Journal of Applied Biomedicine 16 (2018) 111–119

Contents lists available at ScienceDirect

Journal of Applied Biomedicine

journal homepage: www.elsevier.com/locate/jab

Original research article

Effect of pramipexole alginate nanodispersion (PAND) on the transgenic *Drosophila* expressing human alpha synuclein in the brain

Yasir Hasan Siddique[a,*], Falaq Naz[a], Wasi Khan[b], Smita Jyoti[a], Braj Raj Singh[c], Alim Hussain Naqvi[d]

[a] *Aligarh Muslim University, Faculty of Life Sciences, Department of Zoology, Section of Genetics, Drosophila Transgenic Laboratory, Aligarh, India*
[b] *Aligarh Muslim University, Faculty of Science, Department of Physics, Aligarh, India*
[c] *TERI-Deakin Nanobiotechnology Centre, TERI Gram, The Energy and Resources Institute, Gurgaon, Haryana, India*
[d] *Aligarh Muslim University, Interdisciplinary Nanotechnology Centre, Aligarh, India*

ARTICLE INFO

Article history:
Received 27 December 2016
Received in revised form 5 October 2017
Accepted 21 November 2017
Available online 1 December 2017

Keywords:
Pramipexole
Parkinson's disease
Oxidative stress
Drosophila melanogaster
Nanocomposite

ABSTRACT

In the present study the effect of pramipexole alginate nanodispersion (PAND) was studied on the transgenic *Drosophila* exhibiting the PD symptoms. The PD flies were allowed to feed on the diet having PAND at final concentration of 1, 2 and 3 μM. A dose dependent significant delay in the loss of climbing ability and improvement in the activity was observed in PD flies. A dose dependent significant change in the oxidative stress markers and dopamine content was also observed in the PD flies exposed to various doses PAND. The improvement in the PD symptoms was more in PD flies exposed to PAND compared to PD flies exposed to pramipexole alone.

Introduction

Parkinson's disease (PD) is a common neurodegenerative disorder characterized by the loss of dopaminergic neurons in the substantianigra of the mid brain (Feany and Bender, 2000). It is also classified as a movement disorder, although it may lead to several non-motor types of symptoms such as sensory deficits, cognitive difficulties or sleep problems (Barnett-Cowan et al., 2010). Dopamine agonists help PD patients by reversing the dopamine depletion and related motor deficits (Iravani et al., 2006). Besides having protective effects against the PD symptoms the side effects of the dopamine agonists cannot be ruled out (Castro-Hernandez et al., 2015; Cedarbaum, 1987; Gallant et al., 2016; Patterson et al., 2010; Rasmussen et al., 2011; Tan et al., 2009). The side effects of the dopamine agonists have been reported due to overdose or prolonged use of the drugs. The most frequent adverse effects are sleep disturbances, aggravation of dyskinesias, nausea, orthostatic hypotension, hallucinations, headache, dizziness (Degim et al., 2003; Rektorová et al., 2003). The advancement of nanotechnology has led to formation of drug nano composites that not only increase the half-life, but also the solubility, stability and permeability of drugs (Siddique et al., 2016a; Yang, 2010). Expression of α-synuclein in all *Drosophila* neurons induces progressive locomotor deficits (Feany and Bender, 2000). The disruption of a group of 15 dopaminergic neurons per hemisphere in the anterior medial region of the brain has been correlated with the climbing impairments in this model (Riemensperger et al., 2013). Due to ethical issues, *Drosophila* has been worldwide accepted as an alternative to mammals. It has 80–90% of similarity in the conserved domains and 75% of the disease related genes in humans have functional orthologs (Pandey and Nichols, 2011). The yeast based UAS (Upstream Activation Sequences)-GAL4 system is a method for activating gene expression (Brand and Perrimon, 1993). In this system, the UAS is an enhancer that is specifically targeted by the GAL4 protein (Lee et al., 2013). On the basis of the bipartite approach of gene activation the transgenic *Drosophila* models for disease causing genes, such as human α-synuclein, Parkin, DJ-1, Pink 1 and LRRk2 have been generated with their specific phenotypes (Guo, 2012). In the present study the transgenic flies expressing the human alpha synuclein under the UAS-GAL4 system was used to study the effect of PAND.

* Author for correspondence: Aligarh Muslim University, Faculty of Life Sciences, Department of Zoology, Section of Genetics, Drosophila Transgenic Laboratory, Aligarh, Uttar Pradesh 202002, India.
E-mail address: yasir_hasansiddique@rediffmail.com (Y.H. Siddique).

https://doi.org/10.1016/j.jab.2017.11.002
1214-021X/

Materials and methods

Synthesis of pramipexole-alginate nanodispersion (PAND)

To prepare pramipexole-alginate nanodispersion (PAND), 40 mg of pramipexole (Sigma, USA) was dissolved in 2 ml of milliQ water. Then this solution was added drop-wise into the warm 0.20% (123 ml) aqueous solution of sodium alginate (Ottokemi, India). The mixture was then ultra-sonicated at 100 W with 30 kHz frequency for 5 min and subjected to continuous stirring at 50 °C for 1 h. The synthesized PAND was stored in refrigerator under dark conditions (Siddique et al., 2016a).

Characterization

Pramipexole and synthesized pramipexole-alginate nanodispersion (PAND) were characterized through Transmission electron microscopy (TEM) and Fourier transform infrared (FTIR) spectroscopy. TEM images of pramipexole drug and its nanodispersion with alginate were captured with JEOL, JEM-2010 Transmission electron microscope for the confirmation of nanostructure formation. The FTIR spectra of the samples were observed in the wavenumber range from 400 to 4000 cm^{-1} using Perkin Elmer spectrometer.

Drosophila culture and crosses

The flies were cultured on standard *Drosophila* food containing agar, corn meal, sugar and yeast at 25 °C (Siddique et al., 2011). Crosses were set up using six virgin females of UAS-Hsap/SNCA. F5B and mated to three males of elav-GAL. The progeny expressed the human α-synuclein in the neurons and the flies were referred as Parkinson's disease (PD) flies (Feany and Bender, 2000). All flies taken for performing experiments were 1–3 day old. The PD flies were also exposed separately to different doses of PAND mixed in the culture medium. PAND was added in the medium having the final concentrations of 1, 2 and 3 μM of pramipexole. As a negative control the PD flies were allowed to feed on the diet supplemented with 10^{-3} M of dopamine and 3 μM of pramipexole. The control flies (UAS-Hsap/SNCA.F) were also exposed to the selected doses of PAND to see any negative effects. All the tests were performed after 24 days of the exposure.

Dopamine content

Dopamine content was measured as per the method described by Schlumpf et al. (1974). Fifty heads of flies from each group were taken in 500 μl of HCl-butanol (0.85 ml of 37% HCl in 1 l *n*-butanol). After homogenization the samples were centrifuged at 3000 rpm for 5 min. After collecting the supernatant, 250 μl of heptane and 100 μl of 0.1 M HCl were added. The samples were vortexed and centrifuged at 3000 rpm for 5 min. The upper organic phase was discarded and the lower aqueous phase was kept for dopamine assay. To 100 μl of aqueous phase, 50 μl of 0.4 M HCl, 100 μl of sodium acetate buffer (pH 6.9), 100 μl of iodine solution was added and kept for 2 min. The reaction was stopped by adding of 100 μl of sodium sulphite solution. After 2 min, 100 μl of acetic acid (10 M) was added and then the mixture was heated at 100 °C for 6 min. The OD was taken at 375 nm after cooling the samples at room temperature.

Drosophila climbing assay

The climbing assay was performed according to Pendleton et al. (2002). Ten flies were placed in each empty glass vial. After the acclimatization for 10 min at room temperature, every group was assayed at random to a total of 10 trials for each. The number of flies above the mark of the vial were counted after 10 s of climbing and repeated 10 times to get the mean number.

Drosophila activity pattern analysis

From the 12th day the activity of flies (males) in all treated groups were analyzed by using *Drosophila* Activity Monitor (TriTek, USA). The activity was recorded every hour for a total of 288 h and the data was analyzed by Actogram J software. The results were presented as chi-square periodogram (Chiu et al., 2010; Rosato and Kyriacou, 2006).

