7. DISCUSSION

Parkinson disease (PD) is one of the very commonly occurring neurodegenerative disorder especially in elderly patients. Till date there is no proper cure to treat PD, since the currently available drugs in market can neither arrest nor reverse the progression of neurodegeneration which leads to disease. These drugs only give the symptomatic relief by mimicking the effect of dopamine (DA) or boost the level of DA in brain (107). Most of the patients need levodopa along with DA receptor agonist to treat PD (32). But it is observed that, the ‘gold standard’ drug (33) to treat PD levodopa results in fluctuation of motor responses in approximately 30-50 % of patients after 5 years or more treatment (34). Apart from levodopa the drugs which prescribed to treat PD includes DA receptor antagonist, MAO-B inhibitors, COMT inhibitors and cholinergic antagonist drugs have lost of limitation along with side effects (107). Thus by considering above facts it’s a need of time to find a new drug treatment to target different mechanism apart from dopaminergic system to prevent or minimise the neurodegeneration of dopaminergic neurons to manage the PD.

In this context, by extensive literature survey 1,9-Pyrazoloanthrone an c-Jun-N-terminal kinase 3 (JNK-3) inhibitor and Conessine a Histamine H-3 receptor antagonist were selected for study by considering the recent reports which suggest that the highest density of Histamine H3-receptors is found in basal ganglia (42, 43) and H3-antagonists can increase the turnover of dopamine in basal ganglia (38, 39). Few other reports suggest that, c-Jun N-terminal kinase (JNK) pathway have an important role in stress mediated neurotoxicity and inflammation; while blockade of JNK by specific inhibitors could prevent or efficaciously slow-down the progression of PD (108). The extensive study has been carrier out to evaluate the effect of selected molecules on management of PD.

The preliminary in-silico and kinase selectivity studies provided an idea about the selectivity of molecules. It was very important that, selected molecules should be potent and selective towards JNK-3 and H-3 receptor to avoid or minimise the side effects associated with JNK-1, JNK-2, Histamine H-1 and H-2 receptors.

To estimate the 1,9-P and Conessine entrapment in liposomes, drug content in rat plasma and brain a simple, sensitive and rapid analytical and bio-analytical UFLC method was developed. The developed UFLC methods for
estimation of 1,9-P and Conessine were validated as per the USFDA guidelines (75). The 1,9-P was found to be poorly water soluble, sparingly soluble in methanol, ACN and ethanol. The molecular weight of 1,9-P is less than 2000 Dalton and it is partly soluble in water, as it is polar by nature so Hibar C\textsubscript{18} column was used with reverse phase mode for analysis (109). The λ max for 1,9-P was found to be 210 nm by using UV-Visible spectroscopy. Initially mobile phase in combination of ACN: water (40:60) showed very less retention time; however, with same mobile phase at pH 4.0 it showed good resolution between 1,9-P and IS, but IS showed tailing effect. The good resolution factor was observed at a ratio of 47 % ammonium acetate buffer (10 mM, pH 8.0 adjusted with ammonia) and 53 % ACN with a flow rate 1 mL/min. At above mentioned ratio of mobile phase with a run time of 12 min and flow rate 1 mL/min a retention time for 1,9-P and IS as 6.9 min and 8.2 min respectively was observed. The 1,9-P and IS are highly sensitive to a pH and wavelength; a slight change in either leads to major changes in resolution between 1,9-P and IS. In validation process solid phase extraction (SPE), liquid-liquid extraction (LLE) and protein precipitation (PPT) techniques were applied to determine the limit of detection for 1,9-P. The limit of detection of 2 ng/mL was obtained with SPE as well as PPT when ACN was used as an protein precipitating agent, but LLE doesn’t showed good limit of detection for 1,9-P. As PPT technique is easy, less time consuming in comparison to SPE with multiple purification steps and is time intensive, hence further validation was done by using PPT technique (87).

