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Evaluation of the protective role of quercetin and lecithin on Ifosfamide neurotoxicity in rats

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Abstract

Objective: The present study aimed to contribute the ability of quercetin and /or lecithin to attenuate ifosfamide neurotoxicity. Seventy female albino rats were randomly divided into seven groups. Ifosfamide (IFO; 80mg/kg b.wt.) was administrated for five consecutive days intraperitoneally (i.p.), while quercetin (50mg/kg b.wt.) and lecithin (100mg/kg b.wt) were given orally either singly or in-combination with IFO for six consecutive days. **Results:** Ifosfamide induce neural toxicity as indicated by decreased GSH/GSSG ratio and elevated NO level in different brain areas. As well as altering neurotransmitters levels accompanied by inhibition of choline esterase activity in serum. Also, it stimulates an apoptotic signaling program by up-regulation of caspase-3 and assimilation of Bcl-2 gene expression in all tested brain areas. Pretreatment with quercetin and lecithin singly or in-combination could attenuate ifosfamide neurotoxicity by variable degrees of improvement. This improvement seems to be a parameter selective among different brain areas. **Conclusion:** The present study concludes that pretreatment with combined therapy showed more pronounced effect compared to singular one. Therefore, we suggested that the synergistic effect of quercetin and lecithin in the combined treatment results in marked neuroprotective effect in part through their antioxidant properties and intervening in apoptotic pathways.

Keywords: Ifosfamide- neurotoxicity – neurotransmitters -antioxidants- caspase-3 - Bcl-2.

1. Introduction

Several therapeutic agent used in chemotherapy have been reported to produce multiple side effects that decrease patients' quality of life not only during treatment, but also long after the treatment has terminated. The therapeutic value of the alkylating agent ifosfamide has been limited by major side effects including central nervous system (CNS) toxicity. CNS is particularly vulnerable to the effects of chemotherapy. The CNS toxicity ranges from drowsiness and confusion to coma and death (David and Picus, 2005; Schiff and Wen, 2006; Ajithkumar *et al*., 2007 and Brunello *et al*., 2007). Ifosfamide-induced CNS toxicity estimated to occur in 10–30% of adults after administration (Ajithkumar *et al*., 2007). Altered mental status appears to be the most common manifestation among children and adolescents (Kerdudo *et al*., 2006). Cerebellar dysfunction, transient weakness, cranial nerve dysfunction, and seizure activity have been described in rare cases to ifosfamide treatments (Pratt *et al*., 1986). Although the

biochemical processes of the ifosfamide induced-neurotoxic side effects are still unclear, Ifosfamide neurotoxicity could be attributed to metabolites rather than to ifosfamide itself (Chatton *et al*., 2001). Reactive oxygen species (ROS) and oxidative stress have been presumed to be involved in this damage process. It is generally believed that ROS serve as a common initiator of the apoptotic process (Mates, 2000). Most chemotherapeutic agents may induce cell death via caspase activation. The first activation pathway involves the release of mitochondrial membrane molecules such as cytochrome c into the cytosol, a process which is regulated by Bcl-2 and Bcl-2-related proteins (Solary *et al*., 2000 and Kim *et al*., 2002). Quercetin is a ubiquitous flavonoid found abundantly in fruits and vegetables. Recently, numerous studies have revealed the neuroprotective impacts of quercetin. Additionally, evidence supports that quercetin can act as an autophagy enhancer in Parkinson's disease model and modulates the microenvironment that leads to neuronal death (El-Horany *et al*., 2016). Phosphatidylcholine or lecithin (PC, 1, 2-diacyl-sn-glycero-3phosphocholine) is a polyunsaturated fatty acid compound. PC constitutes 45–60% in membranes of cell organelles such as mitochondria, Golgi complex, rough endoplasmic reticulum and nucleus. PC builds nerve cells and neurotransmitters. In fact 17 percent of the brain is lecithin, so lecithin is a brain food. It relaxes the nerves due to improving their protective covering, reduces stress and it hydrates the skin from the inside (Dixon, 2008). It has been reported that administration with PC extends prevalent antioxidant and cytoprotective properties in different pathological events in experimental settings including ischemia / reperfusion insult in brain (Aabdallah and Eid, 2004). It has been thought that PC treatments can protect against different organ injury possibly through its antioxidant, antiinflammatory and antifibrotic actions (Akin *et al.*, 2007). Due to the pervious introductory, the current study hypothesizes that supplements containing quercetin and/or phosphatidylcholine (lecithin) would ameliorate ifosfamide neurotoxic effects.

2. Materials and Methods

2.1. Animals

Animals experiment was performed with approval from the local ethics committee. Adult female healthy rats *Rattus norvegicus* were supplied by National organization of drug control and research, Egypt (NODCAR). Rats of seven weeks old weighing 180–200 g, were housed under controlled environmental conditions, fed standard pellet chew (El-Nasr Chemical Co., Cairo, Egypt) and permitted free excess of tap water ad libitum. Animal protocol was performed in accordance with the slandered procedures laid down by OECD (1997) guidelines 424 and the criteria outlined in the “Guide for the Care and Use of Laboratory Animal”.

2. 2. Chemicals

Haloxan vials contain 1g Ifosfamide in dry lyophilized powder form (Baxter, Germany). The content was dissolved in saline solution (0.9% NaCl) immediately before injection. Quercetin was purchased from Sigma-Aldrich Chemical Company (St. Louis, MO). Phosphatidylcholine (lecithin) from soybean was obtained from Lewis Laboratories International Ltd. (Westport, CT). All other chemicals and reagents used were of analytical grade.

