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Neuroprotective Potential of *Bacopa monnieri, Camellia sinensis* and Curcumin on Chlorpyrifos Exposed *Drosophila melanogaster*

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Abstract

The popular treatment approaches of neurodegenerative diseases are generally for symptom management, but unfortunately, there is no significant curative approach present so far that can signifcantly reduce its progression. Indian traditional medicine is being used alone or in a combination of herbs to fight against these diseases. The commonly used herbs/traditional medicine(s) for this purpose are *Bacopa monnieri* (Brahmi), *Curcuma longa* (curcumin) and *Camellia sinensis* (green tea). In the present study, herbs were judged for its free radical scavenging activity through DPPH free radical scavenging assay, Total Phenolic Content (TPC) and also Total Flavonoid Content (TFC). Among them, green tea exhibited highest total phenolic content and also higher total flavonoid content (99.667±3.547 mg GA/g and with 7.633±2.532 mg QE/g, respectively) followed by *Bacopa monnieri* and *Curcumin*. In this context, Green tea showed maximum free radical scavenging activity than curcumin followed by Brahmi (GT>CUR>BM). These herbs were evaluated to check their potential in the improvement of the climbing ability of flies and acetylcholinesterase enzyme activities using *Drosophila melanogaster* (fruit flies). In this study, fruit flies were fed normal food, chlorpyrifos contaminated food and also chlorpyrifos (Chlor) along with individual herbs as mentioned above (Chlor+GT, Chlor+CUR, Chlor+BM). Interestingly, we found that the free radical scavenging activity and antioxidant activities, of Brahmi (97%) has the highest improvement in climbing activity as compared to green tea (90%) followed by curcumin (86%) and organisms treated with these herbs show a trend BM>GT>CUR. Moreover, Brahmi also exhibited maximum improvement in AChE activities as compared to curcumin and green tea and showed a trend BM>CUR>GT.

Keywords: Antioxidant, *Drosophila melanogaster*, Free Radical Scavenging, Neurodegeneration, Plant Extract, Total Phenolic Content

1. Introduction

Neurodegenerative diseases are one of the groups of human diseases with common characteristics without exhibiting any symptoms throughout its development within the central nervous system; it develops suddenly in an individual[1]. Among neurodegenerative diseases, Alzheimer's Disease (AD) demonstrates reduced cognitive functions akin to learning and memory which has been tested clinically[2]. The triggering event in AD is the accumulation of Aβ peptide that may underlie synaptic failure which results in the decline of cognitive function and neuronal dysfunction[3]. Following AD, second most known disorder of neurodegenerative disease is Parkinson's Disease (PD)[4,5].

Variety of previous studies have shown that environmental risk factors are directly or indirectly answerable for

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neurodegeneration[6]. Irregular unharness of dopamine and above all its release into the cytoplasm of dopaminergic neurons, ends up in oxidative stress that causes the instant death of neurons[7]. Prevalence of neurodegenerative illness is rising because of increasing CNS infections, psychiatrical and neurologic disorders[8]. The phytochemicals with medicinal properties are used[9] to combat these diseases. There is a lot of information and data of phytochemicals developed from a long list of herbs originated from nature, and ancient medicines for thousands of years in developing countries like India[10,11].

These natural remedies include *Withania somnifera, Mucuna pruriens Bacopa monnieri* and *Curcuma longa*. Among these, we aimed to evaluate efficacy of *Bacopa monnieri* as a preventive/therapeutic agent for a neurodegenerative disease in Drosophila model system with different nutraceuticals like tea leaf and curcumin[12]. *Bacopa monnieri* is usually referred to as water hyssop and has been used as a conventional medicinal plant since years[13,14]. The extract of *B. monnieri* displays *in vitro* antioxidants and cell protection effects. In animals, it inhibits acetylcholinesterase (AChE) repressive activity, activates choline acetyltransferase and increases cerebral blood. Various components of the β-amyloid-induced oxidative cascade that can lead to AD and diminish β-amyloid levels in the brain of AD transgenic mouse model (PSAPP mice)[15]. Based on a previous report, *B. monnieri* has been shown to act as memory enhancer, anti-anxiety, anti-epilepsy and against psychological feature dysfunctions since primeval period[16].