The Conessine was found to be slightly water soluble, sparingly soluble in methanol, ACN and ethanol. The molecular weight of Conessine is less than 2000 Dalton and it is partly soluble in water, as it is polar by nature so Hibar C\textsubscript{18} column was used with reverse phase mode for analysis (109). The λ max for Conessine was found to be 210 nm by using UV-Visible spectroscopy. Initial combination of mobile phase ACN: water (70:30) showed good peak area but very low retention time. However, mobile phase Methanol: Ammonium formate (75:25) at pH 6.5 showed good resolution between Conessine and IS, but IS showed tailing effect. The better resolution factor was observed at a ratio of 90 % methanol and 10% hexane sulphonic acid (10 mM, pH 8.4 adjusted with ammonia) with a flow rate 0.9 mL/min. At above mentioned ratio of mobile phase with a run time of 12 min and flow rate 0.9 mL/min a retention time for Conessine and IS as 3.18 min and 8.6 min respectively was observed. The IS is highly sensitive to a pH and wavelength;
a slight change in either leads to major changes in resolution of IS. For extracting the drug from biological samples various extraction procedures have been tried such as solid phase extraction (SPE), liquid-liquid extraction (LLE) and protein precipitation (PPT) to determine the percentage recovery for Conessine. The limit of detection 4.0 ng/mL was obtained with SPE as well as PPT when ACN was used as a protein precipitating agent, but LLE doesn’t showed good limit of detection for Conessine. As PPT technique is easy, less time consuming in comparison to SPE with multiple purification steps and is time intensive, hence further extraction of drug were carried out using PPT technique.

To cross the blood brain barrier (BBB) is one of most challenging task to deliver the drug into brain to treat the PD. Being a most important and vital organ of body it is very important to select biocompatible carriers to deliver the drug into brain. Amongst nanocarriers, liposomes have great important role as brain targeted delivery as it is capable of delivering drug into brain (110, 111). Liposomes carry an outer lipophilic membrane that improves their permeability across cell membranes, thereby making the BBB penetrable (112). Liposomes have been shown to provide stable encapsulation for various drugs and offer distinct advantages over unencapsulated agents (113) also, they are non-toxic, non-immunogenic, non-carcinogenic, non-thrombogenic and biodegradable (114) thus, liposomes have been proposed for use in this study. While preparing different batches of liposomes using various concentrations of phosphatidylcholine, cholesterol and stearic acid, changes in entrapment efficiency and particle size were observed. An increase in lipid concentration increased the particle size and decreased entrapment. The higher concentration of phosphatidylcholine showed better entrapment efficiency 84.56 ± 0.25 % and 88.57 ± 0.62 % with highest particle size (1642.0 ± 4.56 nm, 2584.0 ± 4.56) respectively for Conessine and 1,9-P. The % entrapment efficiency for liposomes was also influenced by cholesterol content. The higher concentration of cholesterol increased the particle size and entrapment efficiency; this may be due to increase in hydrophobicity. Use of ultrasonication, a small modification in preparation showed significance effect on size of liposomes which reduced the particle size of liposomes to 112.33 ± 0.84 nm and 106.2 ± 1.02 nm for 1,9-P and Conessine respectively.

The prepared liposomes exhibited small unilamellar vesicles (SUVs), as the mean particle size of 1,9-P and Conessine liposomes was found to be 112.33 and 106.2 nm with PDI of 0.286, 0.286 respectively which demonstrated in
Figure 24 and 25. For effective targeting of the brain, a formulation is expected to have a particle size of less than 200 nm (115).

The charge of the outer membrane affects the distribution and stability of the liposomes. The overall surface charge of the 1,9-P and Conessine liposomes measured was found to be -19.40, -18.50 mV respectively. Liposomes were prepared by adding the phosphatidylcholine which turn the polar groups towards the surrounding water phase which contains a negative charge. The significance of zeta potential value can be related to the stability of colloidal dispersions which gives long term stability. The stability of small molecules and particles in suspension and emulsion could be maintained by zeta potential. The low zeta potential is responsible for repulsion in contrast with attraction which will break and flocculate the dispersion (116).

The selected batches of developed formulations were subjected to characterization to check the surface morphology using scanning electron microscopy (SEM) and Fourier transform infra-red (FTIR) spectroscopy. The images of prepared liposome formulation appear as numerous scattered, spherical, dark stained particles with unilamellar vesicle structure ranging from 100-150 nm particle size. The liposomes were observed to possess a smooth surface which could contribute to the release of the drug in sustained manner compared to rough surfaces.