2.3. Drug Treatments

Animals were divided into seven groups, each containing ten rats. The first group served as control, which received the vehicle only (0.9% NaCl, 0.25 ml intraperitoneally for 5 days), Groups 2 and 3 were administered orally quercetin at a dose of 50 mg/kg bwt (Francescato *et al*., 2004) or lecithin at a dose of 100 mg/kg bwt (Lee *et al*., 2013) suspended in distilled water, respectively for six days whereas group 4 was used as positive control were administered ifosfamide at a dose of 80mg/kg bwt (Chen *et al*., 2008) intraperitoneally for five days. Groups 5 and 6 were administered either quercetin or lecithin along with ifosfamide by the same treatments regimens, respectively. On the other hand, Group 7 received a combination of both protective agents along with ifosfamide and treated similarly.

2.4. Blood collection and tissue preparation

At the end of the experimental time, the animals were fasted overnight; rats were scarified by decapitation, blood samples and brain tissues were collected. Serum separated by centrifugation at 3000 rpm for 10 min and was stored at -20 ˚C for cholinesterase estimations. The brain of each rat was removed gently and rinsed with cold ice saline to remove excess blood. Then brain areas (cortex, cerebellum, striatum, pons and thalamus & hypothalamus) were separated immediately. Brain areas of half numbers of rats were quickly minced and homogenized for gene expression and oxidative stress parameters according to the method preparation of the tested parameters.

2.5. Determination of cholinesterase

A modification of (Ellman *et al*., 1961) method described by (Gorun *et al*., 1978) was used for serum butulyl cholinesterase activity determination.

2.6. Determination of brain oxidative stress, and nitric oxide

Glutathione (GSH), oxidize glutathione (GSSG) and nitric oxide (NO) as (NO2/NO3) were assayed by HPLC according to Yilmaz *et al*. (2009) and Papadoyannis *et al*. (1999) respectively.

2.7. Determination of brain neurotransmitter content

Second half of brain areas samples were homogenized in 75% methanol for neurotransmitters determination, Norepinephrine (NE), Dopamine (DA) and Serotonin (5-HT) were determined using HPLC coupled with UV detection according to (Pagel *et al*., 2000).

2.8. Gene expression studies

2.8.1. RNA Extraction

Brain area samples of all studied groups were homogenized and total RNA was isolated with GF-1 total RNA extraction kit (Vivantis, California, USA) and further estimated for quantity and quality with Beckman dual spectrophotometer (USA) at 260/280 nanometer wavelengths.

2.8.2. Real Time PCR (qRT-PCR) for Quantitative

The mRNA expression level was quantified by qRT–PCR (Real time PCR). 1000 ng of the total RNA from each sample were used for cDNA synthesis by high capacity cDNA reverse transcription kit (Fermentas, USA). The cDNA was subsequently amplified with the Syber Green I PCR Master Kit (Fermentas) in a 48-well plate using the Step One instrument (Applied Biosystem, USA) as follows: 10 minutes at 95 ºC for enzyme activation followed by 40 cycles of 15 seconds at 95 ºC, 20 seconds at 55-60ºC and 30 second at 72 ºC for the amplification step. Changes in the expression of each target gene were normalized relative to the mean critical threshold (CT) values of β - actin housekeeping gene by the ΔΔ Ct method. The qPCR assay with the primer sets (Table A) were optimized at the annealing temperature. All cDNA including previously prepared samples of all studied groups (for, Bcl-2 and Caspase- 3), internal control (for β- actin gene expression as housekeeping gene), and non-template control (water to confirm the absence of DNA contamination in the reaction mixture), were in duplicate. The PCR product was separated by electrophoresis through a 1% agarose gel, stained, and photographed under ultraviolet light.

Table (A): Primers sequence and annealing temperature specific for each gene

Target gene	Primer sequence: 5`- 3`	Gene bank accession number
Bcl-2	Forward: 5'-GGGAAACACCAGAATCAAGT-3' Reverse:: 5'-AGCCAGGAGAAATCAAACAG-3'	NM_016993.1
Caspase-3	Forward: 5'-GGTATTGAGACAGACAGTGG-3' Reverse: 5'-CATGGGATCTGTTTCTTTGC-3'	XM_006253130.3
β-actin	Forward: 5'-ATGGATGACGATATCGCTGC-3' Reverse: CTTCTGACCCATACCCACCA-3'.	NM_031144.3

2.9. Statistical analysis

The data obtained are represented in tables and figures as Mean ± Standard error (X ± SE). The significance of the difference between the groups was calculated by one-way analysis of variance (ANOVA) followed by Duncan at P <0.05 and were carried out using the SPSS-PC computer software package version 17.

3. Results

The present study showed that ifosfamide (IFO) induced a significant depletion in GSH level ($p<0.05$) in cortex, striatum and thalamus & hypothalamus by 24.4%, 12% and 55.78% respectively, while it caused a significant increase in pons by 22.22% compared to the control group ($p<0.05$). Treatment with either quercetin or lecithin alone or in combination showed a significant increase ($p<0.05$) in GSH level in all brain areas compared to IFO group (Table 1).

Table (1): Effect of quercetin and/or lecithin supplementation on GSH level in different brain areas of Ifosfamide intoxicated rats.

