The Role of Sodium Diclofenac, Taurine and Bismuth Subnitrate Against Neurotoxicity Induced by Cyclosporine in rats: a study of drug interaction.

Suzan F.I. El-Sisi
National Organization for Drug Control and Research

Suzanelsisi@yahoo.com

ABSTRACT

Coadminisration of an immunosuppressive cyclosporine A (CSA) and nonesteriodal antiinfilammatory drug (NSAID), sodium diclofenac (SD) increases the efficacy for relief pain for patients with rheumatoid arthritis. However, clinical studies showed enhancement of cyclosporine toxicity upon this combination. Neurotoxicity is one of the most significant side effects of CSA toxicity. To characterized biochemical parameters of neurotoxicity, the study was assessed the effect of CSA (10mg/kg) alone or in combination with SD (10 mg/kg) for 6weeks on energy metabolism (ATP and glucose metabolism), oxidative stress [MDA (end product of lipid peroxides), NO (nitric oxides, as total nitrate), GSH (reduced glutathione), LDH (lactic dehydrogenase enzyme)], and monoamines [dopamine (DA), noradrenalin (NA) and serotonin (SR)] in five different brain areas (cerebral cortex, cerebellum, Striatum, pons, and thalamus & hypothalamus) of adult albino rats. CSA alone inhibited energy production (ATP and glucose metabolism), alternated oxidative stress through increasing levels of MDA and LDH, and decreasing levels of GSH and NO in blood and tested brain areas tissues as well as it inhibited neurotransmitters releases. When SD combined with CSA, it enhanced CSA-induced inhibition of mitochondrial energy, inhibition of neuroamines release and increase oxidative stress alternations. The study also extended to evaluate and compare the protective effect of taurine (a major intracellular free ß-amino acid and potent endogenous antioxidant) with bismuth subnitrate (BSN), an antiulcer drug and a specific inducer of metalothionine (MT) in infected tissues, against neurotoxicity induced by concurrent administration of CSA and SD. BSN co-administration could somewhat reduced CSA-induced neurotoxicity only through ameliorated oxidative stress state by showing significant increase in the level of GSH, and significant reduction in the level of MDA and LDH activity. Whereas the co-administration of the potent cytoprotective antioxidant, taurine, antagonized all CSA negative effects, by ameliorating CSA-induced mitochondrial dysfunction, improvement oxidative stress and modulated neurotransmitters. [Nature and Science. 2009;7(10):37-48]. (ISSN: 1545-0740).

Key words; cyclosporine A, NSAID, sodium diclofenac, neurotoxicity, taurine, bismuth subnitrate, oxidative stress, neurotransmitters, ATP.

INTRODUCTION

Cyclosporine A is an immunosuppressive undecapeptide drug, which is most frequently used in transplant surgery and in the treatment of autoimmune disease (Allison, 2000). The adverse effects of CSA include acute and chronic nephrotoxicity, neurotoxicity, hypertension, and new-onset diabetes (Kozlowska et al, 2006). Cyclosporine is a calcineurin inhibitor that inhibit nuclear factor of activated T cells results in inhibition of interleukin 2 production in the T cells. The most limiting side effects of calcineurin inhibitors is nephrotoxicity (Dunn et al, 2001) followed by neurotoxicity (Wijdreks, 2001). Even though nephrotoxicity remain the biggest problem with CSA treatment, CSA dependent neurotoxicity occurs up to 60% of organ transplant patients and also can lead to serious life-threatening condition and to withdrawal of CSA from patient's regimen (Helderman et al, 2003). CSA-induced neurotoxicity was reported in stem cell transplant recipient (Raza et al, 2007), in hematopoeitic malignancies after allogenic bone marrow transplantation (Bartynski et al, 2005). Brain is the organ with the highest demand for oxygen and oxidative energy production. The central intermediate substance of oxidative metabolism is acetyl–coenzyme A, which can originate from carbohydrates, fatty acids, or amino acids. Brain, in contrast to other organs, utilizes only glucose to produce ATP and lacks the enzyme for ß-oxidation of fatty acids. (Serkova et al, 2004). Mitochondrial encephalopathies (ME) are a heterogeneous group of metabolic diseases characterized by mitochondrial malfunction that leads to cellular

energy failure and cells damage (Serkova et al, 2004).

Although CSA has been shown in a series of controlled trails to be of benefit, patients continue to require NSAID, as Sodium diclofenac (SD), for relief of joint pain and stiffness (Rossi, 2006). SD is a NSAID, taken to reduce inflammation and an analgesic reducing pain in condition such as in arthritis or acute injury (Rossi, 2006).The adverse effects of SD include, gastrointestinal complaints, liver damages, acute and chronic nephrotoxicity, heart attack, bone marrow depression (Brater, 2002; Rossi 2006 and Solomon et al, 2006), and develop neurological side effects such as; confusion, depression, dizziness, headache, sedation, sleep disturbance, somnolence (Slagle, 2001). In experimental animals, SD was found to decrease neuron number of the rat hippocampus (Gokcimen et al, 2007).

Long term treatment of NSAID leaded to ulceration and gastrointestinal bleeding. Hence most patients must receive ulcer-protection drugs as bismuth subnitrate (BSN) during long term treatment with SD. Many studies showed that BSN can protect tissues against toxicity by inducing metalothionine in these tissues (Kondo et al, 2004).

Taurine is sulfur containing ß-amino acid; it was found to play an important role in the field of cytoprotective through its antioxidant effect (Erdem et al, 2000). Taurine was found to have a modulated action against neurotoxicity (Louzada, 2004).

Based on these observations, the study aimed to study the positive or negative effects upon drug interaction for patients receiving CSA, SD. and BSN, upon neurotoxicity induced by CSA. Also the study extended to compare and through light on the possible ameliorative effect of the potent cytoprotective antioxidant taurine as well as BSN, against neurotoxicity induced by concurrent administration of CSA and SD.

MATERIALS AND METHODS

Animals

A total number of 56 female albino rats weighing 120±20g B.wt were used. The animals were brought from laboratory animal breeding of National Organization for Drug Control and Research (NODCAR), Giza, Egypt. They were kept under strictly hygienic conditions. They were put on a standard basal diet and allowed free access to drinking water.