Preparation of homogenate for biochemical analysis

Fly heads from each group were isolated (50 heads/group; five replicates/group) and the homogenate was prepared in 0.1 M phosphate buffer for the biochemical parameters.

Lipid peroxidation assay

Lipid peroxidation was measured according to the method described by Ohkawa et al. (1978). The reaction mixture consisted of 5 μl of 10 mM butyl-hydroxy toluene (BHT), 200 μl of 0.67% thiobarbituric acid, 600 μl of 1% *O*-phosphoric acid, 105 μl of distilled water and 90 μl of supernatant. The resultant mixture was incubated at 90 °C for 45 min and the OD was measured at 535 nm. The results were expressed as μ moles of TBARS formed/h/gram tissue.

Estimation of glutathione-S-transferase (GST) activity

The glutathione-*S*-transferase activity was determined by the method of Habig et al. (1974). The reaction mixture consists of 500 μl of 0.1 M phosphate buffer, 150 μl of 10 mM CDNB, 200 μl of 10 mM reduced glutathione and 50 μl of supernatant. The OD was taken at 340 nm and the enzyme activity was expressed as μ moles of CDNB conjugates/min/mg protein.

Estimation of glutathione (GSH) content

The glutathione (GSH) content was estimated colorimetrically using Ellman's reagent (DTNB) according to the procedure described by Jollow et al. (1974). The supernatant was precipitated with 4% sulphosalicyclic acid (4%) in the ratio of 1:1. The samples were kept at 4 °C for 1 h and then subjected to centrifugation at 5000 rpm for 10 min at 4 °C. The assay mixture consisted of 550 μl of 0.1 M phosphate buffer, 100 μl of supernatant and 100 μl of DTNB. The OD was read at 412 nm and the results were expressed as μ moles of GSH/gram tissue.

Estimation of protein carbonyl content (PCC)

The protein carbonyl content was estimated according to the protocol described by Hawkins et al. (2009). The brain homogenate was diluted to a protein concentration of approx 1 mg/ml. About 250 μl of each diluted homogenate was taken in Eppendorf centrifuge tubes separately. To it 250 μl of 10 mM 2,4-dinitrophenyl hydrazine (dissolved in 2.5 M HCl) was added, vortexed and kept in dark for 20 min. About 125 μl of 50% (w/v) trichloroacetic acid (TCA) was added, mixed thoroughly and incubated at −20 °C for 15 min. The tubes were then centrifuged at 4 °C for 10 min at 9000 rpm. The supernatant was discarded and the pellet obtained was washed twice by ice cold ethanol: ethyl acetate (1:1). Finally, the pellets were re-dissolved in 1 ml of 6 M guanidine hydrochloride and the absorbance was read at 370 nm.

Estimation of monoamine oxidase (MAO)

The method described by McEwen (1965) was used to estimate the monoamine oxidase activity. The assay mixture consisted of 400 μl of 0.1 M phosphate buffer (pH 7.4), 1300 μl of distilled water, 100 μl of benzylamine hydrochloride and 200 μl of brain homogenate. The assay mixture was incubated for 30 min at room temperature and then 1 ml of 10% perchloric acid was added and centrifuged at 1500*g* for 10 min. The OD was taken at 280 nm.

Assay for caspase-9 (Dronc) and caspase-3 (Drice) activities

The assay was performed according to the manufacturer protocol with some modification (Bio-Vision, CA, USA). The assay was based on spectrophotometric detection of the chromophore *p*-nitroanilide (pNA) obtained after specific action of caspase-3 and caspase-9 on tetrapeptide substrates, DEVD-pNA and LEHD-pNA, respectively. The assay mixture consisted of 50 μl of brain homogenate and 50 μl of chilled cell lysis buffer incubated on ice for 10 min. After incubation, 50 μl of 2× reaction buffer (containing 10 mM DTT) with 200 μM substrate (DEVD-pNA for Drice, and LEHD-pNA for Dronc) was added and incubated at 37 °C for 1.5 h. The reaction was quantified at 405 nm.

Statistical analysis

The statistical analysis was done by post hoc Dunnett's test using SPSS 16.

Results

The results of TEM measurement indicate the successful conversion of the parent pramipexole drug molecules into the nanoparticles of average size ~ 20 nm as displayed in Fig. 1A and B. The various interactions between the drug and its nanodispersion with alginate were established by monitoring shift in the vibrational frequencies of the molecules through IR spectroscopy. The typical IR spectrum of the parent pramipexole drug is presented several characteristics peaks as shown in Fig. 1C. It has a prominent stretching vibrational band at ~1630 cm^{-1}, which is assigned to C=N stretching of azomethine group. While various characteristic peaks of pramipexole drug shifted/modified to the different wavenumber by transforming it into nanoregime with alginate (PAND) (Papadimitriou et al., 2008). This is suggesting the involvement of azomethine group (>C=N) group in the nanodispersion formation *via* the reduction of electron density on nitrogen. In addition, broaden peak corresponds to hydroxyl group (OH) at 2800–3400 cm^{-1} demonstrating a shift to higher wavenumbers in the nanodispersion sample. This may be attributed to the proton displacement from the phenolic OH group on complexion.

The results obtained for the dopamine content are shown in Fig. 2. The PD flies showed a significant decrease of 1.92 fold compared to control flies (Fig. 2; $p < 0.01$). The exposure of PD flies to PAND having pramipexole concentration of 1 μM, 2 μM and 3 μM showed a 1.23, 1.36 and 1.51 folds increase in the dopamine content compared to unexposed PD flies (Fig. 2; $p < 0.05$). The exposure of 3 μM of pramipexole alone showed an increase of 1.22 fold in the dopamine content compared to unexposed PD flies

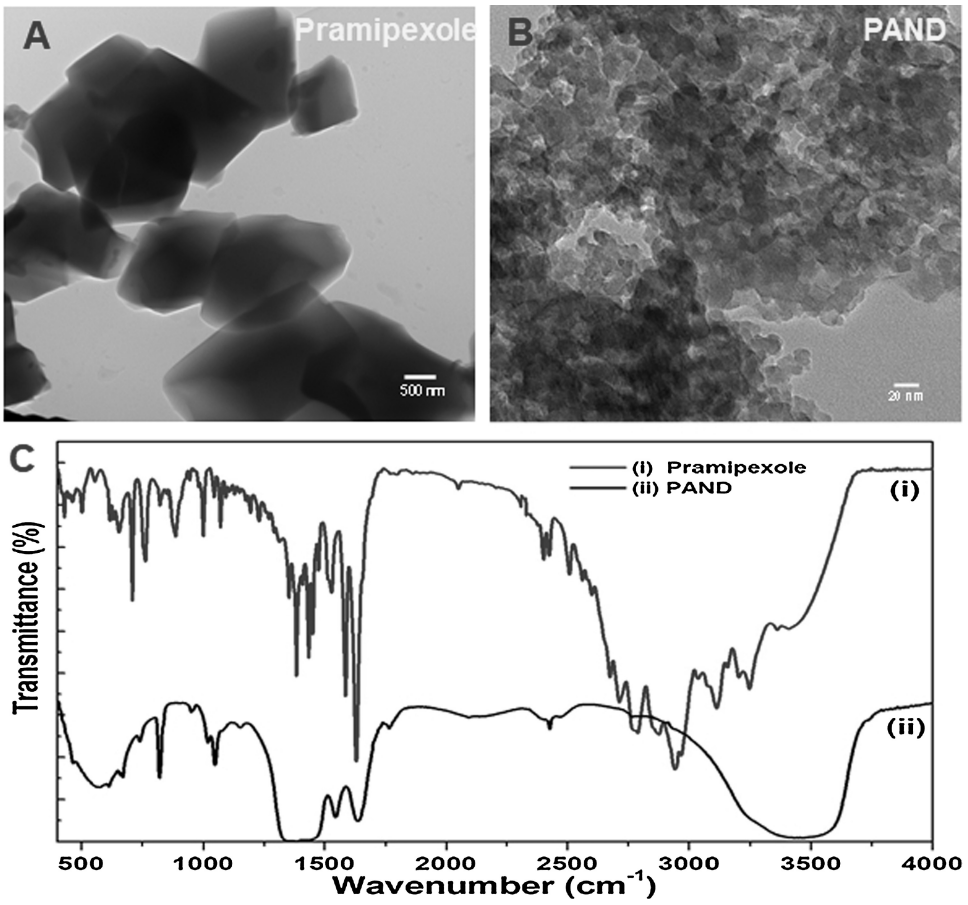


Fig. 1. TEM images (A, B), and FTIR spectra (C) of pramipexole and its nanodispersion with alginate (PAND).