Treatment	GSH (m mol/g tissue)				
	Cortex	Cerebellum	Striatum	Pons	Thalamus& Hypothalamus
Control	1.68±0.028	1.49±0.015	1.75±0.01	1.44±0.018	1.47±0.041
Quercetin	1.74±0.015	1.70*±0.067	1.83±0.039	1.69±0.031	1.4±0.039
Lecithin	1.73±0.048	1.56±0.009	1.76±0.038	1.64±0.063	1.55±0.037
Ifosfamide	1.27*±0.019	1.43±0.037	1.54*±0.003	1.76*±0.092	0.65*±0.018
Quercetin + Ifosfamide	1.96*#±0.038	1.68#±0.038	1.96*#±0.012	1.56±0.131	1.76*#±0.02
Lecithin + Ifosfamide	1.898*#0.019	1.57±0.047	1.56*±0.04	1.92*±0.017	1.70*#±0.013
Combined	1.55*#±0.008	1.49±0.071	1.56*±0.024	1.83*±0.055	1.59#±0.026

Results were expressed as mean±SE for each 5 rats.
* Significance difference versus control at P < 0.05.
# Significance difference of the mixture versus Ifosfamide at P < 0.05.

Ifosfamide produced a significant elevation in GSSG in all brain areas as compared with control group ($p<0.05$), this increase was pronounced in cortex, cerebellum and pons (27.43%, 40.63% and 30.91%) respectively. Treatment with quercetin or lecithin alone or in combination pre IFO administration showed improvements in GSSG in all brain areas; where a significant reduction in GSSG ($p<0.05$) were recorded in all brain areas. L+IFO group showed a significant reduction in all tested brain areas, marked improvement was noticed in cortex, cerebellum and pons by 16.03%, 32.64% and 24.89% respectively as compared to IFO group. Noteworthy that Q+ IFO and combined group showed more improvement (Table 2).

Table (2): Effect of quercetin and/or lecithin supplementation on GSSG level in different brain areas of Ifosfamide intoxicated rats.

Treatment	GSSG (m mol/g tissue)				
	Cortex	Cerebellum	Striatum	Pons	Thalamus& Hypothalamus
Control	4.74±0.208	5.12±0.214	5.66±0.232	5.37±0.195	4.85±0.227
Quercetin	4.81±0.31	5.88±0.271	5.63±0.279	5.05±0.061	4.34±0.17
Lecithin	5.34±0.14	5.34±0.045	5.80±0.105	5.23±0.204	5.41±0.186
Ifosfamide	6.038*±0.058	7.20*±0.22	6.01±0.501	7.03*±0.324	5.70±0.297
Quercetin + Ifosfamide	4.94#±0.128	5.14#±0.149	5.96±0.166	5.91#±0.256	4.57#±0.114
Lecithin + Ifosfamide	5.07#±0.162	4.85#±0.307	3.42*#±0.188	5.28#±0.239	3.88*#±0.165
Combined	4.48#±0.092	5.073#±0.246	4.14*#±0.155	5.35#±0.315	4.90±0.277

Results were expressed as mean±SE for each 5 rats.
* Significance difference versus control at P < 0.05.
# Significance difference of the mixture versus Ifosfamide at P < 0.05.

Concerning GSH /GSSG ratio the results revealed general decrease in all brain areas of ifosfamide group but these decreases were statistically significant in cortex, cerebellum and thalamus & hypothalamus reached 68.44%, 47.23% and 167.27% compared to control ones at $p<0.05$. There are improvement in all treated groups in all brain areas this improvement was remarkable in combined group especially in cortex, cerebellum and thalamus & hypothalamus areas (66.67%, 50% and 175%) respectively compared with IFO group ($p<0.05$) as shown in Table (3).

Table (3): Effect of quercetin and/or lecithin supplementation on GSH/GSSG ratio in different brain areas of Ifosfamide intoxicated rats.

Treatment	GSH /GSSG ratio				
	Cortex	Cerebellum	Striatum	Pons	Thalamus& Hypothalamus
Control	0.36±0.011	0.29±0.009	0.31±0.013	0.27±0.010	0.30±0.006
Quercetin	0.37±0.024	0.29±0.003	0.33±0.011	0.33±0.01	0.32±0.007
Lecithin	0.32±0.008	0.29±0.003	0.30±0.005	0.31±0.001	0.29±0.004
Ifosfamide	0.21*±0.005	0.20*±0.007	0.27±0.023	0.25±0.022	0.12*±0.008
Quercetin + Ifosfamide	0.40#±0.011	0.33#±0.003	0.33±0.011	0.27±0.034	0.39*#±0.013
Lecithin + Ifosfamide	0.37#±0.011	0.33#±0.030	0.46*#±0.013	0.37*#±0.013	0.44*#±0.021
Combined	0.35#±0.007	0.30#±0.030	0.38*#±0.020	0.35#±0.014	0.33#±0.019

Results were expressed as mean±SE for each 5 rats.
* Significance difference versus control at $P < 0.05$.
# Significance difference of the mixture versus Ifosfamide at $P < 0.05$.

Table (4): Effect of quercetin and/or lecithin supplementation on Nitric oxide level in different brain areas of Ifosfamide intoxicated rats.