Materials

-Bismuth subnitrate, taurine and cyclosporine A were purchased from Sigma Co. USA.

-Sodium Diclofenac was purchased from Egyptian market pharmacy, ADWIC.

The tested drug doses were equivalent to the daily human doses, freshly prepared before administration, dissolved in water except BSN dissolved in citrate solution, and given orally.

Experimental design

Rats were classified into 7 equal groups each comprises 8 rats and treated daily for 6 weeks, as follow:

G1; Control group (CN), fed on basal diet and orally administered citrate solution as vehicle.

G2; Taurine control group (T), orally administrated 500 mg/kg of taurine.

G3; BSN control group (B), orally administrated 15 mg/kg of BSN.

G4; Cyclosporine A group (CSA), orally administrated 10 mg/kg of CSA

G5; Combined treated group (CSA+SD), orally administrated 10 mg/kg of CSA plus 10 mg/kg of sodium diclofenac.

G6; Taurine treated group (T+C), treated as in G5 and supplemented with 500 mg/kg of taurine.

G7; Bismuth treated group (B+C), treated as in G5 and supplemented with 15 mg/kg of BSN.

At the end of the treatment schedule, blood samples were taken from each rat and then they were sacrificed, brain tissues were removed, some subjected to histopathological examinations as described by Bancrofet et al. (1996), the others were homogenated in 4 different areas (cerebral cortex, cerebellum, pons, and thalamus & hypothalamus) in iced 10% KOH. Separated sera and supernatant of homogenate tissues were processed for the biochemical analysis; ATP was determined by the method of (Zhang et al, 2000), blood glucose by (Trinder 1969), oxidative stress (MDA, NO, as total nitrate and GSH were determined by HPLC methods of Karatepe (2004); Everett et al, (1995); Jayatilleke & Shaw (1993) respectively. LDH, were determined by the commercial kits of (Buhl and Jackson, 1978).

Monoamines; DA, NA and SR were measured by HPLC chromatography according to the methods of Pagel et al, (2000).

Statistical analysis, were done using SPSS ANOVA test version 11.5, $P \leq 0.05$ was considered significant.

RESULTS AND DISCUSSION

Neurotoxicity is one of the most significant clinical side effects of the immunosuppressive undecapeptide cyclosporine A, CSA, occurring at some degree in up to 60% of transplant patients. The clinical mechanisms of CSA-induced neurotoxicity remain controversial and poorly understood. It was found that the clinical symptoms of CSA neurotoxicity mimic those of mitochondrial encephalopathy (ME) (Beal, 1998; Serkova et al, 2004). ME are a heterogeneous group of metabolic diseases characterized by mitochondrial malfunction that leads to cellular energy failure with increased lactate production through increasing activity of LDH release (Serkova et al, 2004).

Table (1): The effect of taurine and BSN against CSA and SD on the levels of ATP (nmol/gm) in different brain areas after 6 weeks of treatment.

Groups (n=8)	Cerebral cortex	Cerebellum	Striatum	Pons	Thalamus& hypothalamus
CN	1.494 ± 0.047	1.460 ± 0.058	1.391± 0.043	1.354 ± 0.027	1.250 ± 0.047
T	1.515 ± 0.073	1.467 ± 0.063	1.414 ± 0.065	1.346 ± 0.040	1.247 ± 0.044
B	1.482 ± 0.052	1.499 ± 0.073	1.380 ± 0.047	1.349 ± 0.031	1.269 ± 0.070
CSA	1.316 ± 0.031*	1.392 ± 0.066	1.278 ± 0.039*	1.310 ± 0.044	1.135 ± 0.031*
CSA+SD	1.233 ± 0.032*,[a]	1.417 ± 0.034	1.158±0.044*,[a]	1.266 ± 0.017*	0.934±0.045*,[a]
T+C	1.379 ±0.038*,[b,c]	1.426 ± 0.035	1.348± 0.033[b,c]	1.350 ± 0.029[b]	1.166±0.034[b,c]
B+C	1.231 ± 0.051*,[a]	1.428 ± 0.053	1.190 ± 0.063*	1.335 ± 0.022	1.00 ± 0.056*

- Significant difference *vs*. CN: *P<0.05, between CSA & CSA+SD: [a]P<0.05, between CSA+SD and treated groups: [b]P<0.05, between T+C & B+C: [c]P<0.05.

Confirming with the recent study of Leu et al, (2008) who demonstrated sever CSA neurotoxicity including chondoriod encephalopathy, seizures, paralysis, coma, and cerebella ataxia. The brain, in contrast to the other organ utilizes only glucose to produce ATP and lack the enzyme for B-oxidation of fatty acids, Metabolism of one molecule of glucose produces thirty-six molecules ATP: Two ATP through cytosolic glycolysis, two ATP via the mitochondrial Krebs cycle, and thirty-two molecules through mitochondrial oxidative phosphorylation. It should be obvious that disturbances in mitochondrial glucose metabolism would lead to a considerable decrease in energy production, which, in the brain, cannot be compensated by ß-oxidation of fatty acids (Serkova et al, 2004). These later observations were in good keeping with the present study that showed treatment rats with CSA (10 mg/kg/day for 6 weeks), exhibit a significant ($P<0.05$) decrease in ATP concentrations (Table1), accompanied by significant increase ($P<0.05$) in the levels of blood glucose concentrations (Table 3& Figure A).

Table (2.I): The effect of taurine and BSN against CSA and SD on the levels of MDA (nmol/gm) in different brain areas after 6 weeks of treatment.