To investigate the formation of hydrogen bonding amongst lipids and stearic acid, and to obtain the information of interaction, liposomes were examined by FTIR spectroscopy (Figure 28 and 29). The hydrogen bonding between the carbonyl group of stearic acid and hydrogen group of phosphatidylcholine and cholesterol essentially regulates the rigidity of the liposomes. The balance of hydrophilic and hydrophobic groups provides the structural rigidity to liposomes, playing an important role in encapsulation of hydrophilic and hydrophobic drug (82). FTIR spectra of phosphatidylcholine indicates the characteristic spectra at 1744 and 1242 cm\(^{-1}\) for carbonyl group (-C=O) and PO\(_2^-\) anti-symmetry double bond stretching respectively. FTIR spectra of cholesterol shows two characteristic peaks in the region of 2800 and 3200 cm\(^{-1}\), which corresponds to C-H stretching vibration of methyl groups and vibration of cyclic hydrocarbons. The characteristic peaks cantered at 1693 and 2866 cm\(^{-1}\) in stearic acid spectra are associated with carboxylic group and long chain of alkene respectively. 1,9-P pure drug shows the characteristic bond vibration at 3220 cm\(^{-1}\) (NH- secondary amine stretching) and 3104 cm\(^{-1}\) (C-H aromatic stretching). The Conessine pure drug shows the
characteristic peaks of CH\textsubscript{3} bending (1450 cm\textsuperscript{-1}) and aromatic hydrocarbon 2900 cm\textsuperscript{-1}. Liposomes has the characteristic bands of CH\textsubscript{3} bending (1437 cm\textsuperscript{-1}) and conjugated C=O (1707 cm\textsuperscript{-1}). However, the loss of characteristic peaks of phosphatidylcholine, cholesterol and stearic acid shows a strong interaction amongst them which is needed for formation of rigid liposomal vesicles. The characteristic CH aromatic stretching peaks of pure 1,9-P and CH\textsubscript{3} bending 1450 cm\textsuperscript{-1} was absent in liposome spectra suggests that 1,9-P and Conessine was significantly encapsulated in liposomes. In liposomal spectra, there were no major shifting of functional groups which confirms drug and lipids used have no interaction.

The developed formulations were subjected to \textit{in vitro} release kinetic study using the PBS (pH 6.8) at 37 °C. Both the developed formulations followed sustained release of action up to 24 h with maximum release of 84.23 ± 6.00 % and 79.30 ± 4.10 % for 1,9-P and Conessine liposome respectively. The release kinetic data demonstrated various kinetic models amongst that formulations showed Zero order release kinetic which had higher linearity (y = 2.9919x + 31.127, R\textsuperscript{2} = 0.9574 and y = 0.6113x + 1.3554, R\textsuperscript{2} = 0.9632). The n value of formulation was found to be 0.878 (limits 0.45- 1) which shows the mechanism of drug release is by non-Fickian solute diffusion (111, 117). The sustained release of 1,9-P and Conessine from liposomes may result to effective treatment in PD condition for long period of time.

The stability tests performed for 1,9-P and Conessine liposomes revealed minimal degradation at 4 °C (112.33 ± 0.84; 106.2 ± 1.02 on day 0 to 108.75 ± 1.94; 103.02 ± 1.02 after 6 months) and significant degradation at 25 °C (112.13 ± 0.84; 103.44 ± 1.92 on 0 day to 105.86 ± 1.02; 96.56 ± 0.85 after 6 months).

Anemia, jaundice and other diseases are caused by \textit{in vivo} hemolysis, that is, the blood erythrocytes are broken. So an \textit{in vitro} hemolysis test is commonly used to screen certain drugs in early clinical development, it is also used to evaluate the biocompatibility of liposomes. \textit{In vitro} erythrocyte-induced hemolysis is a simple and reliable measure for estimating the biocompatibility of materials (118). It has been reported that up to 5% hemolysis is permissible for biomaterials (119). Initial evaluation of the biocompatibility of the liposomes was performed \textit{in vitro} using hemolysis assays and it showed 1.24 and 1.81 % of hemolysis for 1,9-P and Conessine liposomes which is below acceptable limit.
The obtained pharmacokinetic and biodistribution data indicates that, the 1,9-P liposomes reaches brain rapidly at a maximum concentration (2143.84 ± 126.98 ng/g) after 2.0 h, which is much higher than 1,9-P solution (695.68 ± 59.67 ng/g) after 2.0 h. This indicates that liposomal formulation can easily cross blood brain barrier (BBB) than 1,9-P solution and remains stable for up to 3-4 h. The pharmacokinetic and tissue distribution of 1,9-P was analysed by non-compartment extra-vascular model. The order of AUC was found to be kidney > liver > brain > lungs > spleen > heart. The order of the maximum 1,9-P concentration in tissue was kidney > brain > liver > lungs > spleen > heart.