Natural substances that are present in extracts obtained from plants, fruits, and vegetables corresponding to olive oil, red wine, and tea are well-known polyphenols[17,18]. The first role of polyphenols is to act as an antioxidant and supply cell resistance against aerophilous stress through free radical scavenging activity. Antioxidant properties of polyphenols play a therapeutic role against neurodegenerative dieases like PD and AD[19]. The *C. longa* usually referred to as curcumin derived from turmeric (Haldi), is a yellow seasoning, being used as a food preservative, and flavoring medication in India. *Curcumin longa* has additional antioxidant and anti-inflammatory properties and can defend against pathology of AD. The varied neuroprotective activities of *C. longa* are still restricted, however, *C. longa* influences neuronic proliferation.

Organophosphate compounds are a class of chemicals chiefly used as an insecticide and an industrial effluent. The hepato-toxic action of organophosphate compound by inhibition of acetylcholinesterase (AChE)[20] that ends up in the intoxication of the central and peripheral nervous system. It is a neurotoxic compound inflicting inhibition of cholinesterase leading to acute toxicity. In this context, chlorpyrifos toxicity could affect cholinesterase inhibition together with the inhibition of mitochondrial ATP production through uncoupling of oxidative phosphorylation that leads to the ROS generation[18].

Chaudhuri *et al.* (2007)[16] demonstrated the correlation of environmental and genetic factors in PD model of *Drosophila melanogaster* and exhibited higher probability of PD in males and found earlier symptoms in them as compared to females. A recent study exhibited the role of *B. monnieri* within the regulation of aerophilic stress, in clock mutant (cryb) of *Drosophila melanogaster*[19]. When treated there is an elimination of 24 h rhythms in negative geotaxis, aerophilic stress markers and antioxidants below aerophilic stress are evaluated.

2. Material and Methods

2.1 Fly Stock

Drosophila melanogaster (Oregon R^+) fly stock was procured from D.A.V Women's College Chandigarh, Punjab, INDIA and culture media was prepared according to Singh *et al.*, (2009, 2010, 2011)[23–25]. *Drosophila* flies and larvae were cultured/reared at 25±1°C on standard *Drosophila* diet containing agar-agar, sugar, maize powder, yeast, nepagin (methyl-p-hydroxy benzoate) and propionic acid. Additional yeast suspension was used for the healthy growth of the organism.

2.2 *Bacopa monnieri* (Brahmi)

Two varieties of *Bacopa monnieri* were used namely Kashmiri Brahmi and commercial Brahmi (Purchased from a local market, Jalandhar). Kashmiri Brahmi was purchased from Agro Food processing emporium Peerbagh, Srinagar, J&K (Jammu and Kashmir). The extract was prepared according to Hosamani and Muralidhara (2009)[26]. Sample (soaked and boiled) was prepared by dissolving 1gm in 10ml of distilled water. One sample was only soaked overnight and another was only boiled for 15 minutes. Both were centrifuged at 3000 rpm for 10 minutes. The supernatant was used for further analysis.

2.2 *Camellia sinensis* (Green Tea))

Green tea (*Camellia sinensis*) was procured from Bud White Tea Ltd (New Delhi, India) and green tea (2%) sample was prepared by boiling 0.2 weight unit of tea leaf in 10 ml of distilled water for five minutes at 95°C. After five minutes it was left to cool down further at room temperature and then the extract was filtered through Whatman's filter paper. There after, filtrate was used for further analysis[27].

2.4 Curcumin (An active ingredient of *Circuma longa*)

Analtyical grade *Curcumin* was procured from Sigma (MO, USA) and 0.1mM solution was prepared by dissolving 36.8mg of curcumin in 1ml of dimethyl sulfoxide (DMSO) to carried out different assays.

2.4.1 Total Phenolic Content

Total phenolic content was determined according to Baba and Malik (2015)[28], using gallic acid as standard. The same concentration of different extracts was taken to make the volume 1ml with distilled water. To this was added 5ml of 0.2 N Folin-ciocalteu's reagent and incubated at room temperature for five minutes. After this added 2.0 ml 7.5% of sodium carbonate. After incubation for thirty minutes at 37oC absorbance was taken at 650nm.