Groups	Cerebral cortex	Cerebellum	Striatum	Pons	Thalamus& Hypothalamus
CN	39.43 ± 0.92	40.87 ±1.12	41.18 ±11.14	51.60 ±0.97	44.18 ± 0.97
T	37.21 ± 1.11	39.43 ± 0.37	40.22 ± 13.01	52.90 ± 0.90	42.19 ± 1.12
B	38.80 ± 0.89	38.60 ± 1.00	42.17 ± 1.06	49.09 ±1.27	42.50 ± 0.72
CSA	39.37 ± 0.63	50.04 ± 0.82*	61.49 ± 1.13*	63.09 ± 0.37*	43.01 ± 1.06
CSA+SD	47.27 ± 1.09*,[a]	48.95 ± 0.76*	59.58 ± 0.72*	63.01 ± 0.92*	45.39 ± 0.67
T+C	30.68 ± 0.97[b,c]	35.11 ± 1.09[b,c]	41.08 ± 0.86[b,c]	52.94 ± 1.01[b]	42.39 ± 0.80
B+C	40.58 ± 1.15[b]	42.40 ± 1.23[b]	47.60 ± 1.10[b]	54.91 ± 1.27[b]	43.84 ± 0.68

- Significant difference *vs*. CN: *P<0.05, between CSA & CSA+SD: [a]P<0.05, between CSA+SD and treated groups: [b]P<0.05, between T+C & B+C: [c]P<0.05.

This interpreted the finding of earlier investigators who demonstrated that the cerebral energy metabolism is the most sensitive indicator of CSA neurotoxicity in vitro, even 100 ng/ml CSA added to incubation medium or perfusate reduces high-energy phosphate (ATP) concentrations by 20% (Serkova et al, 1999). Inhibition of mitochondrial glucose metabolism (the Krebs cycle and oxidative phosphorylation) was accompanied by increased reactive oxygen species (ROS) production (Christian, 2004) that it considered to be another responsible factor for CSA-induced neurotoxicty. The same pattern of metabolic changes was found in the present study, that showed significant increasing ($P<0.05$) in the level of serum and brain MDA (end product of lipid peroxides), accompanied by significant decrease ($P<0.05$) in the level of non enzymatic antioxidant reduced glutathione *vs.* non treated control (Table2$_{I, II}$ & 3,)

Table (2.II): The effect of taurine and BSN against CSA and SD on the levels of GSH (umol/gm) in different brain areas after 6 weeks of treatment.

Groups (n=8)	Cerebral cortex	Cerebellum	Striatum	Pons	Thalamus& Hypothalamus
CN	3.94 ± 0.09	4.07 ± 0.06	3.82 ± 0.07	4.60 ± 0.07	4.84 ± 0.09
T	3.97 ± 1.11	4.14 ± 0.37	4.01 ± 0.14	4.59 ± 0.09	4.90 ± 0.11
B	3.88 ± 0.09	3.86 ± 0.14	3.89 ± 0.06	4.79 ± 1.27	4.69 ± 0.07
CSA	3.67 ± 0.05*	3.21 ± 0.08*	3.04 ± 0.09*	3.09 ± 0.17*	4.41 ± 0.06*
CSA+SD	3.07 ± 0.09*,a	3.12 ± 0.07*	2.88 ± 0.22*	3.01 ± 0.09*	4.33 ± 0.07*
T+C	3.68 ± 0.07*,b,c	3.59 ± 0.09*,b	4.08 ± 0.09b,c	3.94 ± 0.06*,b,c	4.79 ± 0.10b
B+C	3.41 ± 0.05*,b	3.43 ± 0.11*,b	3.55 ± 0.10*,b	3.45 ± 0.07*,b	4.58 ± 0.09b

- Significant difference *vs*. CN: *P<0.05, between CSA & CSA+SD: [a]P<0.05, between CSA+SD and treated groups: [b]P<0.05, between T+C & B+C: [c]P<0.05.

Table (2.III): The effect of taurine and BSN against CSA and SD on the levels of NO as total nitrate (nmol/gm) in different brain areas after 6 weeks of treatment.

Groups (n=8)	Cerebral cortex	Cerebellum	Striatum	Pons	Thalamus& hypothalamus
CN	51.75 ± 1.66	45.50 ± 1.02	62.11 ± 1.22	55.41 ± 2.11	43.45 ± 0.77
T	47.89 ± 1.93	45.87 ± 1.04	65.22 ± 1.91	57.85 ± 1.98	41.74 ± 0.79
B	53.74 ± 2.20	44.78 ±1.11	61.52 ± 2.12	59.00 ± 2.22	45.98 ± 0.02
CSA	43.14 ± 1.64*	36.37 ± 1.41*	51.11 ± 0.99*	50.87 ± 1.14*	36.74 ± 1.01*
CSA+SD	32.04 ± 1.79*,a	34.99 ± 1.41*	49.74 ± 0.88*	46.46 ± 1.11*,a	37.00 ± 0.87*
T+C	41.88 ± 1.12*,b,c	42.18 ± 1.71b,c	56.15 ± 1.94b,c	53.22 ± 0.95b	44.41 ±1.21b
B+C	34.11± 1.15*,a	37.01 ± 1.54*	47.33 ± 1.01*	48.73 ± 1.17*	39.69 ± 1.94

- Significant difference *vs*. CN: *P<0.05, between CSA & CSA+SD: [a]P<0.05, between CSA+SD and treated groups: [b]P<0.05, between T+C & B+C: [c]P<0.05.

Treatment with CSA has been shown to increase O^{-}_{2}, H_2O_2 and $OH^{\cdot -}$ radicals production (Hagar, 2004). The main detoxifying system for lipid peroxides is GSH. The decrease in GSH following CSA observed in this study greatly supported the role of ROS in CSA neurotoxicity. Cyclosporine is a calcineurin inhibitor, the most limiting side effects of calcineurin inhibitors is inhibition of NO production, through a calcinurin-regulating eNOS dephosphorylation (Kou et al, 2002)., which lead to vasoconstriction (Gitanbeek et al, 1999). So it was postulated that CSA can induce neurotoxicity indirectly through its vasoconstriction (Serkova et al, 2004). Another side effect of calcineurin inhibitors is increased activity of LDH and lactate accumulation (Higginson et al, 2002).

Table (3):The effect of taurine and BSN against CSA and SD on the levels of serum MDA (umol/ml), GSH (nmol/dL), NO (nmol/ml), LDH (umol/ml) and glucose (mg/dL) after 6 weeks of treatment.