Treatment	NO (m mol/g tissue)				
	Cortex	Cerebellum	Striatum	Pons	Thalamus& Hypothalamus
Control	14.33±0.396	16.55±0.787	16.27±0.693	14.34±0.340	16.92±0.65
Quercetin	18.10*±0.14	17.79±0.11	16.61±0.22	15.28±0.232	14.62±0.497
Lecithin	16.59*±0.342	16.27±0.267	15.73±0.272	14.49±0.447	16.69±0.381
Ifosfamide	18.75*±0.712	17.67±0.35	18.6±1.003	24.34*±0.704	27.81*±0.855
Quercetin + Ifosfamide	19.24*±0.362	15.91#±0.262	19.70*±0.424	20.58*±1.993	20.11*#±0.887
Lecithin + Ifosfamide	22.36*#±0.569	17.57±0.092	17.58±0.65	15.23#±0.909	20.58*#±0.407
Combined	15.05#±0.259	16.31±0.338	16.84±0.47	16.94#±0.510	15.51#±0.286

Results were expressed as mean±SE for each 5 rats.
* Significance difference versus control at $P < 0.05$.
# Significance difference of the mixture versus Ifosfamide at $P < 0.05$.

The results in Table (4) showed that ifosfamide administration caused elevation in NO level in all brain areas, these elevations was significant ($p<0.05$) in cortex, pons and thalamus & hypothalamus areas as compared with control group (30.84%, 69.74% and 64.36%) respectively. Pretreatment with quercetin, lecithin singly or combined showed a general reduction in NO level. Cortex area of L+IFO treated rats showed significant increase as compared to IFO group ($p<0.05$). In Q+IFO treated group NO level reduced in cerebellum, pons and thalamus & hypothalamus as compared to IFO group by 9.96%, 15.45% and 27.69% respectively ($p<0.05$). In combined group NO reduced significantly in all brain areas this reduction was significantly in cortex, pons and thalamus & hypothalamus by 19.73%, 30.4% and 44.23% respectively as compared to IFO group at $p<0.05$. The combined group showed more improvement than each supplement alone.

It can be noticed from Table (5) that IFO administration showed variable response in brain area, where no significant change in NE content after IFO administration in cortex, pons and thalamus & hypothalamus, while decreased significantly in cerebellum by 37.42% and increased significantly in striatum by 10.39% as compared to control group ($p<0.05$). Improvement was noticed in striatum of Q+ IFO and combined group 31.76% and 24.71% respectively as compared to IFO group ($p<0.05$).

IFO treatment reduced DA level in all brain areas, this decrease was statistically significant in cortex, cerebellum and pons 21.89%, 29.75% and15.09%, respectively ($p<0.05$), while striatum area showed non-significant increase versus control group.

Table (5): Effect of quercetin and/or lecithin supplementation on Norepinephrine level in different brain areas of Ifosfamide intoxicated rats.

Treatment	NE (µg /g tissue)				
	Cortex	Cerebellum	Striatum	Pons	Thalamus& Hypothalamus
Control	0.55±0.013	1.71±0.028	0.77±0.009	0.56±0.007	0.56±0.015
Quercetin	0.51±0.009	1.38*±0.055	0.86*±0.024	0.57±0.011	0.72*±0.006
Lecithin	0.52±0.019	1.31*±0.030	0.79±0.018	0.58±0.008	0.59±0.043
Ifosfamide	0.54±0.018	1.07*±0.019	0.85*±0.017	0.58±0.009	0.53±0.017
Quercetin + Ifosfamide	0.57±0.011	1.04*±0.004	0.58*#±0.009	0.58±0.018	0.33*#±0.015
Lecithin + Ifosfamide	0.54±0.004	1.17*±0.016	0.82±0.021	0.58±0.024	0.39*#±0.013
Combined	0.58±0.020	0.96*±0.024	0.64*#±0.006	0.57±0.006	0.32*#±0.008

Results were expressed as mean±SE for each 5 rats.
* Significance difference versus control at $P < 0.05$.
# Significance difference of the mixture versus Ifosfamide at $P < 0.05$.

Selective improvement was estimated among animal groups supplemented with Q or L singly pre IFO administration. Where, L+IFO group showed an improvement in DA level in cortex, cerebellum and striatum 20.45%, 14.12% and 3.45% respectively as compared with IFO group. While in Q+IFO group there are improvements in cerebellum and pons 10.59% and 13.33% respectively. In combined group we noticed non improvement in all brain areas except pons there are non-significant increase as compared to IFO group ($p<0.05$) (Table 6).

Table (6): Effect of quercetin and/or lecithin supplementation on Dopamine level in different brain areas of Ifosfamide intoxicated rats.

Treatment	DA (µg /g tissue)				
	Cortex	Cerebellum	Striatum	Pons	Thalamus& Hypothalamus
Control	1.69±0.068	2.42±0.065	0.55±0.037	1.06±0.002	1.26±0.022
Quercetin	1.48*±0.044	2.04*±0.057	0.65±0.037	1.11±0.028	1.53*±0.024
Lecithin	1.76±0.042	2.06*±0.053	0.57±0.027	1.16±0.032	1.38±0.059
Ifosfamide	1.32*±0.03	1.70*±0.036	0.58±0.035	0.90*±0.019	1.17±0.03
Quercetin + Ifosfamide	1.10*#±0.02	1.88*±0.008	0.63±0.011	1.02#±0.044	0.93*#±0.012
Lecithin + Ifosfamide	1.59#±0.033	1.94*#±0.050	0.56±0.028	0.91*±0.026	0.96*#±0.018
Combined	1.08*#±0.044	1.70*±0.020	0.66±0.045	0.95±0.006	0.84*#±0.031

Results were expressed as mean±SE for each 5 rats.
* Significance difference versus control at P < 0.05.
# Significance difference of the mixture versus Ifosfamide at P < 0.05.