2.4.2 Total flavonoid content

Total flavonoid content was determined according to Baba and Malik (2015)[28], using quercetin as standard. The same concentration of different extracts was taken to make the volume 2 ml with distilled water. To this added 0.1ml of aluminum chloride followed by 0.1ml of potassium acetate. Mixture was incubated at room temperature for 30-minutes then absorbance at 510nm was recorded.

2.4.3 DPPH Radical Scavenging Assay

Free radical scavenging activity was determined according to Zhu *et al.,* (2006)[29], using ascorbic acid as standard. Different concentrations of extract were added to 2ml of DPPH each and incubated at 37°C for 30 minutes. Then the OD was checked at 520nm.

2.4.4 Negative Geotaxis Assay

A climbing assay was performed according to the procedure of Sharma *et al* (2012)[30]. The scores represent the mean of a number of flies above 10cm (n_{top}) and below 10cm (n_{bot}), expressed as the percentage of total number of flies (n_{tot}). Performance index (PI) was also calculated for each experiment as

$$PI = ½[(n_{tot} + n_{top} - n_{bot})/n_{tot}].$$

2.4.5 Acetylcholinesterase Activity

Acetylcholinesterase (AchE) activity was measured by following the detailed protocol given by Srikumar *et al.* (2004)[31]. For this 10% homogenate of flies was prepared in 950 µl of 0.1M phosphate buffer (95mg average per 30 flies). Homogenate was centrifuged for 10 minutes at 3000 rpm at 4°C. One hundered µl of supernatant was added to reaction mixture which was containing 650 µl of 0.1M phosphate buffer (pH 7.4) and 25 µl of 5,5-dithiobis 2-nitrobenzoic acid (DTNB). Absorbance was noted at 412 nm after mixing the contents thoroughly[32] (Ellman *et al.,*1960). To this we added 5 µl of substrate i.e., acetylcholine and change in absorbance was measured for 3 minutes with 1-minute interval.

Enzyme Activity was calculated by the following formula;

$$R = 5.74 \times 10^{-4} \times A/CO.$$

where, R=Rate in moles of substrate hydrolyzed/minute/gm/tissue

A = Change in absorbance/minute.

CO = Original concentration of tissue (mg/ml).

3. Results

3.1 Total Phenolic Content

A standard curve of Gallic acid (20-100 µg/ml) was obtained with a coefficient of determination (R^2) equal to 0.963 (standard curve not shown).

The TPC of two types of *Brahmi* (Kashmiri and commercial, purchased from Phagwara market), green tea and curcumin elicited 3.16±1.858, 4.22±2.682, 1.3867±0.252, 4.3707±0.455, 99.667±3.547, 2.834±0.069 mg Gallic acid equivalent (GAE)/g respectively (Table 1).

On comparing our three nutraceuticals on the basis of total phenolic content, green tea (99.667±3.547) showed the highest phenolic content and soaked commercial *B. monnieri* (1.3867±0.22) showed the least.

3.2 Total Flavonoid Content

Flavonoids are a group of polyphenols that reduce the effect of free radicals that cause chronic stress related to human and animal diseases. A standard curve of Quercetin (2-10 µg/ml) with (R^2= 0.974) was obtained (standard curve not shown). The TFC in two types of *B. monnieri* (Kashmiri and commercial), green tea and *Curcumin* elicited 2.066±0.553, 3.6561±0.635, 38.14±1.1112, 4.1±0.0724, 97.633±2.532, 81.166±0.069 respectively (Table 1B). In the present study Kashmiri (soaked and boiled) and commercial (soaked and boiled), *B. monnieri* in aqueous extract showed the total flavonoid content as 2.066±0.553, 3.656±0.603, 38.14±1.1112, 4.1± 0.0724 mg Quercetin (QE)/g respectively.

3.3 1, 1– Diphenyl-2-picryl-hydrazyl (DPPH) Radical Scavenging Assay

DPPH is an oxidizing radical that can be reduced by the antioxidants. Nutraceuticals which includes Brahmi (2 Kashmir/2 Commercial), green tea and curcumin are a rich source of antioxidants. Ascorbic acid was used as standard (5-10 µg/ml).