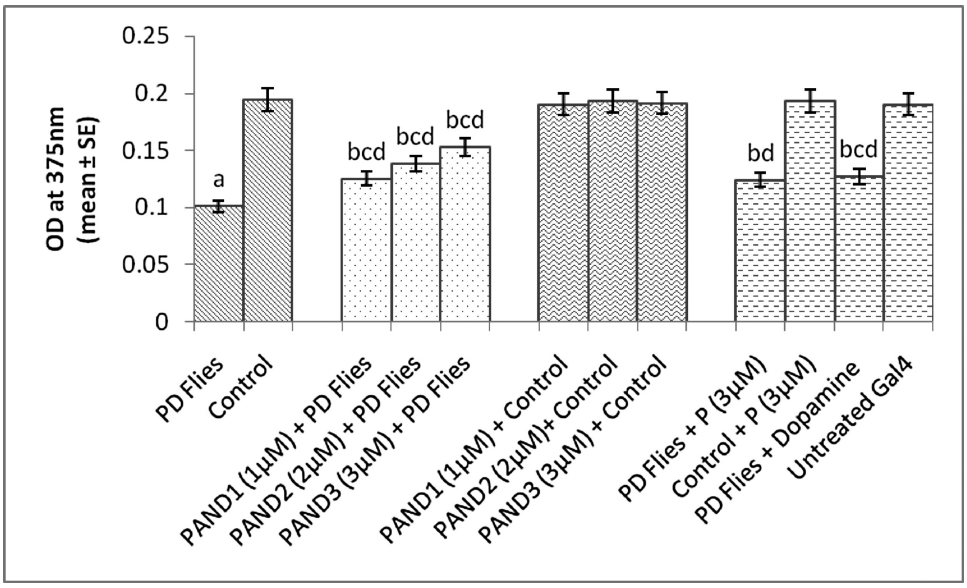


Fig. 2. Effect of pramipexole alginate nanodispersion (PAND) on the dopamine content in the brains of flies. The flies were allowed to feed on the diet supplemented with PAND for 24 days and then assayed for dopamine content. ([a] significant difference with respect to control, $p < 0.01$; [b] significant difference with respect to PD flies $p < 0.05$; [c] significant difference with respect to PD flies exposed to only pramipexole $p < 0.05$; [d] significant difference with respect to control, $p < 0.05$; P: pramipexole; Dopamine: 10^{-3} M; $N = 50$).

(Fig. 2; $p < 0.05$). The exposure of 10^{-3} M of dopamine showed an increase of 1.25 fold compared to unexposed PD flies (Fig. 2; $p < 0.05$).

The results obtained for climbing ability are shown in Fig. 3. The PD flies showed a 4.73 fold decrease in the climbing ability compared to control flies (Fig. 3; $p < 0.01$). The PD flies exposed to PAND having the pramipexole concentration of 1 μM, 2 μM and 3 μM also showed a 2.73, 3.15 and 3.36 folds delay in the loss of climbing ability compared to PD flies (Fig. 3; $p < 0.05$). The exposure of PD flies to 3 μM of pramipexole alone showed a 2.42 fold delay in the loss of climbing ability compared to unexposed PD flies (Fig. 3; $p < 0.05$). The exposure of PD flies to 10^{-3} M of dopamine showed a 3.10 fold decrease compared to unexposed PD flies (Fig. 3; $p < 0.05$). The results obtained on the activity pattern are shown Figs. S1–S11. The PD flies showed a decline in the activity (Fig. S2) as the age of the PD flies increases compared to the control flies (Fig. S1). The PD flies exposed to various doses of PAND showed a dose dependent delay in the activity (Figs. S3–S5) compared to unexposed PD flies (Fig. S2). No significant change in the activity was observed in the control flies exposed to various doses of PAND.

The results obtained for lipid peroxidation (LPO) are shown in Fig. 4A. The PD flies showed a 3 fold increase in LPO compared to control flies (Fig. 4A; $p < 0.01$). The exposure of PD flies to PAND having pramipexole concentration of 1 μM, 2 μM and 3 μM showed a dose dependent decrease of 1.50, 1.95 and 2.43 folds respectively compared to unexposed PD flies (Fig. 4A; $p < 0.05$). The exposure of PD flies to 10^{-3} M of dopamine showed a 1.5 fold decrease in LPO compared to unexposed PD flies (Fig. 4A; $p < 0.05$). The exposure of PD flies to 3 μM of pramipexole showed a 1.34 fold

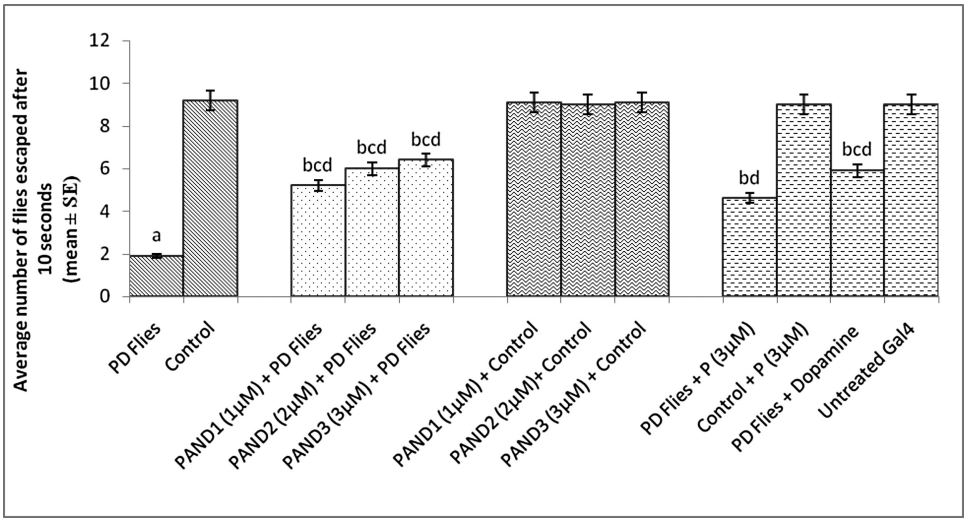
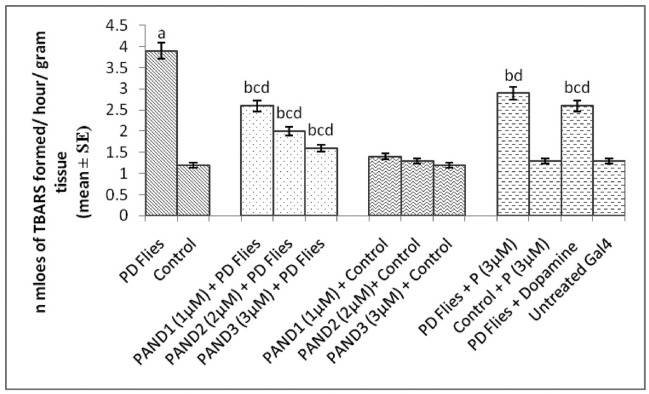
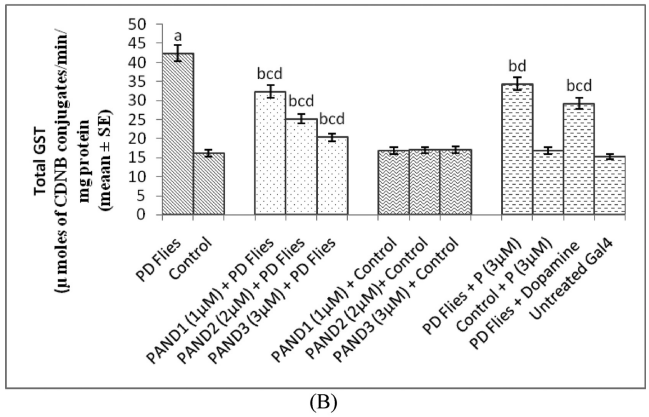