The estimated values in Table (7) showed a significant increase in serotonin (5-HT) level among ifosfamide group, this increase was remarkable in cortex, pons and thalamus & hypothalamus 320%, 19.23% and 13.46% respectively, while cerebellum and striatum revealed a significant decrease by 40% and 40% respectively as compared to control group. There are improvement in 5-HT content in all groups in cortex, cerebellum and striatum ($p<0.05$). 5-HT content reached the control level in striatum of Q+IFO group and in pons of L+IFO group.

Table (7): Effect of quercetin and/or lecithin supplementation on Serotonin level in different brain areas of Ifosfamide intoxicated rats.

Treatment	5-HT (µg /g tissue)				
	Cortex	Cerebellum	Striatum	Pons	Thalamus& Hypothalamus
Control	0.15±0.009	0.30±0.006	0.20±0.004	0.78±0.011	1.04±0.016
Quercetin	0.14±0.004	0.22*±0.004	0.19±0.005	0.75±0.008	1.32*±0.037
Lecithin	0.16±0.006	0.22*±0.007	0.23*±0.002	0.73±0.037	1.23*±0.035
Ifosfamide	0.63*±0.034	0.18*±0.005	0.12*±0.004	0.93*±0.018	1.18*±0.018
Quercetin + Ifosfamide	0.24*#±0.008	0.22*#±0.006	0.20#±0.006	0.91*±0.026	0.66*#±0.019
Lecithin + Ifosfamide	0.23*#±0.008	0.23*#±0.008	0.15*#±0.011	0.79#±0.022	0.78*#±0.027
Combined	0.26*#±0.021	0.21*#±0.004	0.14*±0.002	0.94*±0.008	0.55*#±0.020

Results were expressed as mean±SE for each 5 rats.
* Significance difference versus control at P < 0.05.
# Significance difference of the mixture versus Ifosfamide at P < 0.05.

IFO significantly reduced the activity of serum cholinesterase (434.30 ± 8.94) at $p<0.05$ as shown in Figure (1). Treatment with quercetin or lecithin singly or in combination does not restore this reduction.

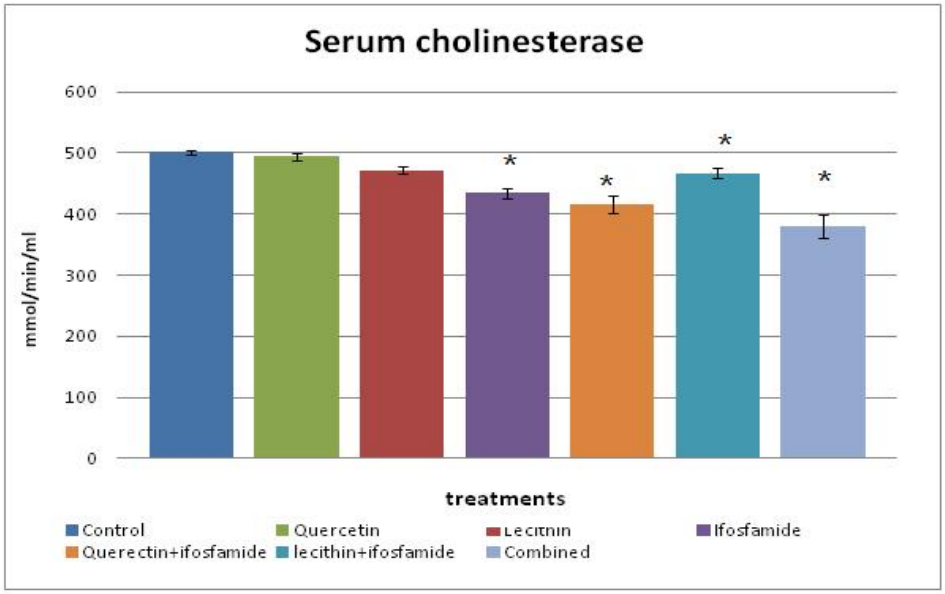
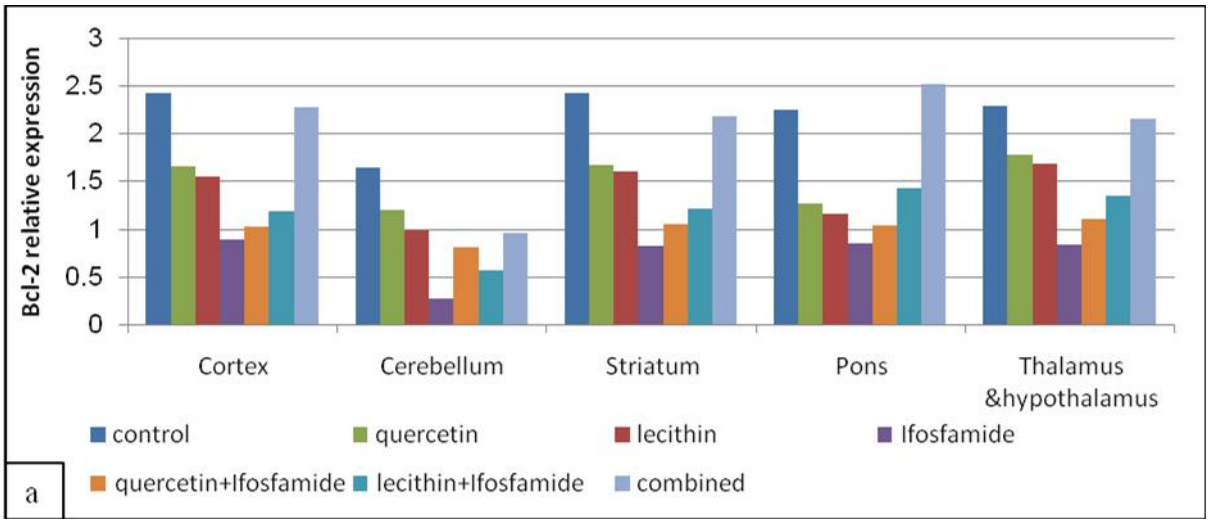