The percentage scavenging effect of *B. monnieri* on DPPH free radical was enhanced with the increase in the

concentration of each extract from 1.25–2.5 mg/ml that is clearly shown in Figure 1A and Table (1C). The percentage inhibition was varying from 28.395% - 81.1343% of *B. monnieri* (Brahmi) extract.

In the comparison of the above results for *B. monnieri*, green tea and *Curcumin* for DPPH we found that the green tea showed maximum free radical scavenging activity with an IC_{50} value of 0.2569 and boiled commercial *B. monnieri* has the least with an IC50 value of 1.265mg/ml. The percentage scavenging effect of curcumin was increased on DPPH radical as the concentration of each extract was raised from 0.46–0.92 mg/ml. The percentage inhibition was varying from 44.63% - 66.46 % of curcumin extract.

Table 1. Total phenolic content (TPC), total flavonoid content (TFC) and *in vitro* antioxidant through DPPH radical scavenging activity of Brahmi, green tea and curcumin (Table 1A).

1A: Total Phenolic Content		
Sample	**Sample (µl)**	**TPC mg GAE/g)**
Soaked Kashmiri *Brahmi*	SKB (50 µl)	3.16±1.858
Boiled Kashmiri *Brahmi*	BKB (50 µl)	4.22±2.682
Soaked commercial *Brahmi*	SCB (50 µl)	1.3867±0.252
Boiled Commercial *Brahmi*	BCB (50 µl)	4.3707±0.455
Green Tea	GT (50 µl)	9.667±3.547
Curcumin	Cur (1 µl)	2.834± 0.069
1B: Total Flavonoid Content		
Sample	**Sample (µl)**	**TFC mg/QE/g**
Soaked Kashmiri *Brahmi*	SKB (50 µl)	2.066±0.553
Boiled Kashmiri *Brahmi*	BKB (50 µl)	3.6561±0.635
Soaked commercial *Brahmi*	BKB (50 µl)	4±1.1112
Boiled Commercial *Brahmi*	BCB (50 µl)	1.1± 0.0724
Green Tea	GT (50 µl)	7.633±2.532
Curcumin	Cur (1 µl)	1.166 ± 0.069

1C: DPPH Radical Scavenging Activity			
Sample (µl)	**Con.mg/ml**	**% Scav. activity**	**IC 50**
Soaked Kashmiri *Brahmi* 25	1.25	38.696±0.9844	1.6814
Soaked Kashmiri *Brahmi* 50	2.5	65.123±0.1768	
Boiled Kashmiri *Brahmi* 25	1.25	49.421±1.562	1.265
Boiled Kashmiri *Brahmi* 50	2.5	81.1343±7.287	
Soaked Commer *Brahmi* 25	1.25	28.395±2.199	2.193
Soaked Commer *Brahmi* 50	2.5	55.015±0.347	
Boiled Commer *Brahmi* 25	1.25	45.640±2.624	1.395
Boiled Commer *Brahmi* 50	2.5	73.032±1.504	
Green Tea	0.25	48.66±10.416	0.2569
Green Tea	0.50	82.47±0.973	
Curcumin	0.46	44.63±3.903	0.545
Curcumin	0.92	66.46±7.883	

Data represents mean± SD, n=3 and experiment was performed three times independently.

3.4 Climbing/Negative Geotaxis Assay

Negative geotaxis assay was performed according to the treatment protocol per triplicates for 6h, 24h, and 48h. During this time interval, locomotor activity was determined among the untreated control flies and treated flies [chlorpyrifos (Chlor), chlor+BM, Chlor+GT, Chlor+Cur] shown in Table 2.

