Influence of Hesperidin combined with Sinemet on genetical and biochemical abnormalities in rats suffering from Parkinson's disease

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Abstract: Parkinson's disease (PD) is a progressive disabling neurodegenerative disorder characterized by severe difficulties with body motions and associated with autonomic dysfunction, depression, and dementia. Oxidative stress is thought to play an important role in the pathogenesis of PD and oxidative damage characterizes proteins, lipids, and DNA in the substantia nigra pars compacta (SNc) of PD patients. To date, L-dopa is the most effective medication for controlling PD symptoms, although long-term treatment can enhance oxidative stress and accelerate the degenerative process of residual cells. Thus, the inhibition of oxidation of L-dopa and the inhibition of reactive oxygen species formation are important strategies for neuro-protective therapy. Therefore, efforts are made not only to improve the effect of L-dopa treatment for PD, but also to investigate new drugs with both antioxidant and neuro-protective effects. Hespiridin (HDN), a naturally occurring flavonoid presents in fruits and vegetables, has been reported to exert a wide range of pharmacological effects including antioxidant, anti-hypercholesterolemic, anti-hyperglycemic, anti-inflammatory, anti-carcinogenic and neuro-protective actions. Chlorpyrifos (CPF) was used in this study as an animal model of PD. Model of CPF-induced Parkinsonism in rats produced neurotoxicity, oxidative stress, hyper-lipidemia, hyperglycemia and DNA damage. Seventy male rats were used in this study and divided into seven equal groups. After 6 weeks, the following groups were studied, control group, CPF group, HDN group, Sinemet group, CPF+HDN group, CPF+Sinemet group, and CPF+HDN+Sinemet group. Here in the present study, the treatment of parkinsonism with HDN alone or combined with sinemet provided a neuroprotection effect when given early in the course of the disease. In conclusion, HDN could be recommended as a disease-modifying therapy when given alone or mixed with L-dopa in course of Parkinson's disease.
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1.Introduction

Parkinson's disease (PD) is the second most common neurodegenerativedisease after Alzheimer's disease (AD). According to many epidemiological data, the number of individuals affected by PD in the most populous nations worldwide is expected to rise and will double within the next several decades ***(Dorsey et al., 2007)***. This development constitutes an enormous public health challenge for the future concerning about the medical care and treatment cost ***(Löhle and Reichmann, 2010)***.Pathologically, PD is a neurodegenerative disease characterized, in part, by the death of dopaminergic neurons (DAergic) in the substantia nigra pars compacta (SNc). Clinically, PD has traditionally been defined by the presence of cardinal motor signs such as tremor, rigidity, bradykinesia, akinesia and postural instability. Other symptoms include fatigue, sleep abnormalities, and depression ***(Jankovic, 2008)***. Prominent pathological manifestations associated with degeneration of SNpc-DAergic neurons include mitochondrial abnormalities ***(Dagda and Chu, 2009 ;Nishioka et al., 2010)***, excessive cytosolic dopamine (DA) oxidation, α-synuclein aggregates, autophagolysosome dysfunction, defects in the ubiquitin-proteasome system (UPS), oxidative stress, nitrosative stress, iron released from bound storage and a gradual loss of neuromelanin (NM) ***(Tofaris and Spillantini, 2005; Levy et al., 2009)***.

PD is associated with both genetic and non-genetic contributing factors, with aging as the most prominent risk factor ***(Thomas and Beal, 2007)***. PD brains exhibit a reduction in mitochondrial complex I activity, which is both the rate-limiting step for mitochondrial respiratory chain activity and an important site for generation of reactive oxygen species (ROS). Reduction of complex I activity may lead to accumulation of ROS, which can further induce mitochondrial permeability transition, ATP depletion, and damage of DNA, lipids and proteins ***(Keeney et al., 2006)***.

The oxidation hypothesis suggests that the free radicals damage, resulting from DA's oxidative metabolism through deamination by monoamine oxidase (MAO) enzyme, plays a role in the development or progression of PD. MAO activity

increases with the natural process of aging and can yield toxic products such as hydrogen peroxide, ammonia, and aldehydes ***(Bortolato et al., 2008;Naoi and Maruyama, 2010)***. Hydrogen peroxide normally is cleared rapidly by glutathione (GSH) but if it is not cleared adequately as in PD, it may lead to the formation of highly reactive hydroxyl radicals that can react with lipids of cell membrane to cause lipid peroxidation and cell damage. In PD, levels of glutathione are decreased, suggesting a greater oxidative damage ***(Bharath et al., 2002; Johnson et al., 2005)***.

Environmental factors have been shown to contribute to the incidence of PD by inducing mitochondrial dysfunction ***(Schneider and Zhang, 2010)***. Pesticides represent one of the primary classes of environmental agents that associated with PD ***(Hatcher et al., 2008)***. Organophosphate (OP) compounds are potent neurotoxic chemicals widely used in agriculture, industry ,households and even as chemical weapons ***(Moreno et al., 2008)***. Chlorpyrifos (CPF)[O,O-diethyl-*O*-(3,5,6-trichloro-2-pyridyl) phosphorothionate] is a crystalline broad spectrum organophosphate insecticide utilized extensively in agriculture and for residential pest control throughout the world under the registered trademarks LORSBAN Insecticide and DURSBAN Insecticide ***(Saulsbury et al., 2009)***. CPF has been reported to exert their primary neurotoxic effects by inhibiting the acetylcholinesterase (AChE), the enzyme responsible for degradation of the neurotransmitter, acetylcholine (ACh), during neurotransmission. It phosphorylates the serine residue at the active site of AChE and thus inhibiting it causing accumulation of acetylcholine at the cholinergic synapses ***(Costa, 2006)***.

In addition to cause neurotoxicity, OPs are also related to a variety of physiological abnormalities including immunotoxicity ***(Galloway and Handy, 2003)***, oxidative stress ***(Kamath et al., 2008;Wu et al., 2011)***, alterations in glucose homeostasis ***(Kamath and Rajini, 2007)*** and hyperglycemia ***(Joshi and Rajini, 2009)***. Also, some studies have shown that these compounds can also induce a disturbance in the lipid status, such as an increase of cholesterol and triglycerides levels that represents a risk factor for premature atherosclerosis ***(Çetin et al., 2010; Lasram et al., 2009)***. However, there is little information in literature concerning the mechanisms involved in hyperlipidemia induced by OPs.

To date, 3,4-dihydroxyphenyl-L-alanine (levodopa/L-dopa) is the most effective medication for controlling PD symptoms, particularly those related to bradykinesia ***(Nagatsua and Sawadab, 2009)***. However, the long-term treatment with L-dopa can generate motor fluctuations, i.e. wearing off and dyskinesia ***(Schapira, 2008)***, since L-dopa and its metabolite dopamine (DA) can enhance oxidative stress and accelerate the degenerative process of residual cell lines ***(Blessing et al., 2003;Hattoria et al., 2009)***. L-dopa is a natural dopamine precursor that can cross the blood–brain barrier (BBB) to reach the brain where it is converted into DA by peripheral decarboxylase and stored in vesicles in order to be progressively released onto postsynaptic receptors. Peripherally, L-dopa is rapidly metabolized to DA by the enzyme aromatic amino acid decarboxylase (AADC) ***(Hardie et al., 1986)***.