Groups (n=8)	MDA (umol/ml)	GSH (nmol/dL)	Nitric oxide (NO2&NO3) (nmol/ml)	LDH (umol/ml)	Glucose (mg/dl)
CN	0.73 ± 0.014	85.50 ± 3.42	32.11 ±1.52	168.25 ± 6.54	87.33 ± 2.87
T	0.68 ± 0.018	85.87± 2.04	29.82 ±1.41	170.88 ± 6.69	91.22 ± 1.98
B	0.76 ± 0.040	84.18 ± 4.11	31.52 ±1.12	171.33 ± 4.79	92.11 ± 2.72
CSA	1.33 ± 0.030*	73.87 ± 4.41*	25.21 ±1.99*	243.88 ± 5.87*	114.13 ± 3.08*
CSA+SD	1.62 ± 0.047*,[a]	64.99 ± 2.41*,[a]	21.87±1.18*	335.88 ± 7.05*,[a]	121.78±3.11*,[a]
T+C	0.97 ± 0.049[b,c]	79.18 ± 2.77[b]	29.15 ± 1.44[b]	214.13 ± 6.76*,[b]	93.78 ± 3.04[b]
B+C	1.13 ± 0.057[b]	77.01 ± 4.54[b]	23.33 ±1.81	212.13 ± 5.56*,[b]	95.56 ± 3.17[b]

- Significant difference *vs*. CN: *P<0.05, between CSA & CSA+SD: [a]P<0.05, between CSA+SD and treated groups: [b]P<0.05, between T+C & B+C: [c]P<0.05.

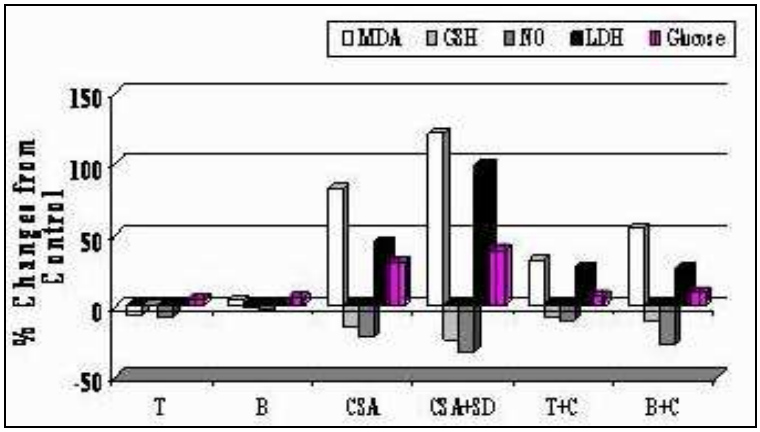

Figure (A): % Changes from control values to show the effect of taurine and BSN against CSA and SD on the levels of serum MDA, GSH, NO, LDH and glucose after 6 weeks of treatment.

These results are in agreement with the data of the present study that revealed a significant decrease in the level NO as well as increase in activity of LDH release (Table3 & figure A, P<0.05 *vs.* to untreated control group). In agreement with the earlier studies of Serkova et al, (2001) who found a decrease in ATP concentrations accompanied by increased lactate concentration in blood and rat brain after six day of CSA treatment. Another study in the skeletal muscle cells showed increased activity of LDH after CSA treatment, which was related to calcineurin inhibition by CSA (Higginson et al, 2002). The data depicted in Table ($4_{I, II, III}$) showed significant inhibition (P<0.05) of the neurotransmitters (AD, DP & SR) generation. In agreement, it was found that CSA-induced inhibition of energy production was accompanied by reduction of glutamate and γ-amino butyric acid (GABA), neurotransmitters generated from Krebs cycle which identified by MRS in rat brain extract (Serkova et al 2001). This result suggests that CSA induced neurotoxicity through its exictotoxic damage of neuron by inhibiting GABAnergic transmission. The histopathological examination made on the brain of CSA-treated rats, showed structural changes include congestion in choroids, in med brain, in blood capillaries of cerebral cortex, edema of the meninges and hemorrhage in the fissure between the two hemisphere (Fig $2_{a, b}$)

Table (4.I): The effect of taurine and BSN against CSA and SD on the levels of NA (ug/gm) in different brain areas after 6 weeks of treatment.

Group (n=8)	Cerebral cortex	Cerebellum	Striatum	Pons	Thalamus& hypothalamus
CN	0.483 ± 0.026	0.643 ± 0.021	1.450 ± 0.056	1.127 ± 0.041	1.311 ± 0.048
T	0.465 ± 0.031	0.639 ± 0.020	1.425 ± 0.070	1.136 ± 0.045	1.305 ± 0.040
B	0.458 ± 0.041	0.658 ± 0.023	1.471 ± 0.046	1.193 ± 0.035	1.278 ± 0.087
CSA	0.427 ± 0.025	0.570 ± 0.029*	1.275 ± 0.067*	0.919 ± 0.059*	1.166 ± 0.052*
CSA+SD	0.372 ± 0.030*	0.498 ± 0.032*,a	1.198± 0.087*	0.876 ± 0.046*	1.145 ± 0.054*
T+C	0.458 ± 0.031b,c	0.589 ± 0.022b,c	1.363 ± 0.071b	1.006 ± 0.039b,c	1.249 ± 0.059
B+C	0.380 ± 0.042*	0.518 ± 0.020*	1.213 ± 0.058*	0.889 ± 0.064*	1.197 ± 0.073

- Significant difference *vs*. CN: *P<0.05, between CSA & CSA+SD: [a]P<0.05, between CSA+SD and treated groups: [b]P<0.05, between T+C & B+C: [c]P<0.05.

Table (4.II): The effect of taurine and BSN against CSA and SD on the levels of DP ((ug/gm)) in different brain areas after 6 weeks of treatment.