Figure (1): Effect of quercetin and/or lecithin supplementation on serum cholinesterase activity in Ifosfamide intoxicated rats.

Administration of IFO 80 mg/kg body weight for 5 days induced significant elevation in caspase-3 activity and reduced Bcl-2 protein expression in all tested brain areas when compared to control group at $p<0.05$. However, cerebellum was more susceptible area to IFO toxicity. Where, the alteration in caspase-3 and Bcl-2 reached about 6 fold than control. Pretreatment with Q and/or L reduced this alteration with the priority of the combined treatment (1.71 and 2.2 fold, respectively) from the control group. As shown in Fig.2 (a & b) and illustrated in Fig.3 of gel electrophoresis.



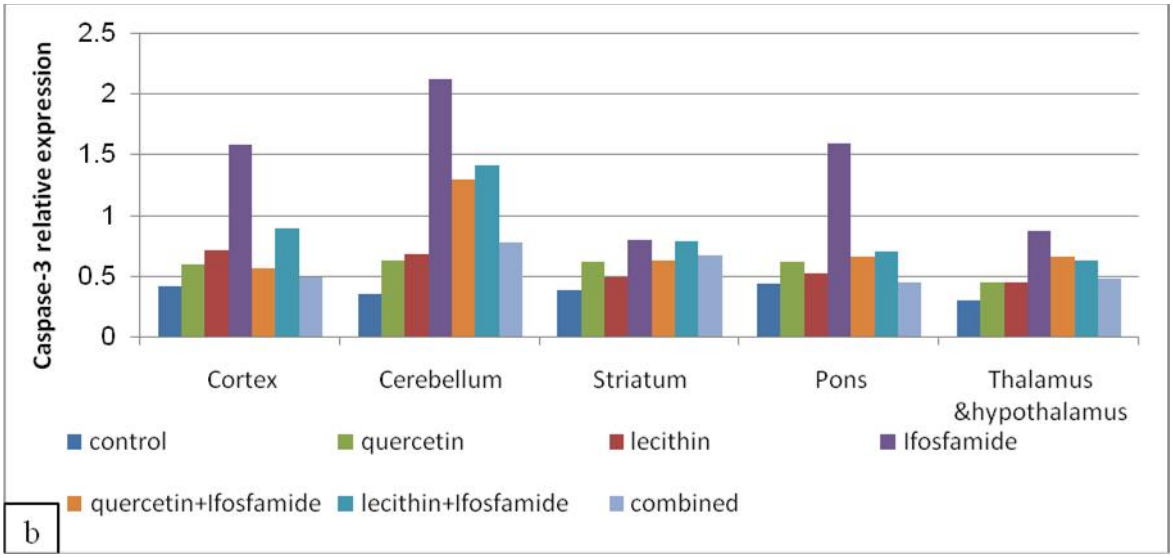
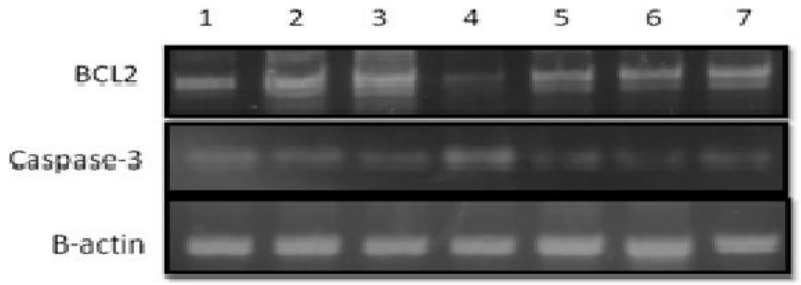


Figure (2): Effect of quercetin and/or lecithin supplementation on mRNA expression of Bcl-2 (a) and Caspase-3 (b) in different brain areas of Ifosfamide intoxicated rats.



Lane 1: Control group
Lane 2: Quercetin group
Lane 3: Lecithin group
Lane 4: Ifosfamide group
Lane 5: Quercetin+ Ifosfamide
Lane 6: Lecithin+ Ifosfamide
Lane 7: Combined group

Figure (3): Agarose gel electrophoresis showed cerebellum PCR product of Bcl-2, Caspase-3 and β-actin genes in all studied groups.