Concomitant administration of inhibitors of extra-cerebraldopa decarboxylase (IEDD) e.g. carbidopa (CD) which do not cross the blood–brain barrier, have permitted the peripheral conversion into DA to be blocked and allowed a fourfold reduction in the L-dopa dose requirement as its plasma elimination half-time has been shown to increase from 60 to 90 min ***(Leppert et al., 1988)***. The first marketed product containing a combination of levodopa and carbidopa was an immediate-release (IR) oral dosage form under the trade name of Sinemet and now it is the main treatment for PD ***(Goole and Amighi, 2009)***.

Efforts are made not only to improve the effect of L-dopa treatment for PD, but also to investigate new drugs with both antiparkinsonian and neuroprotective effects ***(Yuan et al., 2010)***. Nowadays, different natural supplements from plant origin are used as a complementary supplement in the treatment of many diseases by improving the efficacy of drug used or by minimizing the toxic side effect and so enhancing the state. Hesperidin (HDN), a flavonone glycoside, is an inexpensive and abundant byproduct of citrus cultivation (***Susana et al., 2008)***. HDN was reported to have many biological effects including anti-inflammatory, antimicrobial, anticarcinogenic, antioxidant effects and neuroprotective actions ***(Garg et al., 2001 ;Ebrahimi and Schluesener, 2012)***. It exhibits anti-oxidative properties by several different mechanisms, such as scavenging of free radicals, chelation of metal ions such as iron and copper which are of major importance for the initiation of radical reactions, inhibition of enzymes responsible for free radical generation and facilitation endogenous antioxidative defense system ***(Cai et al., 2006)***.

Hence the present study was aimed to investigate the protective efficacy of HDN as a complementary supplement combined with Sinemet to improve genotoxicity and biochemical abnormalities induced by CPF in the Swiss albino male rats.

2.Materials & Methods

2.1.Materials:

2.1.1.Chemicals

- Chlorpyrifos(CPF) was insecticide purchased from the Egyptian company for pesticides under the trade name "*Drusban"*.
- Hesperidin (HDN) was purchased from Sigma-Aldrich Company-Chemicals Private Ltd., India. It was suspended in distilled water and administered orally to rats.
- Sinemet was obtained as tablets manufactured by Global Napi Pharmaceuticals – Egypt under license from Merck & Corporate Inc., Whitehouse Station, New Jersey, USA. All other chemicals used in the experiment were of analytical grade.

2.1.2 Experimental animals

Seventy adult male Swiss albino rats weighing 150-200 g of the same age were used throughout this study. Animals were obtained from the animal house colony of National Research Center, Dokii, Giza, Egypt. Animals were maintained under standard conditions of ventilation, temperature (25±2°C), humidity (60-70%) and light/dark condition (12/12h). The rats were housed in stainless steel cages and provided with free access to drinking water and food *adlibitum*.

Rats were orally administered their respective doses by gavage every day throughout the study. The local committee approved the design of the experiments, and the protocol confirms the guidelines of the National Institute of Health (NIH).

After two weeks of acclimatization, animals were divided into 7 groups (n=10).

- ***Control group***; rats were fed normally for 6 weeks.
-***Chlorpyrifos group*** *(CPF)*; rats were orally administered with CPF (2mg/kg b.wt./day) for 6weeks.
-***Hesperidin group*** *(HDN)*; rats were orally administered with HDN (50mg/kg b.wt./day) according to the method of *Balakrishnan and Menon, (2007)* for 6weeks.
-***Sinemet group;*** rats were orally administered with sinemet (100mg/kg b.wt./day) according to *Lindner et al., (1996)* for 6weeks.
-***Chlorpyrifos plus hesperidin group*** *(CPF+HDN)*; rats were orally administered with CPF (2mg/kg b.wt./day) for 6weeks then orally administered with HDN (50mg/kg b.wt./day) for another 6 weeks. -***Chlorpyrifos plus sinemet group*** *(CPF+Sinemet)*; rats were orally administered with CPF (2mg/kg b.wt./day) for 6weeks then administered with sinemet (100mg/kg b.wt./day) for another 6 weeks.
-***Chlorpyrifos plus hesperidin and sinemet group*** *(CPF+HDN+Sinemet)*; rats were orally administered with CPF (2mg/kg b.wt./day) for 6weeks then administered with HDN (50mg/kg b.wt./day) mixed with sinemet (100mg/kg b.wt./day) for another 6 weeks.

2.2. Methods:

2.2.1.Toxicity studies

The procedure for the determination of LD_{100} and LD_{50} of tested CPF compound was carried out according to ***Reed and Muench, (1938)***.

A total of 60 adult male Swiss Albino rats were used to determine the LD_{100} and LD_{50} of CPF. The rats were divided equally into 6 groups representing doses from 0.25 to 8mg of CPF/ kg b.wt./day for 6weeks with an increasing factor of 2. All animals were administrated by oral gavage with CPF at different doses. Mortality was recorded after 24 hours, and the LD_{50} was calculated as follows:

Log LD_{50}= log LD next below 50% + (log increasing factor x proportionate distance).

Proportionatedistance=

$$\frac{50\% - \%\ \text{mortality next below } 50\%}{\%\ \text{mortality above } 50\% - \%\ \text{mortality below } 50\%}$$

2.2.1.Biochemical analysis

At the end of the experiment, animals were anaesthetized and sacrificed by cervical dislocation after 24 hours fasting period from the final administration. Blood samples were collected in heparinized tubes and centrifuged at 5000 rpm for 10 min to separate plasma samples. Plasma was separated by aspiration, transferred into micro-centrifuge/eppendorf tubes and stored at -80ºC for evaluating of oxidative stress, lipid levels, and glucose. The brain was removed and cleared off blood and transferred immediately to ice cold container containing a sterile 0.9% NaCl and stored at -80ºC for the cholinesterase estimation and comet assay. Bone marrow samples were collected on slides by flushing at least one femur per animal with fetal calf serum for micronucleus assay:

a-Blood analysis

a.1.Estimation of lipid peroxidation:

The quantitative measurement of lipid peroxidation in plasma was performed according to method of ***(Ohkawa et al., 1979)***. The malondialdyhyde (MDA) content, a measure of lipid peroxidation, was assayed in the form of thiobarbituric acid reacting substances (TBARS).

a.2.Estimation of antioxidant enzymes:

The activity of reduced glutathione (GSH) was assayed in the plasma according to the method of ***(Beutler et al., 1963)***. Total (Cu–Zn and Mn) superoxide dismutase (SOD; EC 1.15.1.1) was assayed according to the previous described method ***(Nishikimi et al., 1972)***. Catalase enzyme (CAT; EC 1.11.1.6) was measured according to the method of ***(Aebi, 1984)***. Finally, Glutathione S-transferase (GST; EC 2.5.1.18) was measured according to the method of ***(Habig et al., 1974)***.

a.3.Estimation of total cholesterol, triglycerides, and glucose:

Total cholesterol (TC) levels were assayed in the plasma according to the method of ***(Richmond, 1973)***. Triglycerides (TG) levels were determined by enzymatic colorimetric methods according to the previous described method ***(Fassati & Prencipe, 1982)***. Finally, plasma glucose levels were determined by an enzymatic method based on the oxidase/peroxidase system according to the method of ***(Trinder, 1969)***.

b-Brain tissue analysis

b.1.Estimation of acetyl cholinesterase (AchE):

On the day of biochemical estimation, the entire brain was minced and homogenized with (10%w/v) cold phosphate-buffered saline (pH 7.4) by using a glass homogenizer. The homogenates were centrifuged at 5000 rpm for 5 min at 4° C to separate the nuclear debris. AchE (AChE; EC 3.1.1.7) was assayed by the method of ***(Henry, 1974)***.