Table 2. Locomotor assay: Total percent of *Drosophila* flies above 10cm after exposure to Chlorpyrifos and modulation of Chlorpyrifos induced toxicity through Brahmi (BM), Green Tea (GT) and curcumin (Cur) for 6h, 24h and 48h (Mean±SD, N=20 repeated three time independently)

	Control	Chlorpyrifos	Chlor+BM	Chlor+GT	Chlor+Cur
6 h	97±2.309	71±2.88[a]	97±1.154[b]	90±7.637[b]	86±5.77
24 h	95±0	48.33±2.88[a]	93±5.77[b]	62±2.88[a,b]	83±2.88[a,b]
48 h	95±2.30	5±0.577[a]	75±5[a,b]	42±7.63[a,b]	57±5.77[a,b]

Data represents mean± SD (n=3) and experiment was performed three times independently. Significance ascribed P<0.05, [a] = vs control, [b] = vs chlorpyrifos treated groups.

Data observed in a negative geotaxis among chlorpyrifos revealed time-dependent locomotor deficits. After exposure of flies for 6 hrs, (Table 2) 97% of control untreated flies were able to cross 10 cm within 20 seconds, while as in chlorpyrifos treatment, flies showed a significant decrease in climbing ability (71%) and also a significant increase in mortality. Flies co-treated with BM, GT and Cur improved the locomotor ability of flies to 97%, 90% and 86% respectively indicating clearly their neuroprotective effect. The flies exposed for 24 hrs revealed that among untreated control, 95% of flies were able to cross 10 cm within 20 seconds, while as in chlorpyrifos treatment flies show decreased climbing ability and increased mortality rate among flies. Flies co-treated with BM, GT and Cur improved the performance of flies to 93%, 62%, and 83%. The green tea-treated flies showed a synergetic effect as there was a higher mortality rate as compared to BM and Cur after 24 hrs. Flies treated with BM (93%) showed better locomotor ability as compared to GT (82%) and Cur (83%).

3.5 Performance index

The Performance Index for each experiment was calculated by;

$$PI = \frac{1}{2}[(ntot + ntop - nbot)/ntot]$$

In the present study the performance index was calculated in each experiment for 6, 24 and 48h as shown in Figure 3 and Table (3). After comparing the control and treated flies the performance index was higher in BM treated flies as compared to GT and Cur-treated flies.

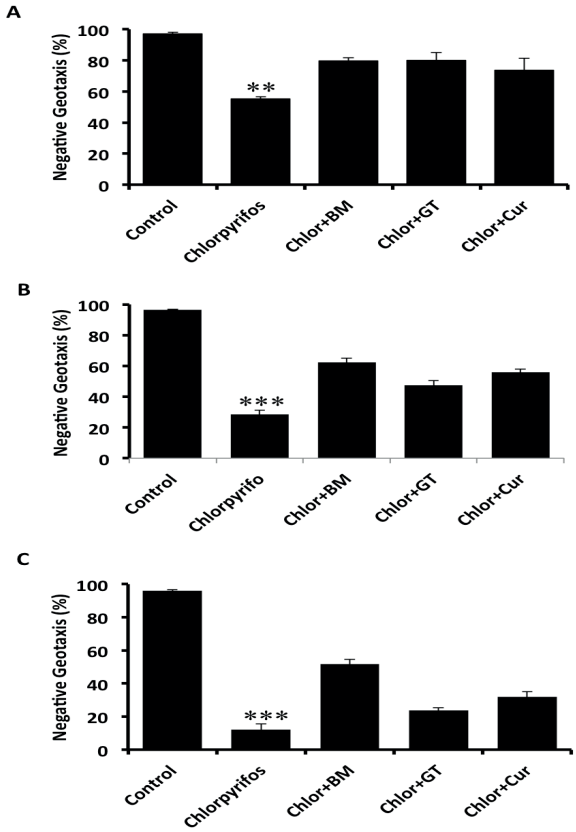


Figure 1. Negative geotaxis assay in chlorpyrifos, Chlor+BM, Chlor+GT and Chlor+Cur treated groups after 6h, 24h and 48h of treatment. Percent of flies above 10 cm (ntop) after 6h (A), 24h (B) and 48h (C) of treatment. Data is represented in mean ± SD (n=10) and experiment was repeated independently three times. Significance ascribed $^{**}P<0.001$ vs control, $^{***}P<0.0001$ vs control.