Group (n=8)	Cerebral cortex	Cerebellum	Striatum	Pons	Thalamus& hypothalamus
CN	0.574 ± 0.014	0.994 ± 0.028	0.925 ± 0.030	0.789 ± 0.017	0.855 ± 0.026
T	0.549 ± 0.021	1.007 ± 0.021	0.918 ± 0.035	0.778 ± 0.025	0.903 ± 0.015
B	0.557 ± 0.022	0.992 ± 0.025	0.911 ± 0.031	0.792 ± 0.035	0.836 ± 0.030
CSA	0.437 ± 0.014*	0.721 ± 0.037*	0.816 ± 0.013*	0.559 ± 0.036*	0.797 ± 0.031
CSA+SD	0.415 ± 0.018*	0.682 ± 0.043*,a	0.732 ± 0.023*,a	0.594 ± 0.035*	0.785 ± 0.022*
T+C	0.590 ± 0.012b,c	0.976 ± 0.022b,c	0.819±0.016*,b,c	0.651±0.024*,b	0.891 ± 0.019b,c
B+C	0.436 ± 0.014*	0.715 ± 0.021*	0.712 ± 0.020*	0.617 ± 0.024*	0.769 ± 0.030*

- Significant difference *vs*. CN: *P<0.05, between CSA & CSA+SD: [a]P<0.05, between CSA+SD and treated groups: [b]P<0.05, between T+C & B+C: [c]P<0.05.

Table (4.III): The effect of taurine and BSN against CSA and SD on the levels of Serotonin (ug/gm) in different brain areas after 6 weeks of treatment.

Group (n=8)	Cerebral cortex	Cerebellum	Striatum	Pons	Thalamus& hypothalamus
CN	0.394 ± 0.017	0.660 ± 0.018	0.511 ± 0.018	0.352 ± 0.011	0.850 ± 0.027
T	0.365 ± 0.013	0.647 ± 0.023	0.534 ± 0.021	0.348 ± 0.010	0.887 ± 0.020
B	0.382 ± 0.022	0.639 ± 0.023	0.495 ± 0.007	0.349 ± 0.013	0.889 ± 0.020
CSA	0.356 ± 0.021	0.592 ± 0.016*	0.487 ± 0.009*	0.350 ± 0.014	0.855 ± 0.021
CSA+SD	0.283 ± 0.012*,a	0.627 ± 0.024*	0.454± 0.014*,a	0.333 ± 0.010	0.834 ± 0.025
T+C	0.379 ± 0.008b,c	0.666 ± 0.025b,c	0.488 ±0.009b,c	0.350 ±0.009	0.861 ± 0.024
B+C	0.301 ± 0.011*	0.608 ± 0.023*	0.450 ± 0.013*	0.330 ± 0.009	0.815 ± 0.016

- Significant difference *vs*. CN: *P<0.05, between CSA & CSA+SD: [a]P<0.05, between CSA+SD and treated groups: [b]P<0.05, between T+C & B+C: [c]P<0.05.

Studies demonstrated a correlation between clinical symptoms of CSA-dependent neurotoxicity and morphological changes in the brain, such as hypodensity of white matter, cerebral edema, metabolic encephalopathy, and hypoxic damage (Gopal et al, 1999; Shah, 1999).

Another principal mechanism for CSA neurotoxicity is that CSA-mediated neurotoxicity caused by a direct effect of CSA on nervous tissue, earlier reports demonstrated that CSA was present in cerebrospinal fluids of patients treated with the drug (Wijdiks, 2001).