b.2.Cytogenetic studies:

b.2.1.Bone marrow micronucleus (MN) assay:

To study micronucleus assay, rats were sacrificed 24 h after treatment. Immediately after the animals were sacrificed, both femurs of the rat were removed and freed from the extra muscles. The epiphyses were cut and the bone marrow was flushed out by gentle flushing and aspiration with fetal calf serum ***(Valette et al., 2002)***.The cell suspension was centrifuged at 1200 rpm for 10 min and the supernatant was discarded. A small drop of the re-suspended cell pellet was spread on clean glass slides and left till air-dried. The bone marrow smears were made in five replicates and fixed in absolute methanol for 10 minutes and stained with Giemsa at pH 6.8 ***(D'Souza et al., 2002)***.The number of micro-nucleated polychromatic erythrocytes (MNPCEs) and the percentage of micro-nucleated cells were scored from the smeared bone marrow slides. The micronucleus frequencies (expressed as percent micro-nucleated cells) were determined by analyzing the number of MNPCEs from at least 3000 polychromatic erythrocytes (PCEs) per animal.

b.2.2.Determination of DNA damage (Comet assay):

The comet assay was performed according to reagent Kit for Single Cell Gel Electrophoresis Assay ***(Angelis et al., 1999)***. A small piece of brain tissue was placed into 1–2 ml of ice cold 1X PBS and20 mM EDTA. Cell suspension was combined with low melting-point agarose (at 37°C) at a ratio of 1:10 (v/v) and immediately 50 µl was pipetted onto Comet Slide. Slides were placed flat at 4°C in the dark for 10 minutes. Slides were immersed in prechilledLysis Solution (2.5 M NaCl, 100 mM EDTA, 10 mMTris, and 1% Triton X-100, pH 10) for 1 h at 4°C in the dark. After lysis, the slides were transferred to a horizontal electrophoresis tank containing freshly prepared pre-cooled (4°C) alkaline electrophoresis buffer pH>13 (200 mMNaOH, 1 mM EDTA) and leaved for 20 min at 4°C in the dark for DNA unwinding. After DNA unwinding process, electrophoresis was carried out in the same buffer for 30min at 0.7V/Cm (20–30 cm between electrodes) at 4˚ C. Excess electrophoresis solution was gently drained and slides were immersed twice in dH_2O for 5 minutes each, then in 70% ethanol for 5 minutes. Samples were dried at ≤ 45°C for 10-15 minutes. 100 µl of diluted SYBR Green I was placed onto each circle of dried agarose and placed in refrigerator for 5minutes. After staining, the slides were dipped in chilled distilled water to remove excess stain and subsequently the covers lips were placed over the slides.The slides were examined under a fluorescent microscope. The length of DNA migration, image length, and DNA damage parameters were calculated.

3.Results

3.1. Single dose acute oral toxicity study of CPF

Table (1):Acute lethal dose (LD_{100}) and median lethal dose (LD_{50}) of CPF in rats

Dose (mg/kg b. wt.)	Number of animals	Survivals (S)	Deaths (D)	Mortality %
0.25	10	10	-	-
0.5	10	8	2	20%
1	10	6	4	40%
2	10	5	5	50%
3	10	3	7	70%
8	10	-	10	100%

Following administration of a single oral gavage dose of CPF, the rats were monitored daily for mortality. The results presented in Table (1) revealed that, the acute lethal dose (LD_{100}) was 8mg/kg body weight, while the median lethal dose (LD_{50}) was 2mg/kg body weight for CPF after oral administration in Swiss Albino rats.

3.2.Clinical signs:

No signs of toxicity were observed in the control, HDN, Sinemet groups. Toxic signs recorded in the CPF group include tremor, weakness, bradykinesia and death in animals Table (2). Animals in the CPF+HDN and CPF+Sinemet groups manifested mild tremor, improvement in the motor coordination and less number in death.

Table (2): Effect of different treatment on the clinical signs in male rats through 6weeks.

Treatment		Week1	Week2	Week3	Week4	Week5	Week6	Total of death	Total number of rats
Control	Clinical signs	-	-	-	-	-	-	0	10
	Number of death	-	-	-	-	-	-		
CPF	*Clinical signs*	*Slow motion*	*Slow motion & Tremor*			*Slow motion & excessive tremor*		6	10
	Number of death	1	-	1	1	1	2		
HDN	*Clinical signs*	-	-	-	-	-	-	0	10
	Number of death	-	-	-	-	-	-		
Sinemet	Clinical signs	-	-	-	-	-	-	0	10
	Number of death	-	-	-	-	-	-		
CPF+HDN	*Clinical signs*	*Slow motion & excessive tremor*	*Mild tremor*			-	-	1	10
	Number of death	1	-	-	-	-	-		
CPF+Sinemet	*Clinical signs*	*Slow motion & excessive tremor*	*Mild tremor*					2	10
	Number of death	-	-	1	1	-	-		
CPF+HDN+ Sinemet	*Clinical signs*	*Slow motion & excessive tremor*	*Mild tremor*				-	1	10
	Number of death	-	1	-	-	-	-		

3.3. Biochemical parameters

Concerning with the drugs used in this study, HDN treated group did not show statistically significant changes in MDA, GSH, TC, TG and glucose levels and SOD, CAT, GST and AchE activities compared to control group. Also, sinemet group showed no significant changes in SOD, CAT activities and TC levels compared to control group, however, GSH, MDA, GST, TG, glucose and AchE showed less significant changes (P<0.05) (Figures 1–9).

3.3.1. Malondialdehyde (MDA) levels:

The data summarized in Figure (1) indicated that, MDA levels were significantly increased (p<0.05) in rats received CPF alone or in combination with HDN and/or sinemet (CPF+HDN, CPF+Sinemet and CPF+HDN+Sinemet groups) when compared to control group. However, the same parameter of the latter three groups was significantly decreased (p<0.05) when compared to CPF treated group. Also, the positive effect of HDN was detected in CPF+HDN and CPF+HDN+Sinemet groups by decreasing in MDA level while, the sinemet did not show the same effect in CPF+Sinemet group comparing to control.

3.3.2.. Superoxide dismutase (SOD) activity:

Figure (2) showed a significant increase ($p<0.05$) in SOD activity in groups treated with CPF, CPF+HDN, CPF+Sinemet and CPF+HDN+Sinemet. Also, the figure clarified the alleviation effect of HDN which exceeded the positive effect of sinemet. On the other hand, SOD activity was significantly sharp decreased in CPF+HDN, CPF+Sinemet and CPF+HDN+Sinemet treated groups compared with CPF-treated group. In addition, Figure (2) demonstrated the positive effect of HDN in CPF+HDN and CPF+HDN+Sinemet groups as detected by the significant decreasing (p<0.05) activity of SOD.

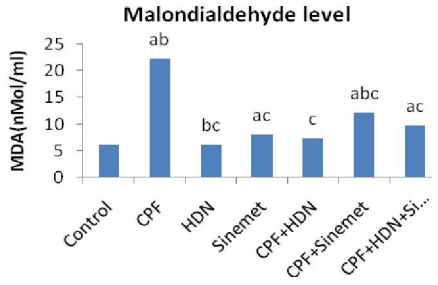

Figure (1): Levels of MDA in the plasma of male albino rats in control and experimental groups. Data are represented as means ± SD; n=10 for each group Significance at p< 0.05. [a] Significant difference from control and other groups.[b] Significant difference from sinemet group and other groups. [c] Significant difference from chlorpyrifos group and other groups. CPF: chlorpyrifos; HDN: hesperidin.