Also, Conessine liposomes reaches brain rapidly at a maximum concentration (3243.8 ± 141.2 ng/g) after 2.0 h, which is much higher than Conessine solution (1095.4 ± 61.25 ng/g) after 2.0 h. This indicates that liposomal formulation can easily cross blood brain barrier (BBB) than Conessine solution and remains stable for up to 3-4 h. The pharmacokinetic and tissue distribution of Conessine was analysed by non-compartment extra-vascular model. The order of AUC was found to be kidney > brain > liver > lungs > spleen > heart. The order of the maximum Conessine concentration in tissue was kidney > brain > liver > lungs > spleen > heart.

The cytoxicity of developed formulation particularly for brain delivery is very important criteria as safety point concerned. Nanoparticles can cause cytotoxicity by adherence of the particles to the cell membrane, internalization of nanoparticles by cells, degradation and subsequent release of cytotoxic degradation product (120). The cytotoxicity study of developed 1,9-P and Conessine liposomal formulations was carried out by MTT assay using SH-SY5Y cell line, a human neuroblastoma cell line (121). The results suggest that, both formulations were non toxic and safe. These results also supported by earlier reports, which suggested that the selected lipids are safe, nontoxic and biodegradable (113). The selected doses of 1,9-P liposome from cytotoxicity study were subjected to evaluate the neuroprotective effect against 6-OHDA and MPTP neurotoxicity. The obtained results showed the neuroprotective effect of 1,9-P a inhibitor of c-jun N-terminal kinase (JNK), which is supported by earlier reports (122).

The apoptotic assay by AO/EB and Hoechst 33342 Staining further verified the neuroprotective potential of the 1,9-P and liposomal formulation. Both live and dead cells had taken up acridine orange and the DNA was stained green, while the dead cell DNA was stained bright orange with ethidium bromide. Using this differential fluorescence between acridine orange and ethidium
bromide, four types of cells were identified; normal viable cells (uniform green staining), the early apoptotic cells (green staining with bright orange condensed nucleus), the late apoptotic cells (orange staining with nuclear fragmentation) and necrotic cells (orange staining without nuclear fragmentation) (127). The 6-OHDA and MPTP treated group showed high number of apoptotic and necrotic cells in contrast to 1,9-P liposome treated group which showed very less apoptotic and necrotic cells. These results suggests that JNK pathway is one of the main intermediator of the neurotoxic effects of 6-OHDA in vitro and by inhibiting it we may have a novel and effective strategy to treat PD.

The Hoechst dye is water soluble dye which can easily cross the cell membrane and binds to minor grooves of DNA with a preference for sequences rich in adenine and thymine. When this dye combines with DNA it produces the fluorescence which indicates the apoptosis of cell (124). In this assay we observed more fluorescent nuclei in 6-OHDA and MPTP treated groups, whereas in treatment group the fluorescence get decreased with increased concentration of test drug.

To investigate the intracellular target and mechanism of 1,9-P, the effect of 1,9-P treatment on 6-OHDA and MPTP induced DNA fragmentation along apoptotic and anti-apoptotic markers were estimated. The DNA fragmentation assay provides the idea about the molecular mechanism of 1,9-P. The present results shows that 6-OHDA and MPTP induced DNA fragmentation can be attenuated by the treatment of 1,9-P in dose dependent manner which supported by earlier reports suggesting that, 1,9-P inhibit the DNA fragmentation by blocking the induction of c-JUN phosphorylation (125).

The results of estimation 6-OHDA and MPTP induced of apoptotic and anti-apoptotic markers demonstrates that 1,9-P liposomes inhibits the mitochondrial apoptotic signaling pathway. This pathway consists of the anti-apoptotic proteins Bcl2 which act as a repressor of apoptosis which maintains mitochondrial membrane integrity and avoids the release of cytochrome-c into cytosol (126). The cytochrome-c released into cytosol is responsible for activation caspase-3 which is key caspase of apoptotic pathway (127, 128), whereas the apoptotic proteins such as Bax act as promoters of cell apoptosis (129). Our results shows that 1,9-P treatment increased the Bcl2 expression by suppressing the Bax expression which resulted in a net effect of attenuation of cytochrome-c release and decreased caspase-3 mediated cell apoptosis.
In addition to above mentioned mechanism the 1,9-P could provides the neuroprotective action through the inhibition of MAP kinases to evaluate that, the expression of p-38 and JNK was measured. It has been reported that the ROS generation triggers the phosphorylation of p-38 and JNK which further leads to apoptosis of cells (130, 131). Interestingly, this activation was suppressed by 1,9-P treatment which provides the another evidence for neuroprotective action of 1,9-P.