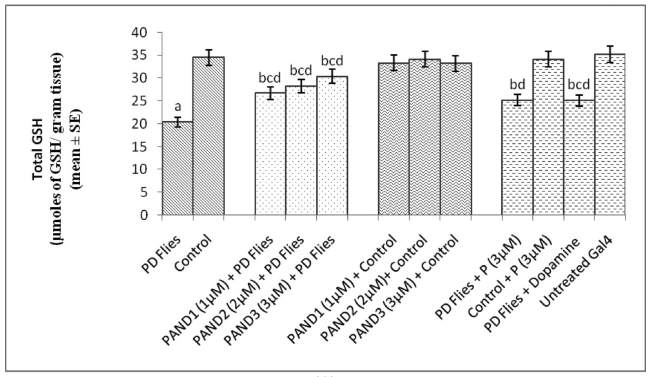
Fig. 3. Effect of pramipexole alginate nanodispersion (PAND) on the climbing ability. The flies were allowed to feed on the diet supplemented with PAND for 24 days and then assayed for climbing ability. The values are the mean of 5 assays. ([a] significant difference with respect to control, $p < 0.01$; [b] significant difference with respect to PD flies $p < 0.05$; [c] significant difference with respect to PD flies exposed to only pramipexole $p < 0.05$; [d] significant difference with respect to control, $p < 0.05$; P: pramipexole; Dopamine: 10^{-3} M; $N = 50$).



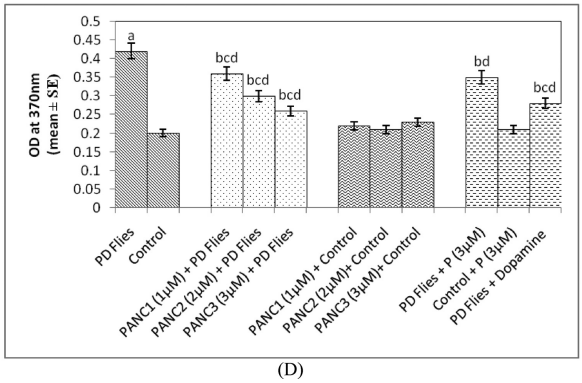
(A)



(B)



(C)



(D)

Fig. 4. Effect of pramipexole alginate nanodispersion (PAND) on the lipid peroxidation (A), GST content (B), GSH activity (C) and PC content (D) and in the brains of flies. The flies were allowed to feed on the diet supplemented with PAND for 24 days and then assayed for LPO, GST activity, PC and GSH content. ([a] significant difference with respect to control, $p < 0.01$; [b] significant difference with respect to PD flies $p < 0.05$; [c] significant difference with respect to PD flies exposed to only pramipexole $p < 0.05$; [d] significant difference with respect to control, $p < 0.05$; P: pramipexole; Dopamine: 10^{-3} M; $N = 50$).

decrease in LPO compared to unexposed PD flies (Fig. 4A; $p < 0.05$). The results obtained for GST activity are shown in Fig. 4B. The PD flies showed a 2.61 fold increase in GST activity compared to control flies (Fig. 4B; $p < 0.01$). The exposure of PD flies to PAND having pramipexole concentration of 1 μM, 2 μM and 3 μM showed a dose dependent 1.30, 1.68 and 2.07 folds decrease respectively in the GST activity compared to unexposed PD flies (Fig. 4B; $p < 0.05$). The exposure of 3 μM of pramipexole alone to PD flies showed a 1.23 fold decrease compared to unexposed PD flies (Fig. 4B; $p < 0.05$). The exposure of PD flies to 10^{-3} M of dopamine showed a 1.45 fold decrease in the activity of GST compared to unexposed PD flies (Fig. 4B; $p < 0.05$). The results obtained for GSH content are shown in Fig. 4C. The PD flies showed a 1.69 fold decrease in the GSH content compared to control flies (Fig. 4C; $p < 0.01$). The exposure of PD flies to PAND having pramipexole concentration of 1 μM, 2 μM and 3 μM showed a 1.30, 1.38 and 1.49 folds dose dependent significantly increase respectively in the GSH content compared to unexposed PD flies (Fig. 4C; $p < 0.05$). The exposure of PD flies to 3 μM of pramipexole showed a 1.23 fold increase in the GSH content compared to unexposed PD flies (Fig. 4C; $p < 0.05$). The exposure of PD flies to 10^{-3} M of dopamine showed a 1.23 fold increase in the GSH content compared to unexposed PD flies (Fig. 4C; $p < 0.05$). The results obtained for PC content are shown in Fig. 4D. The PD flies showed a 2.1 fold increase in PC content compared to control flies (Fig. 4D; $p < 0.01$). The exposure of PD flies to PAND having pramipexole concentration of 1 μM, 2 μM and 3 μM showed a dose dependent significant decrease of 1.16, 1.40 and 1.61 folds respectively, compared to unexposed PD flies (Fig. 4D; $p < 0.05$). The exposure of PD flies to 3 μM of pramipexole showed a 1.2 fold decrease in the PC content compared to unexposed PD flies (Fig. 4D; $p < 0.05$). The exposure of PD flies to 10^{-3} M of dopamine showed a 1.5 fold decrease in PC content compared to unexposed PD flies (Fig. 4D; $p < 0.05$).

The results obtained for MAO activity are shown in Fig. 5. The PD flies showed a 1.87 fold increase in the MAO activity compared to control flies (Fig. 5; $p < 0.01$). The exposure of PD flies to PAND having pramipexole concentration of 1 μM, 2 μM and 3 μM showed a 1.15, 1.28 and 1.50 folds decrease respectively compared to unexposed PD flies (Fig. 5; $p < 0.05$). The exposure of PD flies to 10^{-3} M of dopamine showed a 1.32 fold decrease in the MAO activity compared to unexposed PD flies (Fig. 5; $p < 0.05$). The exposure of PD flies to 3 μM of pramipexole showed a 1.17 fold decrease in MAO activity compared to unexposed PD flies (Fig. 5; $p < 0.05$).

The results obtained for the activity of caspase-9 are shown in Fig. 6A. The PD flies showed a 2.6 fold increase in the activity of caspase-9 compared to control flies (Fig. 6A; $p < 0.01$). The exposure of PD flies to PAND having pramipexole concentration of 1 μM, 2 μM and 3 μM showed a dose dependent significant decrease of 1.23, 1.44 and 1.62 folds respectively compared to unexposed PD flies (Fig. 6A; $p < 0.05$). The exposure of 3 μM of pramipexole showed a 1.3 fold decrease in the activity of caspase-9 compared to unexposed PD flies (Fig. 6A; $p < 0.05$). The exposure of 10^{-3} M of dopamine showed a 1.36 fold of significant decrease in the activity of caspase-9 compared to unexposed PD flies (Fig. 6A; $p < 0.05$). The results obtained for the activity of caspase-3 are shown in Fig. 6B. The PD flies showed a 2.54 fold increase in the activity of caspase-3 compared to control flies (Fig. 6B; $p < 0.01$). The exposure of PD flies to PAND having pramipexole concentration of 1 μM, 2 μM and 3 μM showed a dose dependent decrease of 1.16, 1.53 and 1.64 folds respectively in the activity of caspase-3 compared to unexposed PD flies (Fig. 6B; $p < 0.05$). The exposure of PD flies to 3 μM of pramipexole showed a 1.27 fold decrease in the activity of caspase-3 in PD flies compared to unexposed PD flies (Fig. 6B; $p < 0.05$). The exposure of PD flies to 10^{-3} M of dopamine

showed a 1.21 fold decrease in the caspase-3 compared to unexposed PD flies (Fig. 6B; $p < 0.05$).