3.3.3. Catalase (CAT) activity:

The present study revealed that, CAT activity was significantly decreased (p<0.05) in rats received CPF alone or combined with sinemet and/or HDN when compared with the control group, while the same parameter was significantly increase (p<0.05) in the mixed groups (CPF+HDN, CPF+Sinemet and CPF+HDN+Sinemet) compared to CPF group as shown in Figure (3).

3.3.4. Glutathione-S-transferase (GST) activity:

Figure (4) demonstrated the positive effect of HDN in all groups containing it either alone or mixed with CPF and/or sinemet as detected by the significant increase (p<0.05) of GST activity compared to CPF-treated group. On the other hand, a significant decrease (p<0.05) was detected in the

activity of the same enzyme in CPF and Sinemet groups compared to the control group.

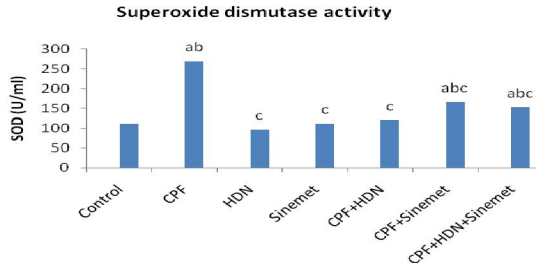

Figure (2): SOD activityin the plasma of male albino ratsin control and experimental groups. Data are represented as means ± SD; n=10 for each group;
Significance at p< 0.05. [a]Significant difference from control and other groups.[b]Significant difference from sinemet group and other groups. [c]Significant difference fromchlorpyrifos group and other groups.
CPF: chlorpyrifos; HDN: hesperidin.

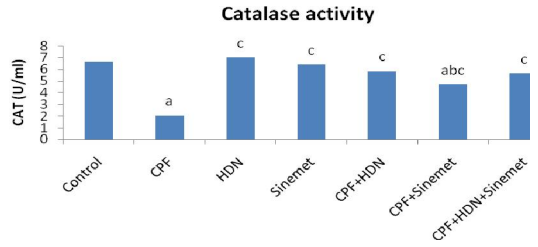

Figure (3): CAT activity in the plasma of male albino rats in control and experimental groups. Data are represented as means ± SD; n=10 for each group;
Significance at p< 0.05. [a]Significant difference from control and other groups.[b]Significant difference from sinemet group and other groups. [c]Significant difference fromchlorpyrifos group and other groups.
CPF: chlorpyrifos; HDN: hesperidin.

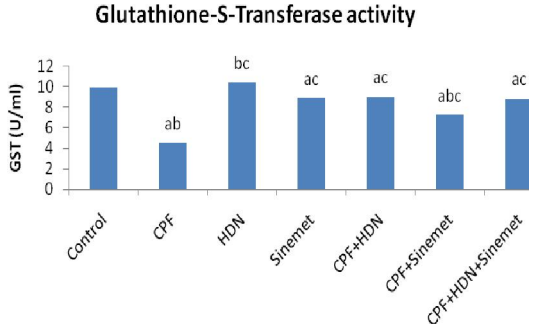

Figure (4): GST activity in the plasma of male albino rats in control and experimental groups. Data are represented as means ± SD; n=10 for each group;
Significance at p< 0.05. [a]Significant difference from control and other groups.[b]Significant difference from sinemet group and other groups. [c]Significant difference from chlorpyrifos group and other groups.
CPF: chlorpyrifos; HDN: hesperidin.

3.3.5. Reduced glutathione (GSH) levels:

Figure (5) indicated that, CPF treatment led to a significant decrease (p<0.05) in GSH levels, while HDN showed significant recovery of this parameter (p<0.05) by enhancing its concentration in plasma. Treatment with HDN alone or mixed with CPF and/or Sinemet significantly alleviated the undesirable decreased levels of GSH comparing with CPF treated animals. It was also obvious that, the effect of HDN surpassed the traditional effect of sinemet.

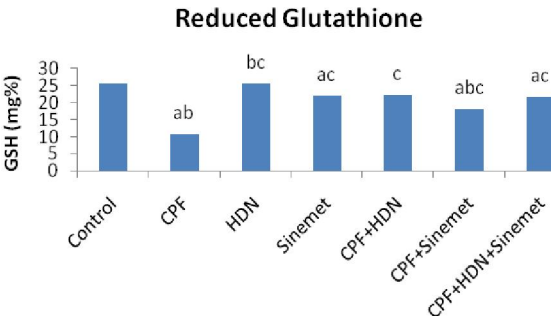

Figure (5): Levels of GSH in the plasma of male albino rats in control and experimental groups. Data are represented as means ± SD; n=10 for each group;
Significance at p< 0.05.
[a]Significant difference from control and other groups.[b]Significant difference from sinemet group and other groups. [c]Significant difference from chlorpyrifos group and other groups.
CPF: chlorpyrifos; HDN: hesperidin.

3.3.6. Total cholesterol (TC) levels:

The data summarized in Figure (6) indicated that, TC levels were significantly increased (p<0.05) in rats received CPF alone or in combination with HDN and/or sinemet (CPF+HDN, CPF+Sinemet and CPF+HDN+Sinemet groups) when compared to control group. However, the same parameter of the latter three groups was significantly decreased (p<0.05) when compared to CPF treated group. Also, the positive effect of HDN was detected in CPF+HDN and CPF+HDN+Sinemet groups by decreasing in TC levels while, the sinemet showed the same effect in CPF+Sinemet group with less degree comparing to control.

3.3.7. Triglycerides (TG) levels:

Figure (7) demonstrated the positive effect of HDN in all groups containing it either alone or mixed with CPF and/or sinemet as detected by the significant decrease (p<0.05) of plasma TG levels compared to CPF-treated group. On the other hand, a significant increase (p<0.05) was detected in the TG level in CPF and Sinemet groups compared to the control group

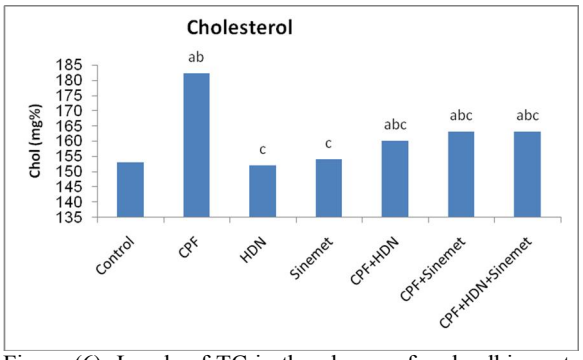

Figure (6): Levels of TC in the plasma of male albino rats in control and experimental groups. Data are represented as means ± SD; n=10 for each group;
Significance at p< 0.05.
[a]Significant difference from control and other groups.[b]Significant difference from sinemet group and other groups. [c]Significant difference from chlorpyrifos group and other groups.
CPF: chlorpyrifos; HDN: hesperidin.

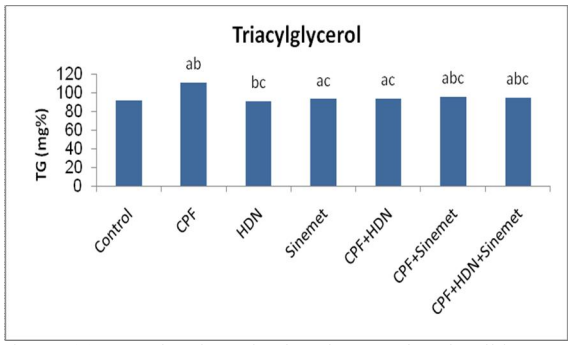

Figure (7): Levels of TG in the plasma of male albino rats in control and experimental groups. Data are represented as means ± SD; n=10 for each group;
Significance at p< 0.05. [a]Significant difference from control and other groups. [b]Significant difference from sinemet group and other groups. [c]Significant difference from chlorpyrifos group and other groups.
CPF: chlorpyrifos; HDN: hesperidin.