The 6-OHDA and MPTP lesioned rat model is most commonly used animal model to induce the early phase of PD. Both neurotoxins have significant effect on dopaminergic neurons and leads to reduction of dopamine in SNC, but 6-OHDA shows more intense and wide cell loss with mortality than MPTP. The studies suggested that, the 1-methyl-4-phenylpyridinium cation (MPP⁺), a oxidative product of MPTP which inhibits the mitochondrial complex-I activity, leads to decrease the cellular ATP level and cell death (54). However, the 6-OHDA leads to neurotoxicity by generating the ROS (54, 132). The site of target for both neurotoxins is mitochondria where they disturb the mitochondrial membrane and subsequently leads to apoptosis of cells. In the current study both model of PD has been selected to find the exact mechanism of selected drug candidates.

Evaluation of anxiety or fear is one of the very important psychological parameter in Parkinson’s disease, because anxiety is directly proportional to dopamine level in basal ganglia (96). The 6-OHDA and MPTP treated groups showed an increase the number of open arm entry and percentage of time spent in the open arm. Whereas, the 1,9-P and Conessine treatment groups showed significant reduction in percentage preference to open arm after the 28 days treatment. But the combination of 1,9-P and Conessine testament showed the effectively increased the anxiety which suggests the combination of both drugs gives synergistic effect in increasing the turnover of dopamine in SNC in both models of PD.

Locomotor dysfunction is a main clinical symptom of PD which is linked to death of dopaminergic cells projecting from SN to striatum. The previous research showed that 6-OHDA and MPTP lesion in rat brain leads to depletion of DA which impairs the central motor function (95). Treatment with 1,9-P and Conessine alone and in combination improved the movement dysfunction significantly compared to 6-OHDA and MPTP lesioned group. Amongst the treatment groups the combination of 1,9-P and Conessine showed improved muscle co-ordination which suggests the combination of
both drug could reverse or protect the neurons from 6-OHDA and MPTP toxicity.

The 6-OHDA lesion depletes the dopamine in addition with noradrenaline and 5-hydroxydopamine. Since MPTP lesion selectively decrease the dopamine level which disturbs the nigrostriatal as well as in certain level the mesolimbic or mesocortical dopamine neurotransmission which causes memory impairment (133, 134). The evaluation of working memory in rats was evaluated on day 1, day 14 and day 28 after 6-OHDA and MPTP lesion in animals. The working memory represents the memory capacity to remembering the clues to escape out from water. The lesioned groups as well as treatment group did not show any changes on day 1. Whereas, after the treatment on day 14 and 28 the latency time to escape from water is still same in lesioned groups. But treatment groups showed significant reduction in latency time as time goes on. The combination of 1,9-P with Conessine group showed a significant improvement in working memory, which may resulted due to restoration of nigral dopaminergic neurons and prefrontal cortex region (135).

Dopamine neurotransmitter plays important role in body movement and motor control. The reduced level of catecholamines and oxidative stress leads neurodegeneration in PD and these causes to the loss of motor function in the patients with PD (136, 137). So it is very important to measure the striatal dopamine level. The striatal dopamine level in 6-OHDA and MPTP lesioned group largely depleted which supports by earlier studies demonstrating that 6-OHDA and MPTP produces ROS with oxidative stress which leads to neurodegeneration of dopaminergic cells. Whereas, the treatment groups showed significant improvement in striatal dopamine level compared to lesioned animals. The combination of 1,9-P with Conessine showed impressive results even better than standard L-Dopa treatment which is well supported by above results.

The oxidative damage along with ROS and aging leads to protein, lipid damage which participates in the pathological process of PD (138, 139). The estimation of total protein concentration was carried out by Lowry’s method to assess two factors one was to know the extent of protein degradation or catabolism that has taken place in the brain and the other is for estimation of DA present in the protein of brain homogenates. The level of protein concentration in 6-OHDA and MPTP lesioned rats decreased drastically as compare to sham operated control treatment group. Interestingly the 1,9-P
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and Conessine alone treatment does not shown significant effect, even though the L-Dopa did not shown more significant effect on protein concentration. Only the combination of 1,9-P with shown promising improvement in total protein concentration.