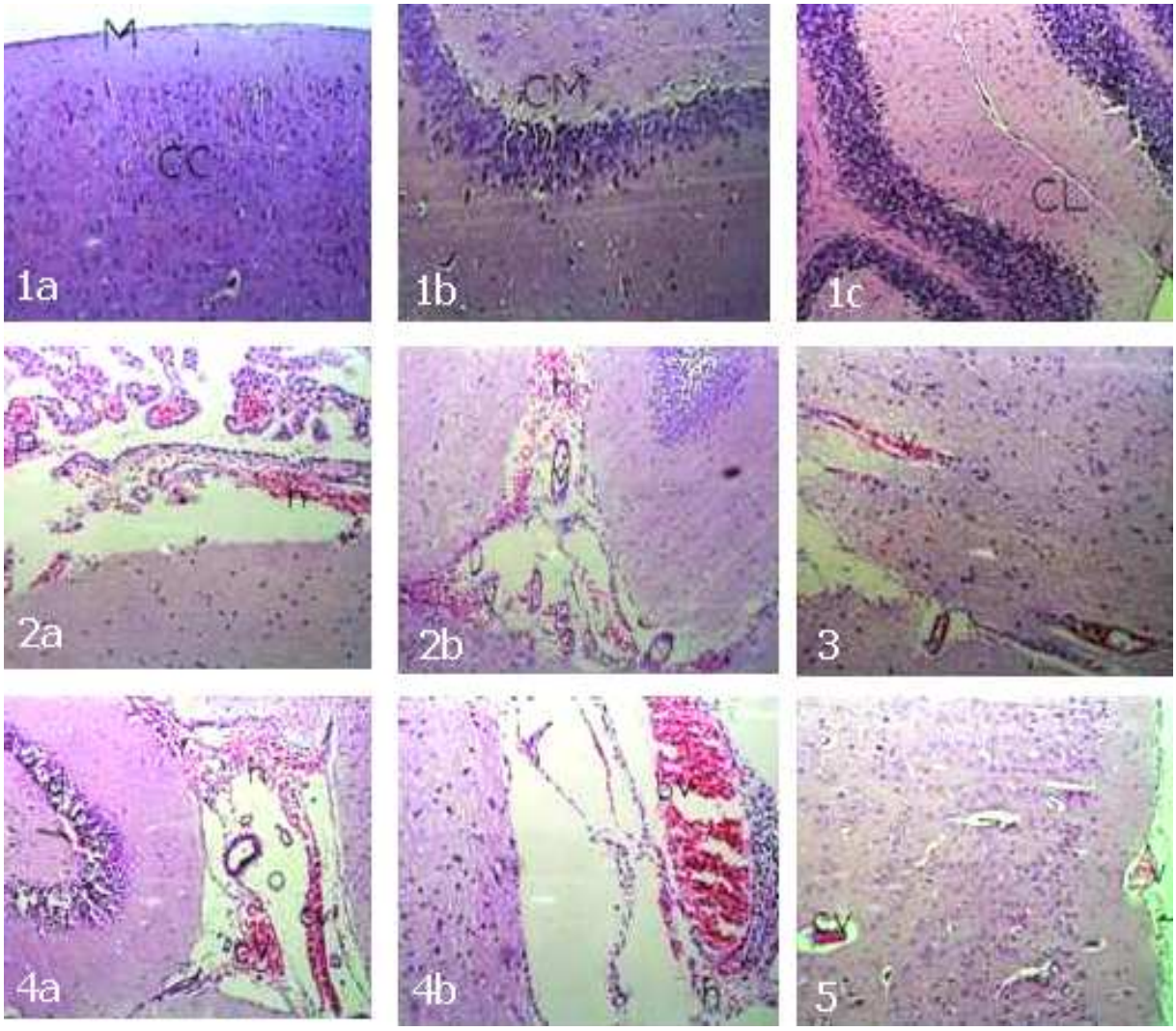

Fig (1a): Brain of CN group showing the normal histological structure of meninges (M) and the cerebral cortex (CC). (H&E X 64).

Fig (1b): Brain of CN group showing the normal histological structure of the cells in the corpus callosum (CM) of med brain. (H&E X 64).

Fig (1c): Brain of CN group showing the normal histological structure of the cerebellum (CL).(H&E X64).

Fig (2a): Brain of CSA group showing congestion in choroids plexus (p) and hemorrhage in the fissure between the two hemisphere (h). (H&E X 64).

Fig (2b): Brain of CSA group showing capillaries congestion with hemorrhage (h) in the two cerebral hemis-phere. (H&E X 64).

Fig (3): Brain of T+C group showing congestion in blood vessel of med brain (v). (H&E X 64).

Fig (4a): Brain of CSA+SD group showing focal hemorrhage in between degenerated cells of corpus callosum as well as hemorrhage (h) oedema (o) and congested blood capillaries.(CV) in the fissure between corpus callosum and cerebral cortex**.** (H&E X 64).

Fig (4b): Brain of CSA+SD group showing severs dilatation and congestion of the blood vessels (bv) with hemorrhage in the fissure between the cerebral tissue and med brain. (H&E X 64).

Fig (5): Brain of B+C group showing congestion in blood capillaries (v) and oedema (arrow) of the meninges as well as congestion in blood capillaries (cv) of cerebral cortex. (H&E X 64).

In the brain of lung-transplanted monkeys treated with CSA, low CSA concentrations were found in the brainstem (tissue-to-blood partition coefficient: 0.3), cerebellum (0.2), and cerebrum (0.3) (Serkova et al, 2004). Even though distribution of CSA into the brain was less than its distribution into other target organs of CSA toxicity (i.e., kidney; kidney-to-blood distribution coefficient: 4.3), CSA was detected in the brain of each study animal. Finally, studies of Sercova et al, (2002*)* have shown that in the rat, CSA not only penetrates brain tissue, but the drug is also found in mitochondria isolated from CSA treated brain, in vivo as well as in vitro. These results were in good keeping with the present study which showed that all tested brain areas affected by CSA toxicity and exhibited marked inhibition of mitochondrial energy, inhibition of neuroamines release and generation of free radicals result in increase oxidative stress alternations. The potency of affected brain area were arranged of ordered; cerebral cortex > striatum > cerebellum = pons > thalamus &hypothalamus (Table 5 & Figure B).

Table (5): The percentage differences from the control values to show the potent effected brain areas [Cerebral cortex (CT), Cerebellum (CR), Striatum (ST), Pons (P), and Thalamus & hypothalamus (H)] against CSA and SD-induced neurotoxicity.

Parameters	CT	CR	ST	P	H
MDA	↑ 20%	↑ 22%	↑ 45%	↑ 22%	↑ 3%
GSH	↓ 22%	↓ 23%	↓ 25%	↓ 35%	↓ 11%
NA	↓ 23%	↓ 23%	↓ 17%	↓ 22%	↓ 13%
DP	↓ 28%	↓ 31%	↓ 21%	↓ 25%	↓ 8%
SR	↓ 28%	↓ 5%	↓ 11%	↓ 8%	↓ 2%
NO	↓ 38%	↓ 23%	↓ 20%	↓ 16%	↓ 15%
ATP	↓ 17%	↓ 3%	↓ 17%	↓ 6%	↓ 25%
Average change /area	25%	19%	22%	19%	11%

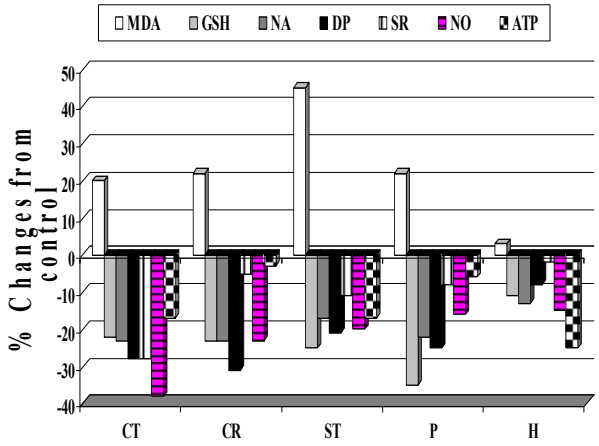

Figure (B): The percentage differences from control values to show the potent effected brain areas against CSA&SD-induced neurotoxicity.