3.3.8. Glucose levels:

The present study revealed that, glucose level was significantly increased (p<0.05) in rats received CPF alone or combined with sinemet and/or HDN when compared with the control group, while the same parameter was significantly decrease (p<0.05) in the mixed groups (CPF+HDN, CPF+ Sinemet and CPF+HDN+Sinemet) compared to CPF group as shown in Figure (8).

3.4.Brain tissue

3.4.1.Estimation of acetyl cholinesterase (AchE):

The activity of AchEin brain tissues was highly significantly decreased in all CPF groups compared to the control group, while a significant increase (P < 0.05) was detected in all sinemt groups compared with CPF-treated group Figure (9).Simultaneous treatment with HDN (HDN, CPF+HDN and CPF+HDN+sinemt groups) significantly abolished the inductive effect of CPF on AchE activity, this process expressed asenhancing the level of the enzyme in brain tissue (p<0.05).

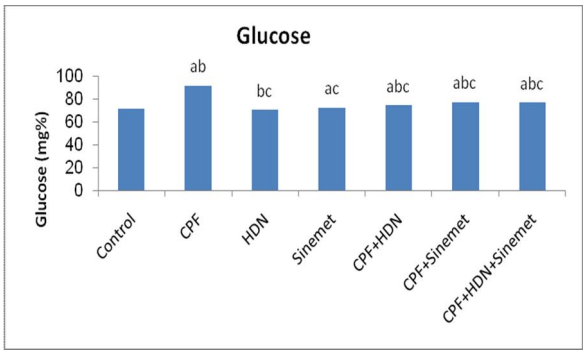

Figure (8): Levels of glucose in the plasma of male albino rats in control and experimental groups. Data are represented as means ± SD; n=10 for each group;
Significance at p< 0.05. [a]Significant difference from control and other groups.[b]Significant difference from sinemet group and other groups. [c]Significant difference from chlorpyrifos group and other groups.
CPF: chlorpyrifos; HDN: hesperidin.

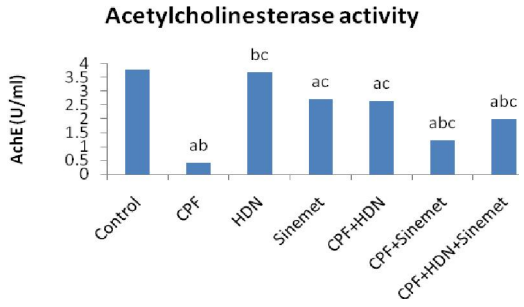

Figure (9): AchE activity in the brain tissue of male albino rats in control and experimental groups.Data are represented as means ± SD; n=10 for each group;
Significance at p< 0.05. [a]Significant difference from control and other groups.[b]Significant difference from sinemet group and other groups. [c]Significant difference from chlorpyrifos group and other groups.
CPF: chlorpyrifos; HDN: hesperidin.

3.4.2.Cytogenetic studies:

3.4.2.-Bone marrow micronucleus (MN) assay:

At the end of the treatment, there were no statistically significant changes in number of MNPCEs in HDN treated group compared with the control group. While, the comparison between the sinemet treated group and control group showed less significant changes in these parameters. On the other hand, CPF-treated group showed a sharp significant increase in the number of MNPCEs, indicating the occurrence of chromosome damages, compared with the control group Table (3).

Table(3): Results of bone marrow micronucleus assay in control and experimental groups

Groups	Number of MNPCEs per 3000 PCEs
Control	(11±2.5)
CPF	(115±20)[ab]
HDN	(10±2)[c]
Sinemet	(14±2.6)[c]
CPF+HDN	(23±3.4)[abc]
CPF+Sinemet	(46±8.4)[abc]
CPF+HDN+Sinemet	(30±4.3)[abc]

Data are represented as means ± SD; n=10 for each group; Significance at $p< 0.05$.
[a]Significant difference from control and other groups.[b] Significant difference from sinemet group and other groups. [c] Significant difference from chlorpyrifos group and other groups.
CPF: chlorpyrifos; HDN: hesperidin; MNPCEs: micronucleated polychromatic erythrocytes

3.4.3.Determination of DNA damage (Comet assay):

Compared to the control group, there was a significant increase in the frequency (percentage) of MNPCEs in CPF-treated groups indicating the occurrence of chromosome damages, while comparing to CPF treated group, there was a significant decreased in the number of MNPCEs in all HDN and sinemet groups even those treated with CPF but the decrease in the CPF+HDN group was significantly sharp supporting that HDN was more effecting in treatment than sinemet.

In cytogenetics study, the DNA damage was assigned in the different groups by the comet assay. Following single-cell electrophoresis, the lengths of the comets (DNA tails) relied on the treatment, where longer tails indicating more DNA damage. Untreated control group and HDN group showed undamaged cell, most of the DNA is located in the head of the comet and no tails are evident,(Fig (10A) and (10B), respectively), which means no DNA damage is observed, while Fig (10C) illustrated the presence of less DNA in the tail (small tail length) in Sinemet.

On the other hand, Fig (10D) showed apoptotic cell, the nucleotide core is not distinguishable and comet tail was sharply longer after treatment of rats with CPF. For mixed groups, Fig (10E) showed a less significant damage of DNA in CPF+HDN treated groups, indicating the positive recovery impact of HDN to counteract the damage effect of CPF. Comet tails were substantially longer in CPF+Sinemet and CPF+HDN+Sinemet treated groups as shown in Fig (10F) and (10G), respectively. These results reflect the less effect of sinemet in ameliorating of the DNA damage.