Oxidative damage has been undoubtedly identified as main reason of degeneration of dopaminergic cells in SNc which leads to PD in 6-OHDA as well as MPTP model (140). Eventhough, Dopamine itself produces the metabolites (quinines) which is prone to produce toxic oxyradicals by autoxidation and enzymatic oxidation (54). The superoxide dismutase (SOD) is main enzyme responsible for detoxification of ROS. GSH is the most important enzyme which contains thiol group occurs mainly in brain (141) and play crucial role in preventing oxidative damage. It has been also used as a biomarker for estimation of oxidative stress in biological system (142). The catalase (CAT) is responsible for clearance of H$_2$O$_2$ by converting it into water and reduces oxidative damage. The researchers demonstrated that the level of SOD, GSH and CAT depleted in PD patients and 6-OHDA and MPTP model (143). All the antioxidant defence mechanisms are related to each other disturbance in one might damage the balance of all (101). Maintenance of balance of antioxidant enzymes is very important to provide neuroprotective action. The 6-OHDA and MPTP lesioned animals showed significant reduction in all anti-oxidant enzyme levels which may be due to production of ROS. The treatment groups show the increase the antioxidant enzyme levels and decreased the oxidative stress this may be due to decrease in ROS and H$_2$O$_2$ production. The combination showed effective increase in antioxidant levels; this shows the combination of 1,9-P with Conessine might be more effective to retain the dopamine level by reducing the oxidative damage of dopaminergic neurons.

The estimation of mitochondrial complex-I activity is very important parameter to assess the mitochondrial integrity. Complex-I is inner part of mitochondrial membrane which is responsible for oxidative phosphorylation system (OXPHOS) which produces the ATP as end product (54). Attenuation of mitochondrial complex-I activity was demonstrated by researchers in PD patients as well as 6-OHDA and MPTP lesioned rats (144, 145). Defects in mitochondrial complex-I functions leads to decreased the production of ATP levels and impaired the proton pumping resulting in mitochondrial membrane impairment which is early stage of apoptosis (146). The electron acceptor compound ubiquinone was used for study by using specific complex-I inhibitor
rotenone to measure the rate of oxidation of NADH. The results showed that 6-OHDA and MPTP lesioned rats significantly decreased the complex-I activity compared to sham operated control. Whereas, the treatment groups showed reversal of 6-OHDA and MPTP altered complex-I activity. Combination of 1,9-P with Conessine showed significant improvement in complex-I activity which supports the earlier result to decrease the apoptosis of dopaminergic cells.

The pathogenic feature of Parkinson’s disease is the destruction of the pigmented substantia nigra, particularly the pars compacta (SNc). The cause of dopaminergic cell death in the SN is still unknown. Recent studies on the pathogenesis of PD have centred on the involvement of environmental and endogenous toxins. Oxidative stress and altered iron content are also thought to be important factors (147, 148). The iron content when increases in SNpc and neuromelanin resulted in generation of free radical generation which is responsible for cell apoptosis. Also, many reports demonstrated the evidence of abnormally increased concentration of iron within the substantia nigra of PD patients (149). The evaluation of iron content in substantia nigra by perl’s DAB staining showed the 6-OHDA and MPTP lesioned groups showed significant elevation of brain iron asymmetry ratio which occurs due to elevation of ROS and oxidative damage of cells due to 6-OHDA and MPTP. Interestingly standard treatment by L-Dopa does not showed any significant effect on brain iron asymmetry ratio. Whereas, the combination of 1,9-P with Conessine treatment group showed significant reduction of brain iron asymmetry ratio which shows the drug treatment inhibits the ROS production with oxidative damage.

The histopathology study performed on brain tissue sample also supports the above findings. The brain slices were stained with Haematoxylin and Eosin stains and the morphological changes in brain striatum were observed. Histological study demonstrated pathological changes in striatum and neuronal cells (150). The 6-OHDA and MPTP lesioned groups showed significant damage in striatum along with decreased the neuronal count. Whereas, the treatment groups with 1,9-P and Conessine shown reconstruction of striatum along with survival of neuronal cells. The combination of 1,9-P along with Conessine protected more neuronal cells which supports the all above finding of study.