4. Discussion

In the current study, we demonstrate the possible protective effects of quercetin and lecithin either singly or in combination against ifosfamide-induced neurotoxicity in rat brain areas. The current results revealed that ifosfamide causes central neurotoxicity and significantly reduces GSH and increases GSSG content therefore decreases GSH/GSSG ratio. Also, increased NO content accompanied with up-regulation of caspase -3 gene expressions and down regulation of Bcl-2 gene expressions among almost brain areas were estimated. Additionally, ifosfamide treatment significantly decreases both of serum cholinesterase and brain areas dopamine content. On the other hand, altered brain areas norepinephrine and serotonin contents were estimated to ifosfamide treatments. Our results supported the theory that IFO induced oxidative stress in the different brain areas of rats. The pathophysiological mechanisms responsible for the development of ifosfamide-induced neurotoxicity can be explained by several hypothesizes but the more sounded hypothesis is that Ifosfamide (IFO) and its metabolites can penetrate through the blood-brain barrier (Shin, *et al*., 2011), where mitochondrial dysfunction causes accumulation of the neurotoxic metabolites of ifosfamide called chloroacetaldehyde, (CAA), (Ajithkumar *et al*., 2007 and Shin *et al*.,

2011). The neurotoxic effects of CAA play a central role due to its direct neurotoxic effect or indirect through glutathione depletion from the CNS when CAA is oxidized to 2-chloroacetic acid. This metabolite has been hypothesized to reduce, or inhibit, mitochondrial activity through impairment of electrons transfer mechanisms (Visarius *et al*., 1998); inhibition of mitochondrial oxidative phosphorylation resulting in impaired fatty acid metabolism (Ajithkumar *et al.*, 2007). The presence of oxidative tissue damage as a result of impaired antioxidant defense mechanism in rats treated with IFO was indicated by Şehirli, *et al*. (2007). Reduced glutathione (GSH) is considered to be one of the most important scavengers of reactive oxygen species (ROS), and its ratio with oxidized glutathione (GSSG) may be used as a marker of oxidative stress (Zitka *et al*., 2012). As mentioned above, glutathione is an important antioxidant system. Under certain circumstances, such as in the onset of apoptosis, glutathione could efflux from the cells (Oda *et al*., 1999), which lowers the reducing capability of the cells and can consequently result in oxidative stress without interference of ROS generation. Therefore, GSH depletion is often taken as a marker of oxidative stress (Franco and Cidlowski, 2012). Oxidative stress causes profound alterations of various biological structures, including cellular membranes, lipids, proteins and nucleic acids, and it is involved in numerous malignancies.

Although many factors can induce apoptosis, it is generally believed that ROS serve as a common initiator of the apoptotic process. During the apoptotic process initial stress-induced damage does not kill cells directly, rather it stimulates an apoptotic signaling program that leads to cell death (Mates, 2000). The present results confirm the important role of caspase-3 in the apoptotic cascade leading to IFO-induced cell death and showed that IFO treatment induces brain cell apoptosis by increasing expression level of caspase-3 and decreasing Bcl- 2. Also, the present findings run in parallel with studies by (Schwartz and Waxman, 2001; Becker *et al*., 2002 and Rzeski *et al*., 2004); they found that caspase-9 plays an essential role in the apoptotic cascade leading to caspase-mediated cell deaths contribute to the neurotoxicity of anticancer drugs *in vitro* in neuronal cultures and *in vivo* in the developing rat brain. Under this condition, IFO can activate Bax and Bak leading to cytochrome c release from mitochondria and subsequent caspase activity. Activation of caspases including 3, 8, and 9 occurs early after IFO treatment of cells and inhibition of caspase activity may suppress IFO-induced cell death.

Also, increased brain levels of nitric oxide following treatment with IFO encountered in the current study would represent another approach to explain IFO neurotoxic side effect. Physiological amounts of nitric oxide are normally generated from L-arginine by the action of nitric-oxide synthase (NOS) isoenzymes, present in endothelia (eNOS) and neurons (nNOS). A third isoform is inducible (iNOS) in various inflammatory and tissue cells during pathological states by the action of cytokines (eg., INF-γ, TNF-α, IL-1β) and other inflammatory signals (Moncada *et al*., 1989). Increased brain levels of nitric oxide observed during toxic and inflammatory states can be deleterious to neuronal tissue, where nitric oxide released from activated glia inhibits neuronal respiration resulting in decreased ATP levels, glutamate release and excitotoxicity (Bal-Price and Brown, 2001). One mechanism by which nitric oxide causes oxidative changes to biomacromolecules (proteins, lipids and DNA) is ascribed to its ability to react with many other free radicals including the superoxide radical. This latter reaction results in the formation of the highly reactive peroxynitrite radical, capable of causing oxidative and/or nitrosative damage to tyrosine residues, thiols, DNA and unsaturated fatty-acid containing phospholipids (Szabó *et al*., 2007).

Additionally, in the present study ifosfamide treatment significantly decreased dopamine level and altered norepinephrine and serotonin contents among different brain areas which declared important pathway implicated to ifosfamide neurotoxicity that mediated by monoamine oxidase (Klastersky, 2003). To our knowledge, this is the first published study that investigates neurotransmitters content (norepinephrine, dopamine and serotonin) alteration occurring after chemotherapeutic drug administration. It has been suggested that the inherent toxicity of many chemotherapeutic agents may impair neurotransmitter signaling and release (Ahles and Saykin, 2007 and Kaplan *et al*., 2016). CNS neurotransmitters as Dopamine (DA), serotonin (5-HT) and norepinephrine (NE) are involved in a variety of neurological functions. We would mention here that the cerebellum decrease level of DA and 5-HT encountered to IFO treatment. DA and 5-HT play roles in reward (Schultz, 2002), cognition and locomotor control (Bäckman *et al*., 2006). Alterations in their system function have been observed in response to genetic modifications that model oxidative stress (Ortiz *et al*., 2011 and Kita *et al*., 2003) and neurodegenerative disease (Eisenberger *et al*., 1986; Morgan *et al*., 1987; Kita *et al*., 2003; Kraft *et al*.,