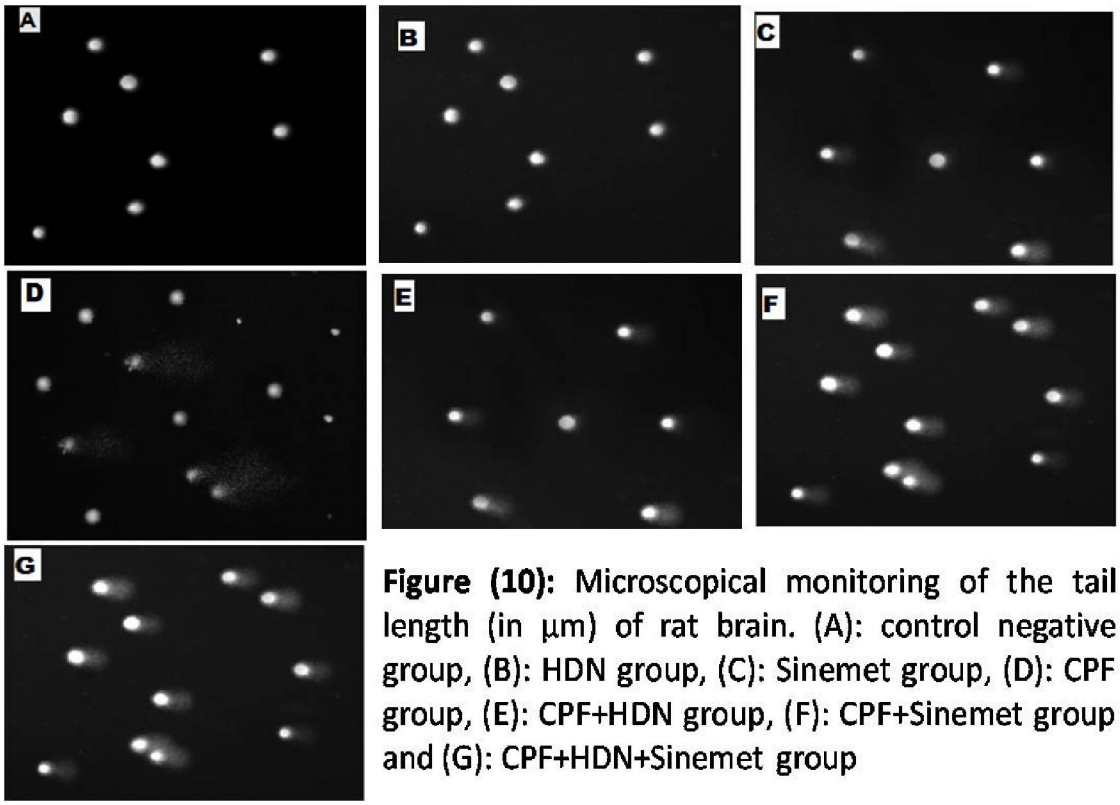

Figure (10): Microscopical monitoring of the tail length (in µm) of rat brain. (A): control negative group, (B): HDN group, (C): Sinemet group, (D): CPF group, (E): CPF+HDN group, (F): CPF+Sinemet group and (G): CPF+HDN+Sinemet group

4. Disscusion

The widespread use of pesticides and herbicides in the environment is of increasing concern because of their effects on the human health, wildlife, and ecosystems. Organophosphate (OP) pesticides are among the leading chemicals widely used for agricultural pest control throughout the world ***(Mehta et al., 2009)***.

Numerous experimental, epidemiological and clinical data showed the connection between both acute and chronic exposure to OP pesticides and damage to the nervous system ***(Lasram et al., 2009;SekarBabu et al., 2010)***. The neuro-toxic action of these compounds has been mainly explained by their ability to inhibit the activities of Ach E which is responsible for hydrolysis of acetylcholine playing a principal role in the transmission of neural impulses in the central and peripheral nervous system ***(Lotti, 2001)***.

An important causative role in the development of diseases of the nervous system has been played by the oxidative /antioxidative imbalance and disorders in lipid metabolism in the nervous tissue, as well as, a decrease in the number of the nervous cells ***(Oliveira and Di Paolo, 2010; Ziv and Melamed, 2010)***.

OP compounds have been reported to cause oxidative stress with consequent lipid per-oxidation and to induce apoptosis and necrosis in the brain under acute and chronic exposure ***(Üner et al., 2006; El-Demerdash, 2011)***. Moreover, it has been reported that OP pesticides can cross the blood–brain barrier (BBB) and cause a loss of neurons in Particular regions of the brain that results in subsequent cognitive decline, impaired memory and attention, and motor function ***(Parran et al., 2005)***. These neuro-behavioral disturbances may eventually lead to Alzhimer's disease, PD and other neuro-degenerative diseases ***(Kamel et al., 2007; Li et al., 2011)***.Epidemiologic studies reported that occupational exposures to OP insecticides are highly associated with increased risk of PD ***(Landrigan et al., 2005;Hancock et al., 2008)***.

One such OP which has spurred renewed interest is chlorpyrifos (CPF). CPF is an organophosphate insecticide known to produce oxidative stress in different human and animal tissues ***(Zama et al., 2007;Uzun et al., 2010)***. In previous studies, it is reported that CPF causes oxidative damage and histopathological changes in various tissues such as brain ***(Giordano et al., 2007)***. Due to the fact that the OPs; particularly CPF, causes oxidative damage in the central nervous system, CPF was used in this current study to be injected in male rats which were used as CPF model of Parkinson's disease. Previous studies showed that exposure of male rats to OP pesticides induced oxidative stress, hyperglycemia, Hypercholesterolemia, hyper-triglyceridemia DNA damage and neurotoxicity in various rat tissues ***(Aly et al., 2010; Acker & Nogueira, 2012).***

Reactive oxygen species (ROS) and its metabolites is the subject of intense research because of their active role in cellular physiology and pathogenesis of number of diseases. In healthy organisms, ROS production is counter balanced by antioxidant defense system to maintain an appropriate redox balance ***(Kruidenier and Verspaget, 2002)***. Organisms possess defense systems to escape the consequences of cell damage caused by ROS including enzymes such as catalase and superoxide dismutase and low molecular compounds, e.g., glutathione. Oxidative stress results when the balance between antioxidant systems and ROS is lost ***(Rao and Chhunchha, 2010)***.

Lipid per-oxidation is the process of oxidative degradation of polyunsaturated fatty acids and its occurrence in biological membranes causes impaired membrane fluidity and inactivation of a several membrane bound enzymes ***(Goel et al., 2005)***. MDA is one of the major oxidation products of peroxidized polyunsaturated fatty acids, and thus increased MDA content is an important indicator of lipid per-oxidation ***(Demir et al., 2011)***. In this study, MDA levels were increased in the CPF treated groups. So, in agreement with ***Gultekin et al., (2000)***, it might be due to potent lipid per-oxidation effects of CPF.

SOD and CAT are the most important defense mechanisms against toxic effects of ROS. SOD catalyzes the conversion of superoxide radicals to hydrogen peroxide and molecular oxygen, also termed as a primary defence, as it prevents further generation of free radicals. CAT converts hydrogen peroxide into water and molecular oxygen, and hence catalyzes its removal ***(Schneider and de Oliveira, 2004)***. It has been reported that the increased activity of SOD is known to serve as protective responses to eliminate reactive free radicals ***(Celik and Suzek, 2009)***. Other studies reported that increased SOD activity may induce cell death through the accumulation of hydrogen peroxide ***(Saggu et al., 1989)***. The present study demonstrated that CPF intoxication induces an elevation in SOD activity as well as a reduction in CAT activity. Similarly, in the previous studies, it has been reported that SOD activity increased in rat tissues by OP pesticides exposure ***(Sarabia et al., 2009)***. ***(Tuzmen et al., 2007; Shittu et al., 2012)*** showed that chronic administration of CPF causes the decrease in pituitary gland and testicular CAT activities. The decline in CAT activity, observed in this study, could be due to the excess production of superoxide radicals ***(Eraslan et al., 2007)*** since superoxide radicals converts feroxy state of CAT to ferryl state, which is an inactive form of the CAT ***(Freeman and Crapo, 1982)***. The increase in SOD activity and decrease in CAT

activity could have been related to increased oxidative stress caused by CPF exposure.

GSH has been reported to act as free radical scavenger and also to replenish intracellular stores of endogenous antioxidants, or as thiol-reducing agents ***(Valko et al., 2007)***. During the metabolic action of GSH, its sulfhydryl group becomes oxidized resulting with the formation of the corresponding disulfide compound, GSSG (oxidized form) ***(Meister and Anderson, 1983)***. In the current study, a significant depletion of GSH was noted in the CPF treated group. The decrease in GSH level could be due to the presence of free radicals produced by CPF. These effects have been previously observed *in vitro* and *in vivo* studies ***(Maran et al., 2009)***. ***Verma et al., (2007)*** proved that rats exposed to CPF exhibited reductions in GSH levels and increases in lipid peroxidation in the brains and livers. Glutathione-S-transferases (GST) are detoxifying enzymes that catalyze the conjugation of a variety of electrophilic substrates to thiol group of GSH, producing less toxic forms ***(Mansour and Mossa, 2009)***. OP pesticides have been reported to significantly inhibit GST activity in various rat tissues ***(Khan and Kour, 2007)***. The results of the present study show a significant decrease in GST activity in CPF group which is in line with previous reported studies (***Kalender et al., 2012)***. The decrease in GST activity can be seen as a result of decreasing levels of GSH following CPF exposure ***(Rai and Sharma, 2007)***. In the present study, the decreased GST activities and GSH level might reflect cellular oxidative stress due to CPF exposure.