Coadminisration of CSA with the nonesteriodal antiinfilammatory drug (NSAID) sodium diclofenac (SD) increases the incidence of neurotoxicity. The data recorded in the combined treated group (CSA+SD), depicted in tables (1, $2_{I, II, III}$ & 3), showed significant decrease in ATP, GSH, and NO production in blood and brain tissue ($P<0.05$ *vs.* CSA treated alone), increment of both serum and brain MDA, LDH release and increase in blood glucose concentration ($P<0.05$ *vs.* CSA treatment). Earlier evidences revealed that SD induced toxicity may involve production of ROS leading to oxidative stress and massive genomic DNA fragmentation and apoptotic cell death (Hickey et al, 2001). In addition, significant increase of brain MDA production accompanied with significant reduction in GSH levels that reflected by significant inhibition of dopanergic neurotransmitter in mice treated with NSAIDs (Salwa et al, 2002). Furthermore, it was found that co-administration of NSAIDs enhanced the excitatory effect of ofloxacin by decreasing the inhibitory amino acid GABA, inhibiting the release of monoamines including NA, DP, and serotonin in rat brain tissue (Nadia et al 2004). It was found that most NSAIDs penetrate poorly into the CNS. However, the cyclooxygenase (COX) enzymes are expressed constitutively in some areas of the CNS, meaning that even limited penetration may cause adverse effects such as

somnolence and dizziness (Rossi, 2006). Confirming the biochemical results in present study, histopathological observations in brain tissues of combined treatment showed structural changes in brain more than observed in CSA alone, it showed severs dilatation and congestion of the blood vessels with sever hemorrhage in the two cerebral hemisphere, cerebellum, cerebral tissue, med brain and in the fissure between them in plus to edema of the meninges than that observed in CSA treatment alone (Fig$4_{a,b}$). The primary mechanism responsible for SD anti-inflammatory/analgesic/antipyretic action is inhibition of prostaglandin synthesis by inhibition of cyclooxygenase (Slagle, 2001) lead to reducing formation of leukotrienes that it might be responsible for its neurotoxicity. It was fount that the decrease in the level of prostaglandin in different tissue might be responsible in the dysfunction of this organ. Decrease prostaglandin in epithelium of stomach cause gastrointestinal bleeding (Rossi 2006). In kidney cause nephrotoxicity (Brater, 2002; Rossi, 2006), in heart cause heart attacks (Solomon et al 2006). Long term treatment of NSAID develop neurological side effects included; confusion, depression, dizziness, headache, sedation, sleep disturbance, somnolence (Slagle, 2001).These neurological side effects might be due to the inhibition of prostaglandin synthesis in neuron tissue. This postulation can confirmed the result of Whelton, (1999) and Huang, (2000) who demonstrated that neurotoxicity of NSAIDs is due to renal insufficiency of prostaglandin that lead to development of CNS toxicity. On the other hand drug interaction between immunosuppressive and NSAIDs increase the level of immunesupp-ressive in blood. Thus coadministration of SD with SCA might responsible in increasing its level in blood and in turn increased the incidence of neurotoxicity of CSA. This postulation was in good keeping with the study of Mignat (1997), which described an increased risk of CNS toxicity following the combined use of NSAID and immunosuppressive drugs. To interpret the latter observations, the present data strongly explored potential drug interactions when CSA co-administered with SD that cause deleterious effects on brain tissue that enhancement inhibition of mitochondrial energy, inhibition of neuroamines release and increase oxidative stress.

On other hand, BSN co-administration antagonized to a lesser extends some of CSA & SD-induced neurotoxicity. It has been shown to attenuate the level of GSH, decreased MDA production on both blood and tested brain areas (Table$2_{I, II}$) as well as reduces serum LDH activity (Table 3). On contrast, BSN have no effect on NO production and could not succeeded to ameliorate CSA-induced inhibition of both mitochondrial energy and neurotransmitters release (Tables1 & $4_{I, II, III}$). Furthermore the histopathological changes that induced by CSA & SD treatment were still observed and could not be attenuated by BSN treatment (Fig 5). It was found that Bi can produce neurotoxic effects in both humans and animals under certain dosing condition (a single 2500 mg/ kg i.p.), the pattern of regions and cells with the highest Bi accumulation is very similar to pattern reports for xenobiotic metals (i.e. mercury, silver, gold) and supports the hypothesis that these metals may share some mechanisms for entry, distribution and storage in the brain (Ross et al, 1996) on other hand, Noach, et al, (1995) concluded that the normal use of BSN and bismuth subcitrate does not exhibit clinical neurotoxicity. This later finding was in agreement with the present results, which does not exhibit any neurotoxic symptoms, when rats treated with 15 mg/kg of BSN alone. BSN an antiulcer, used to protect against ulceration produced with long treatment of SD. Many studies showed that BSN can protect renal tissues against drug toxicity by inducing metallothionine (MT) in renal tissues (Kondo et al, 2004). MT is a thiol rich protein contain sulfur group that bind with radical peroxide to protect cell from radicals induced damage. Furthermore BSN found to bind to and induce GSH as similar as MT, as seen by the extended x-ray absorption (Sun et al, 1999). To interpret the latter observations, the present result showed that BSN markedly increase ($P<0.05$) the lower level of GSH induced by CSA (Table 2_I). GSH is an efficient endogenous antioxidant defense system operates to compact free radicals and plays a vital role in protection of cells against oxidative stress and detoxification of xenobiotes including CSA. Thus induction for brain GSH by BSN might be the major role of BSN in, somewhat, protecting brain tissue against CSA-induced oxidative damage and neurotoxicity.

On the other hand, the co-administration of the potent cytoprotective antioxidant, taurine,