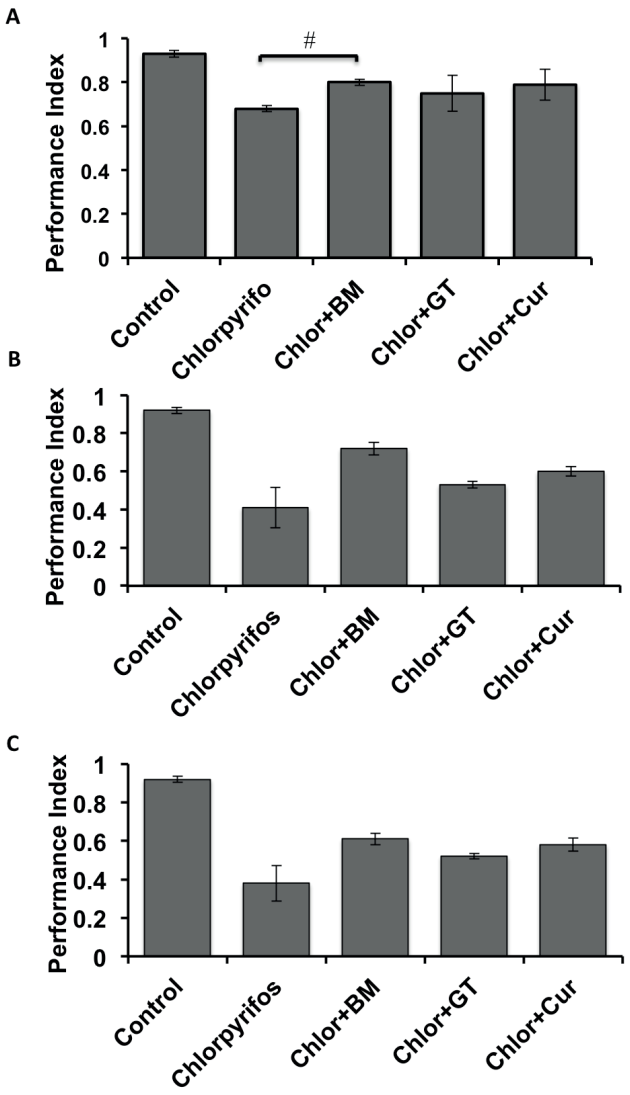


Figure 2. Performance index in chlorpyrifos, Chlor+BM, Chlor+GT and Chlor+Cur treated groups after 48h of treatment. Data is represented in mean SD (n=10) and experiment was repeated independently three times. Significance ascribed $^{\#}P$ <0.001 vs chlorpyrifos.

3.6 Activity of Acetylcholinesterase

Chlorpyrifos treated flies exhibited reduced AChE activity (4.38×10^{-4}M ± 0.93) was dramatically reduced in comparison to the control flies (50.73×10^{-4}M±5.23). The flies treated with Chlor+BM (14.92×10^{-4} M±1.51), Chlor+GT (10.88×10^{-4}M ±2.065) and Chlor+Cur (11.67×10^{-4} M±3.69) showed the AChE activity reduction as compared to control, but elevation in comparison to chlorpyrifos exposed groups.

Table 3. Performance Index of each experiment for negative geotaxis assay and acetylcholinesterase activity in the fly brain of control and treated groups

PERFORMANCE INDEX			
GROUP	**12 h**	**24 h**	**48 h**
Control	0.92± 0.02	0.92± 0.025	0.92± 0.02
Chlorpyrifos	0.675± 0.025[a]	0.41± 0.184[a]	0.38± 0.160[a]
Chlor+BM	0.825± 0.025[a, b]	0.72± 0.057[a,b]	0.61± 0.052[a]
Chlor+GT	0.746± 0.141	0.53± 0.028[a,]	0.58± 0.057[a]
Chlor+ Cur	0.79± 0.123	0.6± 0.04[a]	0.58± 0.057[a]
ACETYLCHOLINESTERASE ENZYME ASSAY			
GROUP	Acetylcholinesterase Activity (after 6 h) (moles/min/g)		
Control	50.73 ±5.23		
Chlorpyrifos	4.38 ±0.93[a]		
Chlor+BM	14.9 ±1.51[a,b]		
Chlor+ GT	10.88 ±2.065[a,b]		
Chlor+Cur	11.67 ±3.69[a,b]		

Data represents mean± SD (n=3) and experiment was performed three times independently. Significance ascribed P <0.05, [a] = vs control, [b] = vs chlorpyrifos treated groups.