In the current study, we reported the hyperglycemic and hyperlipidemic effects of CPF after an administration in rats. Evidence has been found to suggest hyperglycemia as a characteristic outcome of CPF poisoning ***(Rahimi and Abdollahi, 2007)***. Hyperglycemia in experimental animals following acute exposure to OPs appears to be rapid in onset and transient in nature ***(Lasram et al., 2008;Joshi and Rajini, 2012)***. Hypercholesterolemic and hypertriglyceridemic effects indicate the toxicity of CPF. Accordingly, (***Acker & Nogueira, 2012)*** demonstrated that a single acute CPF administration in rats caused hyperglycemic and hyperlipidemic effects in rats.This increased level of cholesterol is attributed to the increased activity of 3-hydroxy-3-methyl-glutaryl CoA reductase (HMG-CoA reductase) and increased incorporation of labelled acetate into cholesterol ***(Brunzell et al., 1983)***.

Neurotoxicity of OP compounds has been mainly explained by accumulation of acetylcholine (Ach), due to the inhibition of AChE activities, that produces cholinergic effects ***(Celik and Isik, 2009)***. AChE activity is known as biomarker of chronic toxicity in human ***(Singh et al., 2011)*** and experimental animals ***(Dwivedi and Flora, 2011)*** following pesticide exposure. The present study reported a significant decrease in brain AChE activity in the CPF group as compared to control. This result is in agreement with the previous studies that reported the inhibition of AChE activity in animals intoxicated with OP insecticides ***(Jintana et al., 2009; Catano et al., 2008)***. ***Darvesh et al., (2003)*** reported that the decreased AChE might be referred to the increase in lipid per-oxidation inducing deformities of cellular membranes, and disturbing metabolic and nervous activity.

CPF-exposed animals were investigated in the current study for genotoxic effects using the comet assay and micronucleus assay. CPF-induced DNA damage was observed in brain tissues, as evidenced by a significant increase in the Comet tail length (µm) in the exposed rats, which is consistent with other reports (***Shadnia et al., 2005;Atherton et al., 2009)***. In this context, a positive correlation was drawn between ROS generation and tail length in the exposed animals of this study. As shown the increase in the comet tail length due to DNA damage in CPF intoxicated rats may be due to the increase in the oxidative stress. ***Bagchi et al., (1995)*** examined that *in vitro* and*in vivo* administration of CPFresulted in two fold increases in DNA single strand breaks which may be due to the generation of ROS. CPF is known to inhibit P_{450} enzyme system and results in free radical production, which causes DNA damage ***(Dinsdale and Verschoyle, 2001)***. The current result was in agreement with ***Ding et al., (2012)*** who reported that the exposure of children to OP was associated with the increased generation of 8-hydroxydeoxyguanosine (8-OHdG), suggesting that exposure to OP may play an important role in DNA damage in children. In the present study, the results of CPF proved its toxic effect by causing of several changes like brain DNA damage, neurotoxicity, oxidative stress, hyperlipidemia, and hyperglycemia.

In the present study, some clinical signs were observed in the CPF group. The deficit in the motor coordination and appearance of tremor were occured in the rats of CPF group as signs of toxicity. Also, a number of rats were dead by administration of this insecticide. This finding is similar to those recorded by ***Ambali et al., (2010)***. Recently, these results were confirmed by ***Ambali and Aliyu, (2012)***. This may be due to damage to the portion of the brain responsible for motor coordination, probably due to oxidative stress as exemplified by increased oxidative stress, hyperlipidemia, hyperglycemia and DNA damage in the group. Also, the locomotor deficits recorded in the CPF group may be partly due to either oxidative damage to the muscle or impairment of neuronal transmission as a result of paralysis of the neuromuscular junction apparently resulting from prolonged AChE inhibition, leading to accumulation of

ACh in the cholinergic receptors in the peripheral and central nervous system. The accumulation of ACh in the cholinergic receptors leads to muscarinic, nicotinic and central nervous cholinergic syndromes ***(Eaton et al., 2008)***.

Although L-dopa remains as the gold standard replacement symptomatic therapy, it could contribute to bad prognosis of PD. A number of papers have suggested that L-dopa promotes neurodegeneration and accelerates progression of PD by way of oxidative metabolism ***(Du et al., 2009; Muller et al., 2004)***. In the present study, sinemet treated group showed a less significant increase in DNA damage with slightly increase in the lipid peroxidation and decrease in the levels of GSH, GST and AchE, compared to control group. It also showed a less significant increase in the levels of glucose and TG with slightly increase in TC levels. Numerous studies reported exogenous L-dopa could contribute to neurotoxicity via either oxidative stress as it has the potential to auto-oxidize to quinone and semiquinone, generating ROS and depleting striatal GSH ***(Hattoria et al., 2009)***, or by induction of apoptosis, as evidenced by accelerated activity of caspase-3 and DNA damage by a mechanism independent of oxidative stress ***(Maharaj et al., 2005; Pedrosa and Soaresda-Silva, 2002)***. The quinone products of L-Dopa auto-oxidation could also bind to and deplete GSH levels, further reducing antioxidant defenses available to dopamine neurons. But, there were no changes in the activities of SOD and CAT in sinemet treated group compared to control group. Moreover, L-Dopa treatment showed a hyperglycemic effect in the plasma of fasted wister rats ***(Furman and Wilson, 1979)***. Also, in this study, the treatment of CPF-intoxicated rats with sinemet showed a less significant amelioration in the antioxidant activities, MDA and GSH levels compared to the CPF treated group.

Dopaminergic neurons oxidize dopamine by monoamine oxidase, a reaction known to cause production of superoxide and hydrogen peroxide. Consequently, dopaminergic neurons are in a perpetual state of oxidative stress, and this imbalance may lead to reduced levels of endogenous antioxidants. The brain is only 2–3% of the total body mass, but it consumes 20% of body oxygen. Cells in the brain are particularly susceptible to oxidative damage due to high levels of polyunsaturated fatty acids in their membranes and relatively low activity of endogenous antioxidant enzymes ***(Miller et al., 2009)***. Recent studies demonstrated that adding L-dopa to the mesencephalic cultures showed a decrease in the number of viable cells, reduction in neurite length, DNA damage, and distinct signs of oxidative stress ***(Mosharov et al., 2009;Sabens et al., 2010)***. L-dopa toxicity was reduced in mesencephalic cultures obtained from parkin knockout mice that have increased content of glutathione ***(Casarejos et al., 2005)***. A reduction in the number of DAergic neurons was reported after direct intranigral infusion of L-dopa in rats ***(Reksidler et al., 2009)***. Brain infusion or *i.p.* injection of the drug increased the levels of hydroxyl radical ($^{\cdot}OH$) and nitric oxide (NO) in substantianigra and the striatum ***(Itokawa et al., 2006;Golembiowska et al., 2008)***. There were signs of lipid peroxidation in the cortex in monkeys treated with L-dopa (80 mg/kg with carbidopa or entacapone) for 13 weeks ***(Zeng et al., 2001; Lyras et al., 2002)***. ***Isobe et al., (2006)*** observed higher levels of 3- nitro-tyrosine (a marker of oxidative stress caused by $ONOO^{-}$) in the cerebrospinal fluid (CSF) of patients treated with L-dopa (450 mg, 8 weeks). Another effect of L-dopa therapy is an increase of homocysteine in the plasma level. Homocysteine levels correlate with the plasma levels of β-amyloid and α-synuclein, considered the systemic markers of neuro-degeneration ***(Obeid et al., 2009; Zoccolella et al., 2010)***.