Discussion

The image of PAND exhibits well dispersed and uniform particle size throughout the image. Further, no other impurity of secondary phase was observed in the image which confirms the good quality of the synthesised sample. In addition FTIR spectroscopy was performed to identify the important functional groups in the parent pramipexole drug and its nanodispersion (PAND) form. However, the FTIR spectrum of pramipexole alginate nanodispersion (PAND) exhibits a broad band $\sim$3500 cm^{-1}, which can be assigned to OH of water molecules associated with complex formation. The weaker band $\sim$800 cm^{-1} was attributed to OH rocking and twitching vibrations of coordinated water molecules. The data indicates that significant difference in the synthesized pramipexole (PAND) alginate nanodispersion at the molecular level. The results of the present study suggest that PAND successfully reduced the PD symptoms exhibited in our fly model. The fly model used in our study expresses a human alpha synuclein under GAL4-UAS system and also leads to the formation of Lewy bodies in a similar way as in humans (Feany and Bender, 2000). The formation of Lewy bodies not only results in the damage of dopaminergic neurons but also increases the oxidative stress in the brain (Giasson et al., 2000).The damage of dopaminergic neurons results in the decrease production of dopamine there by leading to the motor and cognitive defects (Muñoz-Soriano and Paricio, 2011).

In our present study newly enclosed PD flies (1–3 days old) were exposed to various doses of PAND. The exposed flies not only showed an improved activity but also exhibit the delay in the loss of climbing ability. The delay in the loss of climbing ability was more prominent in the PD flies exposed to PAND compared to the PD flies exposed to pramipexole alone. Pramipexole has been reported to improve the motor and cognitive dysfunctions in humans suffering from PD (Brusa et al., 2013; Burdick et al., 2011; Poletti and Bonuccelli, 2013). In comparing the effectiveness of the drugs (pramipexole, levodopa and amantadine), pramipexole was found to be most effective in improving the motor dysfunctions (Emsaki et al., 2013). The ability of wild type α-synuclein to aggregate presumably explains the formation of insoluble polymers of protein known as fibrils. This fibrillar α-synuclein is the building block of Lewy bodies in association of neurofilaments and other cytoskeletal proteins. The formation of Lewy bodies not only results in the death of dopaminergic neurons but also increases the oxidative stress (generation of free radicals) (Schulz, 2007). The degenerating neurons also produce endogenous toxins and other reactive oxygen species that may further damage normal neurons. The levels of these species are controlled by the antioxidant defence system. LPO, GSH, GST activity, PC content and MAO activity are the reliable markers of oxidative stress. Hence we decided to study these markers in PD model flies (Fatima et al., 2017). An increase in LPO and PC contents in brains of human suffering from PD has been reported earlier (Alam et al., 1997; Cookson, 2005). The PD flies exposed to various doses of PAND showed a dose dependent significant decrease in LPO. The PD flies exposed to pramipexole also showed a decrease in LPO, however this decrease was less compared to the treatment to doses of PAND. Pramipexole has been reported to inhibit lipid peroxidation and reduced injury in substantianigra induced by 1-methyl-4-phenyl-1,2,3,6- tetrahydropyridine (MPTP) in mice (Ramirez et al., 2003; Zou et al., 2000). A depletion in the GSH content and increase in GST activity has been reported in the brains of human suffering from PD and also in other experimental models of PD (Johnson et al., 2012; Zhu et al., 2007). The PD flies exposed to various doses of PAND also showed a dose dependent decrease in the GST and MAO activity.

The PD flies exposed to pramipexole also showed a decrease in GST as well as MAO activity, however the decrease was less compared to the PD flies exposed to PAND. MAOs are mitochondrial enzymes that catalyze the oxidation of monoamines in multiple tissues, including the brain. The elevated level of the enzymes has been implicated in the progression of PD (Dreiseitel et al., 2009). Hence the inhibitors of MAO may act as possible neuroprotective agents (Naoi and Maruyama, 2010). MAO activity has been implicated as a contributor to oxidative neuronal damage associated with various neurodegenerative diseases (Mazzio et al.,

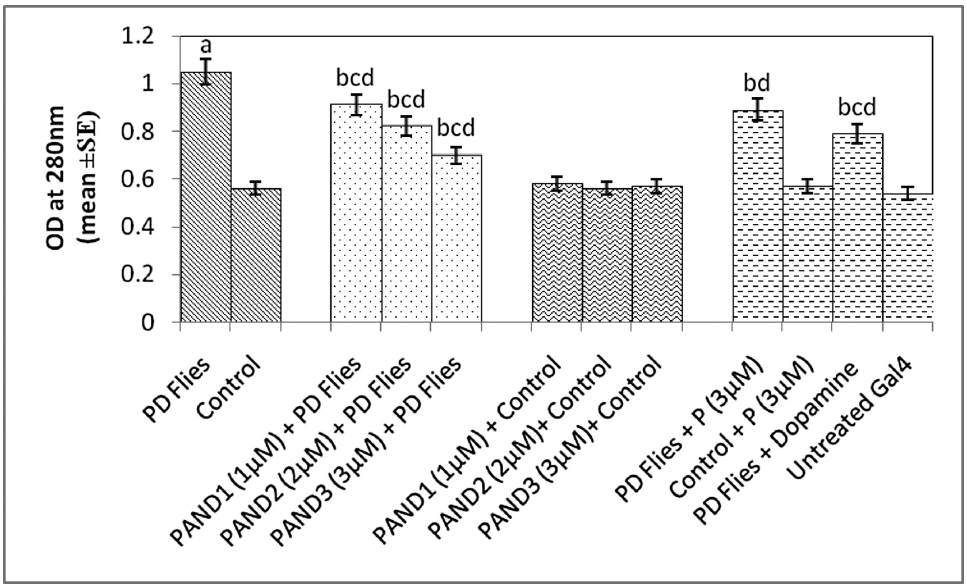


Fig. 5. Effect of pramipexole alginate nanodispersion (PAND) on the monoamine oxidase (MAO) activity in the brains of flies. The flies were allowed to feed on the diet supplemented with PAND for 24 days and then assayed for MAO activity. ([a] significant difference with respect to control, $p < 0.01$; [b] significant difference with respect to PD flies $p < 0.05$; [c] significant difference with respect to PD flies exposed to only pramipexole $p < 0.05$; [d] significant difference with respect to control, $p < 0.05$; P: pramipexole; Dopamine: 10^{-3} M; $N = 50$).