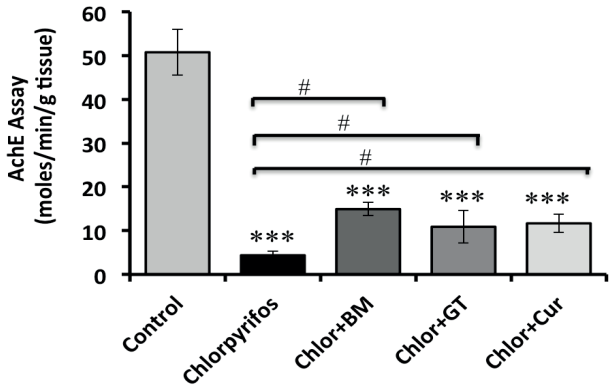


Figure 3. Acetylecholinesterase acitvity chlorpyrifos, Chlor+BM, Chlor+GT and Chlor+Cur dosed groups after 48 h of treatment. Data is represented in mean ± SD (n=3) and experiment was independently performed thrice. Significance ascribed $^{***}P$ <0.0001 vs control, $^{\#}P$<0.001 vs chlorpyrifos

4. Discussion

In the present study Kashmiri (soaked and boiled) and commercial (soaked and boiled), *B. monnieri* aqueous extract showed the total phenolic content as 3.16±1.858, 4.220±2.682, 1.3867±0.252, 4.3707± 0.455 mg GAE /g. In the context of this, a previous report has shown that the total phenolic content in methanolic extract of *B. monnieri* possesses 24.75 mgGAE/g[33] (Jain *et al.* 2017) and phenolic content of *B. monnieri* in the ethanolic and aqueous extract as 3.18 and 3.71 mgGAE/g respectively[34].

The phenolic content in aqueous extract of green tea was 99.67±3.547 mgGAE/g. The phenolic content in methanolic extract of green tea possesses 24.3mg/g[35] (Taheri *et al.,* 2011). This variation may be due to different solvents used for extract preparation. The phenolic content of curcumin is 2.834±0.069 in the present study. Maizura *et al* (2011)[36] found that the phenolic content in curcumin was 0.6789 mg GAE/g (67.89 mg GAE/100g extract). Tanzeela *et al.* (2015)[37] found that the phenolic content of curcumin was 6.787 mgGAE/g, 7.457 mg GAE/g and 0.538 mg GAE/g, 6.824 mg GAE/g in the two solvents at their different concentrations that is ethanol (60% and 80% respectively), and methanol (60% and 80% respectively) and higher phenolic content was found in ethanol (80%).

Previous studies have suggested that the flavonoid content of *B. monnieri* in terms of using Gallic acid as a standard at 10 µg was 6.3 GAE and at 1000 µg was increased to 29.66 GAE[33]. Another report was investigated and it was found that the flavonoid content in methanolic extract of *B. monnieri* was 24.36 mg QE/g[34] (Alam *et al.,* 2012). Flavonoids are secondary metabolites that are necessary for growth and development. In the present study, the flavonoid content of green tea is 97.633±2.532. However previous studies have shown[23].17mgQE/g in aqueous extract of green tea[39]. The flavonoid content of curcumin in the present study is 81.166±0.069 and data from the previous studies did not provide any information for its flavonoid content. On comparing our nutraceuticals the total flavonoid content of green tea (97.633±2.532) has the highest phenolic content and soaked Kashmiri *B. monnieri* (2.066±0.553) the least.

In this study, the IC_{50} value for boiled Kashmiri *B. monnieri* (BKB) was 1.265 mg/ml and soaked Kashmiri *B. monnieri* (SKB) was1.6814 mg/ml. The IC_{50} value for boiled commercial B. monnieri (BCB) and soaked commercial B. monnieri (SCB) was 1.395 mg/g and 2.193 mg/g respectively. This showed that boiled Kashmiri B. monnieri has the highest free radical scavenging activity. Previous studies have shown that the IC_{50} value for Brahmi was 0.45 mg/ml and 0.764 mg/ml using ascorbic acid as standard[34,38]. Variation in the present study may be due to sample extraction in different solvents, fresh leaves were used for the study. On comparing the Kashmiri and commercial *B. monnieri* the highest free radical scavenging activity was found in boiled Kashmiri *B. monnieri* (81.13±7.28) with an IC_{50} value of 1.265.

