimum EC_{50} i.e. maximum scavenging activity as compared to OG and BTOG, which showed similar activity. A dose dependent effect was also observed in this assay. No significant difference was observed after supplementation of milk in BT, OG and BTOG. Previous studies reveal that Milk influenced positively the inhibition of lipid peroxidation by teas (Bourassa *et al.,* 2013; Kaur *et al.*, 2016; Kumar *et al.*, 2019). This may be due to different variety of milk used. From combination index studies it was observed that interaction between BTOG and BTOGM is nearly additive in all performed assays except BTOG in case of ABTS assay showed slightly antagonism.

The overall effect of supplementation of milk and *O. gratissimum* with BT enhances the total antioxidant activity. In comparison with black tea a decrease in DPPH scavenging activity was found for BTM (12.739%) while in case of BTOG (83.007%) and BTOGM (44.257%) whereas an increase was found in ABTS scavenging activity of BTM (9.591%), BTOG (29.361%) and BTOGM (55.772%). An increase in antilipid peroxidation capability was found for BTOG (65.061%) and BTOGM (73.912%) but almost no change was found in BTM (Table 4).

Table 4: Antioxidant activity by supplementing black tea with *O. gratissimum* and milk as standard in different assays.

Assay	Drug/Combo	% Change in radical scavenging activity
DPPH	BT	-
	BTM	12.739 (-)
	BTOG	83.007 (+)
	BTOGM	44.257 (+)
ABTS	BT	-
	BTM	9.591 (+)
	BTOG	29.361 (+)
	BTOGM	55.772 (+)
LPO	BT	-
	BTM	0.0874 (+)
	BTOG	65.061 (+)
	BTOGM	73.912 (+)

BT, OG, BTM, OGM, BTOG and BTOGM represent Black Tea, *O. gratissimum*, Black tea with Milk, *O. gratissimum* with Milk, Black tea with *O. gratissimum*, Black tea in combination with *O. gratissimum* and Milk.

Total phenolic content of different infusions alone and in combinations

The total phenolic content (TPC) calculated on the basis of gallic acid standard curve was found highest in BTM (97.76 mg GAE/g), followed by BT (58.49 mg GAE/g), BTOGM (56.66 mg GAE/g), OGM (55.69 mg GAE/g), BTOG (43.15 mg GAE/g), and lowest in OG (35.24 mg GAE/g), respectively.

Conclusion

In this study, black tea showed more antioxidant potential than *O. gratissimum*. The binary combination of black tea and *O. gratissimum* displayed nealy additive effect that enhanced the antioxidant activity for DPPH, LPO and ABTS as compared to black tea. The addition of milk to black tea, *O.gratissimum* or their combination lead to decrease in antioxidant activity in DPPH assay, but in LPO and ABTS the activity was increased. Further studies are required to confirm the antioxidant effect of black tea and *O. gratissimum* with or without milk by employing in vivo models.

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