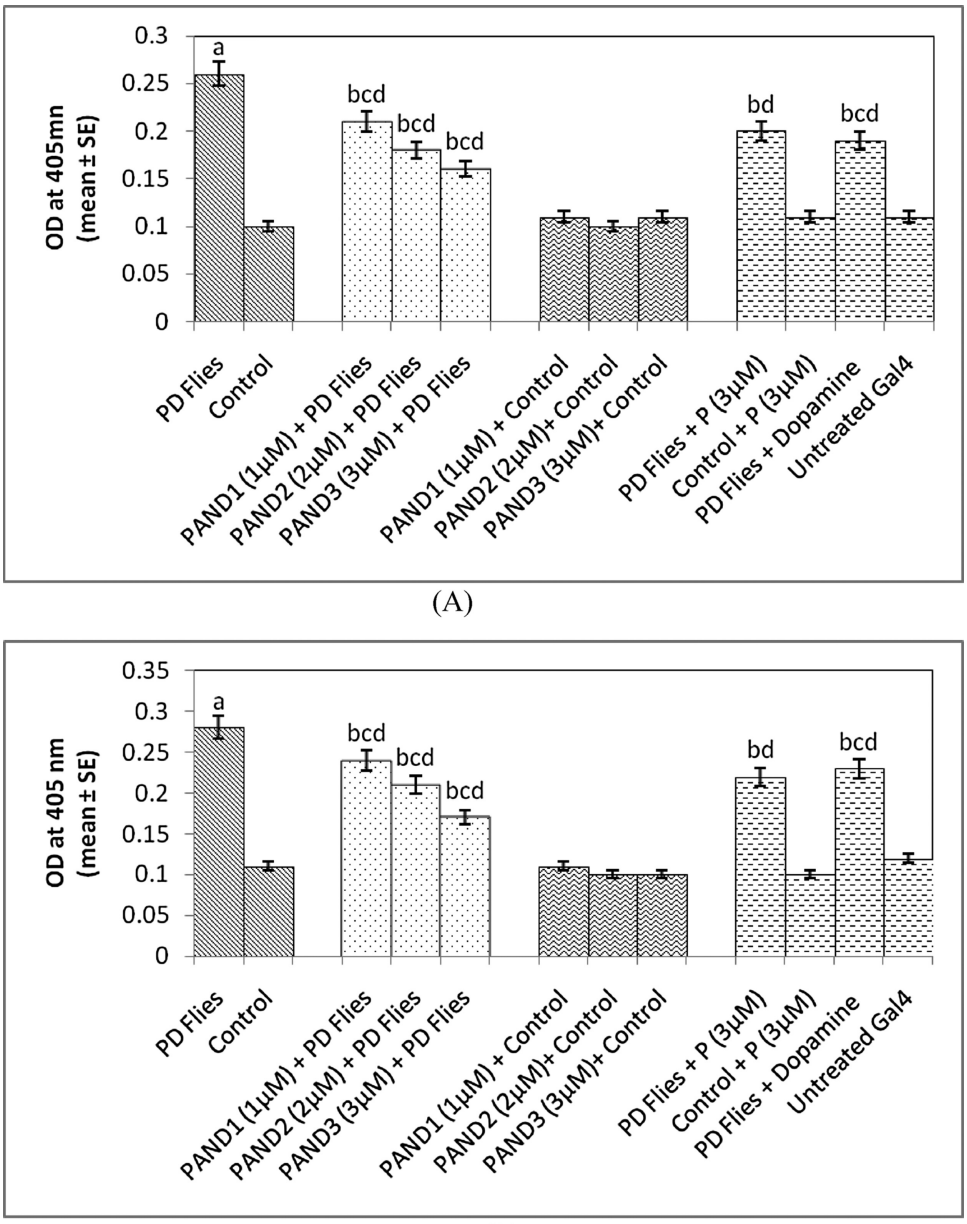


Fig. 6. Effect of pramipexole alginate nanodispersion (PAND) on the caspase-9 (A) and caspase-3 (B) in the brains of flies. The flies were allowed to feed on the diet supplemented with PAND for 24 days and then assayed for caspase 9 & 3 activity. ([a] significant difference with respect to control, $p < 0.01$; [b] significant difference with respect to PD flies $p < 0.05$; [c] significant difference with respect to PD flies exposed to only pramipexole $p < 0.05$; [d] significant difference with respect to control, $p < 0.05$; P: pramipexole; Dopamine: 10^{-3} M; $N = 50$).

1998). MAO inhibitors have been tried to treat the symptoms of PD along with or without levadopa (Riederer and Laux, 2011). The results on MAO activity obtained in our present study suggest that pramipexole could be used as a MAO inhibitor. The PD flies exposed to various doses of PAND showed a dose dependent increase in GSH and reduction in PC content. This could be possible due to the antioxidative property of pramipexole. Some dopamine agonists are antioxidant due to the presence of hydroxylated benzyl ring structure and secondly they also reduce the dopamine turnover, and hence free radical generation, through their action on presynaptic autoreceptor (Iravani et al., 2006). The increase in GSH content and reduction in PC content was lower in PD flies exposed to pramipexole compared to PAND. The PD flies exposed to PAND showed a dose dependent increase in dopamine content. This increase in dopamine content is due to the protection of the neurons provided by pramipexole in the form of reducing the oxidative stress (Carvey et al., 1997; Izumi et al., 2007; Szczudlik and Rudzińska, 2006). Again this increase was more in the PD flies exposed to PAND compared to the PD flies exposed to pramipexole. The results obtained in our study on the activity of caspase-9 and 3 showed a dose dependent decrease in the activity in the PD flies exposed to various doses of PAND. This decrease was more in PD flies exposed to PAND compared to PD flies exposed to pramipexole. There are reports of the inhibition of caspase-3

activity in cultured cells by pramipexole (Gu et al., 2005). In other study pramipexole has been reported to protect the PC12 cell death induced by the treatment of hydrogen peroxide (Fujita et al., 2006).

Human α-synuclein mutation or over expression results in cytotoxicity, with [A53T] α-synuclein being the most toxic variant. Decreased expression of the mitochondrial chaperone protein tumor necrosis factor receptor associated protein-1 (TRAP1) was found to enhance age dependent loss of fly head dopaminergic neurons. The over expression of human TRAP-1 can rescue the loss of dopaminergic neurons thereby reducing the neurotoxicity (Butler et al., 2012). In number of cell lines TRAP1 has been shown to provide anti-apoptotic functions as high levels of TRAP1 reduce the release of key factors responsible for apoptosis including the function of caspase-3 (Gesualdi et al., 2007; Masuda et al., 2004). The over expression of a mutant form of α-synuclein (A30P) dopaminergic GAL4 drivers results in an age dependent loss of neurons in *Drosophila* (Auluck et al., 2002; Botella et al., 2008). Dopamine in synaptic vesicles is not toxic but, due to the accumulation of α-synuclein it is released in the cytoplasm, where it can be easily oxidized and turn into a major source of oxidative stress (Bayersdorfer et al., 2010).

The overall results obtained in our present study confirm that pramipexole not only reduced the oxidative stress markers but also improves the motor dysfunctions of the transgenic flies expressing human alpha synuclein in the brain. Secondly, more desired effects can be obtained by exposing the PD flies to PAND, having the lower doses of pramipexole. It is clearly observed in our study that the PD flies exposed to 3 μM dose of pramipexole showed less improvement but the nanodispersion having the same dose is more effective in reducing the PD symptoms. Thus by creating the nanodispersion the side effects of the high doses of dopamine agonists could be reduced and the desired results could be obtained by exposing the PD patients to this nanodispersion having lower dose of the drug. In our earlier study with bromocriptine alginate nanocomposite (BANC) on a transgenic *Drosophila* model of Parkinson's disease the BANC successfully reduced the oxidative stress markers and delayed the loss of climbing ability of PD flies (Siddique et al., 2016b). It becomes more important if the PD patients are suffering from kidney disease as dopamine agonists are excreted by kidneys (Hong et al., 2008).

The results obtained in our study also validate the use of *Drosophila* for studying the action of drugs and nanodispersion. The use of animals in toxicological research and testing has become an issue of concern for both science and ethics. As a result the emphasis has been given on the use of alternatives to mammals in research and testing (Mukhopadhyay et al., 2003). In this context, *Drosophila* has an advantage in the initial discovery process regardless of raw throughput (Siddique et al., 2012, 2013a,b, 2014, 2016a). As screening is performed directly on the living organism (*Drosophila*), it reduces significantly the post screening cost to identify quality lead from the initial candidate pool (Pandey and Nichols, 2011; Siddique et al., 2015). Concerning our study with nanodispersions the results obtained are in concordance with the results obtained in higher mammalian models. The main limitation in the administration of the drug is the development of suitable carrier that is able to cross the blood brain barrier (BBB), therefore the nano particles are the effective alternative (Leyva-Gómez et al., 2015). The increasing incidence of diseases affecting the central nervous system (CNS) demands the urgent development of efficient drugs (Peluffo et al., 2015).

Conclusion

It is concluded that the drugs in the form nanodispersions could play a pivotal role as it will reduce the toxicity/side effects associated with the excessive use or higher dose of the drug use to treat various CNS disorders.

Conflict of interests

The authors declare no conflict of interests related to this work.

Acknowledgements

We are grateful to Council of Scientific and Industrial Research (CSIR), New Delhi, India for the sanction of the research project (No. 37 (1594)/13/EMR-II) to Dr. Yasir Hasan Siddique. We are also thankful to the Chairman, Department of Zoology, AMU, Aligarh for providing laboratory facilities.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.jab.2017.11.002.

References

Alam, Z.I., Daniel, S.E., Lees, A.J., Marsden, D.C., 1997. A generalized increase in protein carbonyls in the brain in Parkinson's but not incidental Lewy body disease. J. Neurochem. 69, 1326–1329.

Auluck, P.K., Chan, H.Y., Trojanowski, J.Q., Lee, V.M., Bonini, N.M., 2002. Chaperone suppression of alpha-synuclein toxicity in a *Drosophila* model for Parkinson's disease. Science 295, 865–868.