2009 and Ortiz *et al*., 2010 and 2012). Serotonin has been shown to suppress excitatory synaptic transmission (Schmitz *et al*., 1999). The increase in 5-HT level by Q or L could modulate the activation of AMPA/kainate receptors (Licata *et al*., 1998) thereby correct the IFO associated encephalopathy. In the present work, quercetin and lecithin supplementations with IFO significantly attenuate brain area oxidative stress, reversed GSH, GSSG, and GSH/GSSG ratio near the normal control as a result increase Bcl-2 gene expression level which act as inhibitor for caspase-3 by blocking mitochondrial release of cytochrome c. Direct scavenging of reactive oxygen species is one of the many antioxidant actions required to restore oxidative equilibrium once it is lost in different pathologies. The hypothesis that restoring redox equilibrium through activation of intracellular signals is also an important step of the antioxidation process is gaining increasing support (Schoroeter *et al*., 2002). Various reports suggest that quercetin passes through the blood–brain barrier and influences the neuronal cells directly. A higher concentration of quercetin metabolites appear in the brain after several hours of administration of quercetin (Day *et al*., 2001 and Paulke *et al*., 2006). Quercetin metabolites found in brain could attenuate oxidative stress not only through radical scavenging but also through non radical scavenging activities (Williamson *et al*., 2005). A study on healthy P19 neurons reported that quercetin treatment did not affect neuron survival but depletion in intracellular glutathione contents has been observed which can affect working of nervous system. The current protective role of quercetin was in agreement with Denny Joseph and Muralidhara (2013)**,** who stated that quercetin can behave as a neuroprotector in rat brain when used in combination with fish oil and may show beneficial effects against neurodegenerative diseases like Alzheimer's disease as it shows inhibitory affect against acetylcholinesterase (Choi *et al*., 2012). Moreover, quercetin has been reported to reduce the oxidative stress induced by 6-hydroxydopamine in neurons from the brain striatum of rats (Haleagrahara *et al*., 2013). Quercetin are specific NO scavengers (Boullerne *et al*., 1999; López-López *et al*., 2004 and Zhang *et al*., 2011). It offers neuroprotection against Al-induced cognitive dysfunction, cholinergic impairment, and oxidative damage as demonstrated by Sharma, *et al*. (2013). Indeed, another report has described the beneficial effects of quercetin on endotoxic shock (Abd-El-Gawad and Califa, 2001) and the authors explained these effects by lipoperoxidation inhibition and increases in glutathione peroxidase activity.

Also, phosphatidylcholine (PC) or lecithin showed neuroprotective roles against ischemia/reperfusion insult in rat brain (Aabdallah and Eid, 2004). PC has been shown a strong reactive oxygen species scavenging effect and prevented lipid peroxidation in an *in vivo* study of oxidative stress (Navder *et al*., 2000). Indeed, oral pre-treatment with PC decreased ischemia–reperfusion-induced reactive oxygen generation in the small intestine (Ghyczy *et al*., 2008). Furthermore, phosphotidylcholine decreases severe sepsis in rat model, in liver, heart and lung MDA levels (Demirbilek *et al*., 2004). Although PC act as a choline donor, and could inhibit an increase in oxidative damage caused by choline-deficiency (Ossani *et al*., 2007) it failed to restore serum cholinesterase in present study. The molecular and cell signal mechanisms may be responsible for this effect and it was unclear**.**

Although the exact mechanism for the increased risk of encephalopathy with ifosfamide treatment is not clear, we have been suggested that the low serum albumin estimated in our parallel study concerning with Ifosfamide hepatorenal toxicity (data under publication) reflects impairment of hepatic function and, in turns impaired hepatic ifosfamide metabolism. If we supposed that neurotoxic metabolites of ifosfamide are protein bound, the present hypoalbuminaemia may results in increased free metabolite and thereby facilitating metabolite entry into the CNS. The concurrent supplementation with quercetin or lecithin with ifosfamide treatment reduced the hepatic stress and normalized serum total protein level, which in turns enhanced the protein bound capability and decreased ifosfamide metabolites entrance to the CNS. Our hypothesis was supported by previous studies by (Curtin *et al*., 1991 and David and Picus, 2005). Collectively, the present study suggested that quercetin and lecithin limits the development of IFO-induced neurotoxicity mainly via attenuated hepatic and neural oxidative stress concomitants with down regulation of apoptotic signaling in brain area tissues.

The present data showed that the combined therapy including quercetin and lecithin with IFO exhibits the most pronounced effect as compared to each compound alone with IFO as improving in GSH,GSSG, GSH/GSSG ratio, NO, and gene expression changes (Bcl-2 and caspase-3) as compared to IFO group. Our results agreement with (Dajas *et al*., 2002 and 2003) who reported that increased the possibility of quercetin crossing the blood brain barrier by mixing it with lecithin.

(Ramadan, 2008) stated that quercetin increases the antioxidant activity of soy lecithin in a triolein model system.

In conclusion: supplementation with combined quercetin and lecithin in the current study significantly modulated the biochemical markers of ifosfamide intoxicated rats. Therefore, we suggest that the synergistic effect of quercetin and lecithin in the combined therapy results in marked neuroprotective effect in part through its antioxidant properties and down regulation of apoptotic signaling in brain area tissues.

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