The percentage scavenging effect of green tea on DPPH radical was raised with an increase in the concentration of each extract from 0.25–0.5 mg/ml that (which) is clearly seen in Figure 1B. The percentage scavenging activity was varying from 48.66 % - 82.47 % of green tea extract at concentration of 0.25-0.5 mg/ml (table 3b) and the IC_{50} value for green tea is 0.2569 mg/ml.

From the previous reports, it was found that green tea has a % inhibition activity of 80.3 % in the methanolic extract[35]. Two different studies have shown that the IC50 value for green tea on using Gallic acid and Ascorbic acid as a positive control was calculated as 0.02326 mg/ml and 0.0195 mg/ml[40].

The present study suggests that the % scavenging activity of curcumin varies from 44.63±3.903%-66.46±7.883% at a concentration of 0.46 – 0.52 mg/ml and the IC50 value of curcumin 0.545. The study revealed that the curcumin has % scavenging activity of each sample against DPPH ranging from 55.60 to 71.64% at a concentration of 0.001-0.005 mg/ml. The IC50 value was found to be 0.00108 mg/ml [41]. Maizura *et al* (2011)[38] revealed that the curcumin has the scavenging activity of 64.6 ± 2.4%.

After exposure of flies for 48 hrs (table 2) it was observed that among the untreated (control), 95% of flies were able to cross 10 cm within 20 seconds, while as in chlorpyrifos treatment only 5% were able to cross the 10cm within 20 seconds and there was higher mortality rate among chlorpyrifos treated flies. Flies co-treated with *B. monnieri,* green tea and curcumin improved the performance of flies to 75%, 42%, and 57% respectively. The green tea-treated flies showed the significant synergistic effect as there was a higher mortality rate as compared to *Brahmi* and curcumin after 48 hrs while as the locomotor deficit was also improved (42%). Flies treated with curcumin showed better locomotor ability as compared to green tea and also lesser mortality (57%). After 48 hrs Brahmi showed the maximum improvement and green tea the least.

Previous studies have suggested that the rotenone-induced 48% of mortality among flies and caused locomotor deficits by 95% whereas the flies were not able to reach the top of the cylinder for 1 minute. With *B. monnieri* treatment more number of flies (45-65%) showed better negative geotaxis behavior[26]. Another study documented that the flies exposed to paraquat increased locomotor deficits, while untreated flies were able to reach 7 cm height of the test tube. In

contrast, flies pre-fed with curcumin before treatment of paraquat showed higher locomotor activity[40,42]. There is no evidence on the study of negative geotaxis in green tea that might be because of its synergistic effect.

In the present study, comparison of the three nutraceuticals revealed that there was not so much variation among them in inhibiting the chlorpyrifos effect. It was found that the *Brahmi* elevates most of the AChE activity by inhibiting the chlorpyrifos that has dramatically reduced the AChE activity in flies.

Previous studies have shown that the activity level of the AChE enzyme in rotenone-exposed flies treated with *Brahmi* was marginally increased[26]. Another study has shown that the flies treated with dietary supplement curcumin prior to paraquat treatment reduced AChE activity[40].

5. Conclusion

We demonstrate the corroboration on neuroprotective effect of three different nutraceuticals viz., *B. monnieri,* green tea, and curcumin against the chlorpyrifos induced toxicity that caused the locomotor deficits and reduced the acetylcholinesterase activity in the flies. We presented here the antioxidant activity levels of three different nutraceuticals that prevent free radicals and suppress oxidative stress. Use of phytoconstituents to the flies exposed to chlorpyrifos improves impaired locomotory behavior and also increases the enzyme activity in the brain ganglia of flies indicating that these substances possess neuroprotective potential. We concluded that *Brahmi* is the more effective nutraceutical than curcumin and least is the green tea for the prevention of neurodegenerative diseases.

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