Barnett-Cowan, M., Dyde, R.T., Fox, S.H., Moro, E., Hutchison, W.D., Harris, L.R., 2010. Multisensory determinants of orientation perception in Parkinson's disease. Neuroscience 167 (4), 1138–1150.

Bayersdorfer, F., Voigt, A., Schneuwly, S., Botella, J.B., 2010. Dopamine-dependent neurodegeneration in *Drosophila* models of familial and sporadic Parkinson's disease. Neurobiol. Dis. 40, 113–119.

Botella, J.A., Bayersdorfer, F., Schneuwly, S., 2008. Superoxide dismutase over expression protects dopaminergic neurons in a *Drosophila* model of Parkinson's disease. Neurobiol. Dis. 30, 65–73.

Brand, A.H., Perrimon, N., 1993. Targeted gene expression as a means of altering cell fates and generating dominant phenotypes. Development 118, 401–415.

Brusa, L., Pavino, V., Massimetti, M.C., Bove, R., Iani, C., Stanzione, P., 2013. The effect of dopamine agonists on cognitive functions in non-demented early-mild Parkinson's disease patients. Funct. Neurol. 28 (1), 13–17.

Burdick, K.E., Braga, R.J., Nnadi, C.U., Shaya, Y., Stearns, W.H., Malhotra, A.K., 2011. Placebo-controlled adjunctive trial of pramipexole in patients with bipolar disorder: targeting cognitive dysfunction. J. Clin. Psychiatry 73 (1), 103–112.

Butler, E.K., Voigt, A., Lutz, A.K., Torgel, J.P., Gerhardt, E., Karsten, P., et al., 2012. The mitochondrial chaperone protein TRAP1 mitigates α-synuclein toxicity. PLoS Genet. 8 (2), e1002488.

Carvey, P.M., Pieri, S., Ling, Z.D., 1997. Attenuation of levodopa-induced toxicity in mesencephalic cultures by pramipexole. J. Neural Transm. 104 (2–3), 209–228.

Castro-Hernandez, J., Afonso-Oramas, D., Cruz-Muros, I., Salas-Hernández, J., Barroso-Chinea, P., Moratalla, R., González-Hernández, T., 2015. Prolonged treatment with pramipexole promotes physical interaction of striatal dopamine D3 autoreceptors with dopamine transporters to reduce dopamine uptake. Neurobiol. Dis. 74, 325–335.

Cedarbaum, J.M., 1987. Clinical pharmacokinetics of anti-parkinsonian drugs. Clin. Pharmacokinet. 13, 141–178.

Chiu, J.C., Low, K.H., Pike, D.H., Yildirim, E., Edery, I., 2010. Assaying locomotor activity to study circadian rhythms and sleep parameters in *Drosophila*. JoVE 43, e2157.

Cookson, M.R., 2005. The biochemistry of Parkinson's disease. Annu. Rev. Biochem. 74, 29–52.

Degim, I.T., Acarturk, F., Erdogan, D., Lorlar, N.D., 2003. Transdermal administration of bromocritine. Biol. Pharm. Bull. 26 (4), 501–505.

Dreiseitel, A., Korte, G., Schreier, P., Oehme, A., Locher, S., Domani, M., Sand, P.G., 2009. Berry anthocyanins and their aglycons inhibit monoamine oxidases A and B. Pharmacol. Res. 59 (5), 306–311.

Emsaki, G., Asgari, K., Molavi, H., Chitsaz, A., 2013. A comparison of the effectiveness of three drug regimens on cognitive performance of patients with Parkinson's disease. Psicologica: Int. J. Methodol. Exp. Psychol. 34 (2), 273–284.

Fatima, A., Khanam, S., Rahul, R., Jyoti, S., Naz, F., Ali, F., Siddique, Y.H., 2017. Protective effect of tangeritin in transgenic *Drosophila* model of Parkinson's disease. Front. Biosci. 9, 44–53.

Feany, M.B., Bender, W.W., 2000. A *Drosophila* model of Parkinson's disease. Nature 404, 394–398.

Fujita, Y., Izawa, Y., Ali, N., Kanematsu, Y., Tsuchiya, K., Hamano, S., Yoshizumi, M., 2006. Pramipexole protects against H_2O_2-induced PC12 cell death. Naunyn-Schmiedeberg's Arch. Pharmacol. 372 (4), 257–266.