Numerous neurodegenerative diseases such as PD are associated with neurodegeneration; describes the loss of neuronal structure and function. Nowadays, consumption of dietary flavonoids has been associated with reduced risk of neurodegenerative diseases in human ***(Weinreb et al., 2004; Sutherland et al., 2005)*** and considered as a neuroprotective agent against many neurodegenerative diseases in mice and rats ***(Zbarsky et al., 2005***; ***Kumar and Kumar, 2010)***. The antioxidant properties of flavonoids depend on both metal-chelating properties and free radical scavenging of ROS ***(Haller and Hizoh, 2004)***. HDN, a naturally occurring methoxylated citrus flavonoid presents in fruits and vegetables and hence lipophilic in nature, has been reported to exert a wide range of pharmacological effects including antioxidant, antihypercholesterolemic, anti-inflammatory, anticarcinogenic and neuroprotective actions ***(Tommasini et al., 2005; Ebrahimi and Schluesener 2012)***. It is able to penetrate BBB and used as a potential antioxidant neuroprotective agent ***(Youdim et al., 2003;Hwang and Yen, 2008)***. Over all above data suggests that HDN can be added to the growing list of dietary-derived polyphenolic compounds that exert beneficial actions in the central nervous system.In this study HDN was used as a neuroprotective drug alone and with sinemet in a CPF rat model of Parkinson's disease. The direct protective effect of HDN has been described in the various cell types of the central nervous system, increasing neuronal progenitors survival, improving neurobehavioral alterations, attenuating oxidative damage and restoring antioxidant and mitochondrial complex enzyme activities ***(Nones et al., 2010;Gaur et al., 2011)***.

In the present study, administration of HDN to CPF-treated group showed an improvement in the levels of endogenous antioxidant enzymes (SOD, CAT and GST), GSH and AchE. Also, HDN significantly attenuated the oxidative stress along with lipid peroxidation (MDA) compared with CPF-treated group. HDN supplementation lowered the levels of plasma total cholesterol and triglycerides. Antihypercholesterolamic activities of HDN in CCl4 induced hypercholesterolamic rats have been reported ***(Naveen et al., 2005)***. This observation was in line with previous studies that supplementation of HDN has been found to significantly lower the cholesterol level and triglyceride levels in nicotine-treated rats ***(Balakrishnan and Menon, 2007)***.

Hypocholesterolemic effect of HDN may be mediated via reduction in the HMG-CoA reductase activity ***(Lee et al., 1999)***. Moreover, HDN was recorded to ameliorate the hyperglycemic effect of CPF. This is in line with studies that reported the antihyperglycemic properties of HDN against diabetic complications in a rat model of type 2 diabetes mellitus (T2DM) by potentiating the antioxidant defense system ***(Abdel-moneim et al., 2011;Mahmoud et al.,2012)***.

Regarding to cytogenetics studies, HDN significantly decreased the genetic damage which visualized by the decrease in comet tail length in brain tissues and the number of MNPCEs in bone marrow compared with CPF-treated group. Moreover, HDN has a positive effect when combined with sinemet in CPF+HDN+Sinemet group by ameliorating the toxicity of CPF and the side effects of sinemet drug. On the other hand, HDN alone does not show any biochemical alterations or DNA damage which in turn indicating the protective nature of the product. Improvement of these results in CPF+HDN-treated rats in comparison to CPF intoxicated rats demonstrated the effectivness of HDN in the treatment of oxidative stress, DNA damage and neurotoxicity. This observation was in line with previous studies ***(Wilmsen et al., 2005;Balakrishnan and Menon, 2007)***. HDN treatment has been demonstrated to improve GSH levels in liver and kidneys and decrease in levels of 8-hydroxydeoxyguanosine (8-OHdG), a product of DNA damage, in the urine of diabetic rats ***(Miyake et al., 1998)***. A report by ***(Chen et al., 2010)*** showed that HDN was able to prevent tert-butyl hydroperoxide (t-BuOOH)-induced cell damage by augmenting cellular antioxidant defense. HDN has also been reported to act as a powerful consumer of superoxide, singlet oxygen and hydroxyl radicals ***(Pradeep et al., 2008)***, contributing significantly to the intracellular antioxidant defense system. Recently studies demonstrated that HDN has powerful protective effects against DNA damage induced by gamma irradiation in mice by effectively scavenging the free radicals that produces damage to DNA ***(Hosseinimehr et al., 2009; Kalpana et al., 2011)***. Further, ***Hosseinimehr and Nemati (2006)*** have also reported that HDN possesses strong anti-clastogenic activity against γ-irradiation induced bone marrow damage in mice by reducing the micronuclei frequency. ROS has the ability to propagate the initial attack on lipid rich membranes of the brain to cause lipid per-oxidation and hence threat neuronal survival. The neurons are highly vulnerable to oxidative insults due to low level of GSH ***(Dringen, 2000)***. The present study recorded an increase in the content of GSH and decrease in the extent of lipid per-oxidation with the treatment of HDN. These results were in concordance with earlier reports, where flavonoids had been used for the treatment of different type of brain diseases ***(Jungsook, 2006;El-Sayed et al., 2008)***. It was also reported that HDN offers protection by terminating the lipid per-oxidation side chain rather than scavenging extracellular non-lipid radicals that initiate lipid per-oxidation ***(Heffner and Repine, 1989)***.

Treatment with HDN was shown to have Uimpaired by CPF. This reaffirms the fact that oxidative stress is partly involved in the short-term motor coordination deficit induced by acute CPF exposure. HDN was able to improve the efficiency of locomotion partly due to its antioxidant and AChE restoration properties which ultimately improve neuronal activity. Moreover, HDN is a good hypo-triglycerolemic and hypo-cholesterolemic agent ***(Steinberg and Khoo, 1977)*** as recorded in the present study.

Long term treatment with a flavonoid (epigallocatechingallate) increased the life span and enhanced movement abilities in a transgenic Drosophila melanogaster model of PD ***(Ortega-Arellano et al., 2011)***. Recently with respect to the brain, flavonoids, such as those found in berries and citrus, have been shown to be highly effective in preventing neurodegeneration in both animals and humans. Despite the need for these additional studies, the evidence to date strongly suggests that flavonoids represent precursor molecules in the quest to develop of a new generation of drugs capable of counteracting neuroinflammation and associated neurodegenerative disease ***(Spencer et al., 2012)***.

Conclusion:

In conclusion, Overall data suggested that HDN can be used as a therapeutical drug in neurodegenerative diseases by adding it to dietary-derived poly-phenolic compounds that exert beneficial actions in the central nervous system. Also, the results of present investigation suggest that HDN acts an efficient neuro-protector against the neuro-degeneration induced by CPF of Swiss albino rats and it could be recommended

as a disease-modifying therapy when given with sinemet early in course of Parkinson's disease.

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