Gallant, H., Vo, A., Seergobin, K.N., MacDonald, P.A., 2016. Pramipexole impairs stimulus-response learning in healthy young adults. Front. Neurosci. 10, 374.
Gesualdi, N.M., Chirico, G., Pirozzi, G., Costantino, E., Landriscina, M., Esposito, F., 2007. Tumour necrosis factor-associated protein 1 (TRAP-1) protects cells from oxidative stress and apoptosis. Stress 10 (4), 342–350.
Giasson, B.I., Duda, J.E., Murray, I.V., Chen, Q., Souza, J.M., Hurtig, H.I., Lee, V.M.Y., 2000. Oxidative damage linked to neurodegeneration by selective α-synuclein nitration in synucleinopathy lesions. Science 290 (5493), 985–989.
Gu, M., Iravani, M.M., Cooper, J.M., King, D., Jenner, P., Schapira, A.H., 2005. Pramipexole protects against apoptotic cell death by non-dopaminergic mechanisms. J. Neurochem. 92 (1), 215.
Guo, M., 2012. *Drosophila* as a model to study mitochondrial dysfunction in Parkinson's disease. Cold Spring Harb. Perspect. Med. 2, 1–17.
Habig, W.H., Pabst, M.J., Fleischner, G., Gatmaitan, Z., Arias, I.M., Jakoby, W.B., 1974. The identity of glutathione S-transferase B with ligandin, a major binding protein of liver. Proc. Natl. Acad. Sci. 71 (10), 3879–3882.
Hawkins, C.L., Morgan, P.E., Davies, M.J., 2009. Quantification of protein modification by oxidants. Free Radic. Biol. Med. 46 (8), 965–988.
Hong, C., Sun, Y., Lu, C., 2008. Fatal intoxication using amantadine and pramipexole in a uremic patient. Acta Neurol. Taiwan. 17 (2), 109–111.
Iravani, M.M., Haddon, C.O., Cooper, J.M., Jenner, P., Schapira, A.H., 2006. Pramipexole protects against MPTP toxicity in non-human primates. J. Neurochem. 96 (5), 1315–1321.
Izumi, Y., Sawada, H., Yamamoto, N., Kume, T., Katsuki, H., Shimohama, S., Akaike, A., 2007. Novel neuroprotective mechanisms of pramipexole, an anti-Parkinson drug, against endogenous dopamine-mediated excitotoxicity. Eur. J. Pharmacol. 557 (2), 132–140.
Johnson, W.M., Wilson-Delfosse, A.L., Mieyal, J.J., 2012. Dysregulation of glutathione homeostasis in neurodegenerative diseases. Nutrients 4, 1399–1440.
Jollow, D.J., Mitchell, J.R., Zampaglione, N.A., Gillette, J.R., 1974. Bromobenzene-induced liver necrosis. Protective role of glutathione and evidence for 3, 4-bromobenzene oxide as the hepatotoxic metabolite. Pharmacology 11 (3), 151–169.
Lee, W.H., Loo, C.Y., Bebawy, M., Luk, F., Mason, R.S., Rohanizadeh, R., 2013. Curcumin and its derivatives: their application in neuropharmacology and neuroscience in the 21 st century. Curr. Neuropharmacol. 11 (4), 338–378.
Leyva-Gómez, G., Cortés, H., Magaña, J.J., Leyva-García, N., Quintanar-Guerrero, D., Florán, B., 2015. Nanoparticle technology for treatment of Parkinson's disease: the role of surface phenomena in reaching the brain. Drug Discov. Today 20 (7), 824–837.
Masuda, Y., Shima, G., Aiuchi, T., Horie, M., Hori, K., Nakajo, S., Nakaya, K., 2004. Involvement of tumour necrosis factor receptor-associated protein 1 (TRAP1) in apoptosis induced by I-hydroxyisovalerylshikonin. J. Biol. Chem. 279, 42503–42515.
Mazzio, E.A., Harris, N., Soliman, K.F., 1998. Food constituents attenuate monoamine oxidase activity and peroxide levels in C6 astrocyte cells. Planta Med. 64 (07), 603–606.
McEwen, C.M., 1965. Human plasma monoamine oxidase I. Purification and identification. J. Biol. Chem. 240 (5), 2003–2010.
Muñoz-Soriano, V., Paricio, N., 2011. *Drosophila* models of Parkinson's disease: discovering relevant pathways and novel therapeutic strategies. Parkinson's Dis. 2011, 1–14.
Mukhopadhyay, I., Saxena, D.K., Chowdhuri, D.K., 2003. Hazardous effects of effluent from the chrome plating industry: 70 kDa heat shock protein expression as a marker of cellular damage in transgenic Drosophila melanogaster (hsp70-lacZ). Environ. Health Perspect. 111 (16), 1926–1932.
Naoi, M., Maruyama, W., 2010. Monoamine oxidase inhibitors as neuroprotective agents in age-dependent neurodegenerative disorders. Curr. Pharm. Des. 16, 2799–2817.
Ohkawa, H., Nobuko, O., Yagi, K., 1978. Reaction of linoleic acid hydroperoxide with thiobarbituric acid. J. Lipid Res. 19, 1053–1057.
Pandey, U.B., Nichols, C.D., 2011. Human disease models in Drosophila melanogaster and the role of the fly in therapeutic drug discovery. Pharmacol. Rev. 63 (2), 411–436.
Papadimitriou, S., Bikiaris, D., Avgoustakis, K., Karavas, E., Georgarakis, M., 2008. Chitosan nanoparticles loaded with dorzolamide and pramipexole. Carbohydr. Polym. 73, 44–54.
Patterson, T.A., Li, M., Hotchkiss, C.E., Mauz, A., Eddie, M., Greischel, A., Paule, M.G., 2010. Toxicity assessment of pramipexole in juvenile rhesus monkeys. Toxicology 276 (3), 164–171.
Peluffo, H., Unzueta, U., Negro-Demontel, M.L., Xu, Z., Váquez, E., Ferrer-Miralles, N., Villaverde, A., 2015. BBB-targeting, protein-based nanomedicines for drug and nucleic acid delivery to the CNS. Biotechnol. Adv. 33 (2), 277–287.
Pendleton, R.G., Parvez, F., Sayed, M., Hillman, R., 2002. Effects of pharmacological agents upon a transgenic model of Parkinson's disease in Drosophila melanogaster. J. Pharmacol. Exp. Ther. 300 (1), 91–96.
Poletti, M., Bonuccelli, U., 2013. Acute and chronic cognitive effects of levodopa and dopamine agonists on patients with Parkinson's disease: a review. Therap. Adv. Psychopharmacol. 3 (2), 101–113.
Ramirez, A.D., Wong, S.K.F., Menniti, F.S., 2003. Pramipexole inhibits MPTP toxicity in mice by dopamine D3 receptor dependent and independent mechanisms. Eur. J. Pharmacol. 475 (1), 29–35.
Rasmussen, V.G., Østergaard, K., Dupont, E., Poulsen, S.H., 2011. The risk of valvular regurgitation in patients with Parkinson's disease treated with dopamine receptor agonists. Mov. Disord. 26, 801–806.
Rektorová, I., Rektor, I., Bareš, M., Dostál, V., Ehler, E., Fanfrdlová, Z., Svátová, J., 2003. Pramipexole and pergolide in the treatment of depression in Parkinson's disease: a national multicentre prospective randomized study. Eur. J. Neurol. 10 (4), 399–406.
Riederer, P., Laux, G., 2011. MAO-inhibitors in Parkinson's disease. Exp. Neurobiol. 20 (1), 1–17.
Riemensperger, T., Issa, A.R., Pech, U., Coulom, H., Nguyễn, M.V., Cassar, M., Birman, S., 2013. A single dopamine pathway underlies progressive locomotor deficits in a Drosophila model of Parkinson disease. Cell Rep. 5, 952–960.
Rosato, E., Kyriacou, C.P., 2006. Analysis of locomotor activity rhythms in Drosophila. Nat. Protoc. 1 (2), 559–568.
Schlumpf, M., Lichtensteiger, W., Langemann, H., Waser, P.G., Hefti, F., 1974. A fluorometric micromethod for the simultaneous determination of serotonin, noradrenaline and dopamine in milligram amounts of brain tissue. Biochem. Pharmacol. 23 (17), 2437–2446.
Schulz, J.B., 2007. Mechanisms of neurodegeneration in idiopathic Parkinson's disease. Parkinsonism Relat. Disord. 13, S306–S308.
Siddique, Y.H., Ara, G., Jyoti, S., Afzal, M., 2011. The dietary supplementation of nordihydroguaiaretic acid (NDGA) delayed the loss of climbing ability in *Drosophila* model of Parkinson's disease. J. Diet. Suppl. 9, 1–8.
Siddique, Y.H., Ara, G., Jyoti, S., Afzal, M., 2012. Protective effect of curcumin in transgenic *Drosophila melanogaster* model of Parkinson's disease. Altern. Med. Stud. 2 (1), 3.
Siddique, Y.H., Fatima, A., Jyoti, S., Naz, F., Khan, W., Singh, B.R., Naqvi, A.H., 2013a. Evaluation of the toxic potential of graphene copper nanocomposite (GCNC) in the third instar larvae of transgenic *Drosophila melanogaster* (hsp70-lacZ) Bg 9. PLoS One 8 (12), e80944.
Siddique, Y.H., Mujtaba, S.F., Jyoti, S., Naz, F., 2013b. GC–MS analysis of *Eucalyptus citriodora* leaf extract and its role on the dietary supplementation in transgenic *Drosophila* model of Parkinson's disease. Food Chem. Toxicol. 55, 29–35.
Siddique, Y.H., Naz, F., Jyoti, S., Fatima, A., Khanam, S., Ali, F., et al., 2014. Effect of *Centella asiatica* leaf extract on the dietary supplementation in transgenic *Drosophila* model of Parkinson's disease. Parkinson's Dis. 2014, 262058. doi: http://dx.doi.org/10.1155/2014/262058.
Siddique, Y.H., Haidari, M., Khan, W., Fatima, A., Jyoti, S., Khanam, S., Beg, T., 2015. Toxic potential of copper-doped ZnO nanoparticles in *Drosophila melanogaster* (Oregon R). Toxicol. Mech. Methods 25 (6), 425–432.
Siddique, Y.H., Naz, F., Jyoti, S., Ali, F., Fatima, A., Khanam, S., 2016a. Protective effect of Geraniol on the transgenic *Drosophila* model of Parkinson's disease. Environ. Toxicol. Pharmacol. 43, 225–231.
Siddique, Y.H., Khan, W., Fatima, A., Jyoti, S., Khanam, S., Naz, F., Naqvi, A.H., 2016b. Effect of bromocriptine alginate nanocomposite (BANC) on a transgenic *Drosophila* model of Parkinson's disease. Dis. Models Mech. 9 (1), 63–68.
Szczudlik, A., Rudzińska, M., 2006. Neuroprotective effect of dopamine agonists. Neurol. Neurochir. Pol. 41 (2 Suppl. 1), S22–S28.
Tan, L.C.S., Ng, K.K.C., Au, W.L., Lee, R.K.K., Chan, Y.H., Tan, N.C.K., 2009. Bromocriptine use and the risk of valvular heart disease. Mov. Disord. 24, 344–349.
Yang, H., 2010. Nanoparticle-mediated brain-specific drug delivery, imaging, and diagnosis. Pharm. Res. 27 (9), 1759–1771.
Zhu, Y., Carvey, P.M., Ling, Z., 2007. Altered glutathione homeostasis in animals prenatally exposed to lipopolysaccharide. Neurochem. Int. 50 (4), 671–680.
Zou, L.L., Xu, J., Jankovic, J., He, Y., Appel, S.H., Le, W.D., 2000. Pramipexole inhibits lipid peroxidation and reduces injury in the substantianigra induced by the dopaminergic neurotoxin 1-methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine in C57BL/6 mice. Neurosci. Lett. 281 (2), 167–170.