antagonized (P<0.05) all CSA negative effects, by antagonizing CSA & SD-induced mitochondrial dysfunction showed significant increase in serum and brain ATP, and increase in blood glucose concentration (P<0.05 *vs.* CSA & SD treated group), improvement oxidative stress by increasing the level of serum and brain GSH & NO and decline of serum and brain MDA product, and decrease activity of LDH release (P<0.05 vs. CSA & SD treated group), as well as modulated neurotransmitters release by increasing the level of NA, DP & serotonin as regarding to the level recorded in CSA&SD treated group (Tables1, $2_{I, II, III}$, 3 & $4_{I II, III}$). Taurine, a ß-amino acid found at high concentrations in the brain. It was postulated that neuroprotective action of taurine may be through several mechanisms, include; 1) activation of GABA receptors that decreases neural vulnerability to exictotoxic damage (Louzada, 2004) in agreement it was found that CSA-induced exictotoxic damage to neuron through inhibition of glutamate and GABAergic transmission (Serkova et al, 2001) which accompanied by inhibition the release of monoamins (NA, DP & SR). 2) It can interfere with the activity of the renin-angiotensin-aldosterone system and minimized the elevation in serum cytokine, endothelin, and thromboxane B_2 and inhibited the proliferation of vascular smooth muscle cells (Hu et al, 2009), in addition it increased serum levels of nitric oxide and nitric oxide synthase (Fennessy et al, 2003; Hager et al, 2006), which all lead to inhibit vasoconstriction induced by CSA and attenuated its neurotoxicity. 3) taurine is a potent antioxidant and may attenuate tissue lipid peroxication either by scavenging a wide variety of oxygen derivative free radicals or by binding Fe^{2+} like a chelator or forming chloramines with HOCl and HOCl-metalloproteins, or by binding to or complixing the sulfonic acid group (SO3-) to free metal ion species such as Fe^{2+}, Cu^{+} or oxidant metalloprotein (Erdem et al, 2000). Treatment of rats with taurine attenuated CSA-induced depletion of GSH. It was being found that taurine has the ability to increase intracellular and extracellular GSH; this might be a crucial factor in protecting brain tissue in CSA-induced oxidative damage. Finally, the data presented here suggest that concomitant use of the antioxidant taurine might be useful in reducing CSA-mediated neurotoxicity and also postulated that taurine has a protective action against a variety of toxins where cellular damage is a consequence of ROS. Confirming the biochemical results, histopathological changes that induced by CSA & SD treatment showed marked improvement by taurine treatment, except some congestion in blood vessel of med brain was observed (Fig 3).

In conclusions, this study indicated that; there was a negative relationship when CSA co-administered with SD and a positive one when co-administered with taurine and to a lesser extend positive relation when combined with BSN against CSA-induced neurotoxicity. 2) Reactive oxygen species (ROS) is one of the most key roles in mediating the negative effects of CSA. 3) Taurine has more potent effect than BSN. 4) Taurine exhibited protective effect against a variety of CSA side effects, the potent of which exist where cellular damage is a consequence of ROS. 5) Histopathological examination done on brain sections reinforced the results obtained.

REFRENCES

1. Allison AC. Immunosuppressive drugs: the first 50 years and a glance forward. Immunopharmacology. 2000; 47, 63-83.
2. Bancrofet JD, Stevens A, Turner DR. Theory and Practice of Histological Techniques 4th eds., Churchil Livingstone, New York, London, San Francisco, Tokyo 1996.
3. Bartynski WS, Zeigler ZR, Shadduck R K, Lister J. Variable incidence of cyclosporine and Fk-506 neurotoxicity in hematopoitic malignancies and marrow conditions after allogenic bone marrow transplantation. Neurocrit Care. 2005; 3(1): 33-45.
4. Beal MF. Mitochondrial dysfunction in neurodegenerative diseases. Bioch. Biophys. Acta. 1998; (1366): 211-223.
5. Brater DC. Renal effects of cyclooxyenase-2-selective inhibitors. J, Pain Sympt Manage. 2002; 23, S15-S20; discussion S21-23.
6. Buhl SN, Jackson KY. Optimal conditions and comparison of lactate dehydrogenase catalysis of the lactate-to-pyruvate and pyruvate-to-lactate reactions in human serum at 25, 30, and 37 degrees C. Clin.Chem. 1978; 24: 828.
7. Christians U, Gottschalk S, Miljus J, Hainz C, Benet LZ, Leibfritz D, Sercova N. Alterations in glucose metabolism by cyclosporine link to oxidative stress in rat

brain slices: Interaction with mTOR inhibitors. Br. J. Pharmacol. 2004; In press.
8. Dunn CJ, Wagstaff AJ, Perry CM, Plosker GL, Goa KL. Cyclosporine: An updated review of the pharmacokinetic properties, clinical efficacy and tolerability of microemulsion-based formulation (Neoral) organ transplant. Drug 2001; 61, 1957-2016.
9. Erdem A, Gundogan NU, Usubutun A, Kilinc K, Erdem R, Kara A, Bozkurt A. The protective effect of taurine against gentamicin-induced acute tubular necrosis in rats. Nephrol Dial Transplant 2000; 15: 1175-1182.
10. Everett SA, Dennis M.F, Tozer G.M., Prise V.E., Wardman P and Stratford MRL. Nitric oxide in biological fluids" analysis of nitrite and nitrate by high-performance ion chromatography. Journal of Chromatography 1955; 706, 437-442.
11. Fennessy FM, Moneley DS, Wang JH. Kelly CJ, Bouchier-Hayes DJ. Taurine and Vitamin C Modify Monocyte and Endothelial Dysfunction in Young Smokers. Circulation 2003; 107(3):410-415.
12. Gijtenbeek JM, Van Den Bent MJ, Vecht CJ. Cyclosporine neurotoxicity: A review. J. Neurol. 1999; 246, 338-346.
13. Gokcimen A, Ragbetli MC, Bas O, Tune AT, Slan H, Yazici AC, Kaplan S. Effect of prenatal exposure to an anti-inflammatory drug on neuron number in cornu ammonis and dentate gyrus of the rats hippocampus: a stereological study. Brain Res. 2007; 1127(1): 185-192.
14. Gopal AK, Thorning DR, Back AL. Fetal outcome due cyclosporine neurotoxicity with associated pathological findings Bone Marrow Trasplant. 1999; 23, 191-193.
15. Hager HH, El Etter Arafa M. Taurine attenuates hypertension and renal dysfunction induced by cyclosporine A in rats. Clin. and Exp. Pharmac. 2006; 33: 189-196.
16. Heldernman JH, Bennet WM, Cibrik DM, Kaufman DB, Klein A, Takemoto SK. Immunosuppression : Practice and trends Am J. Transplant. 2003; 3, 41-52.
17. Hickey EJ, Raje RR, Reid VE, Gross SM, Ray SD. Diclofenac induced in vivo nephrotoxicity may involve oxidative stress-mediated massive genomic DNA fragmentation and apoptotic cell death. Free Radical Biology and medicine. 2001; 31: 139-152.
18. Higginson J, Wackerhage H, Woods N, Schjerling P, Ratkevicius A, Grunnet N, Quistoff B. Blockades of mitoge-activated protein kinase and calcineurin both change fiber-type markers inn skeletal muscle culture. Pflugers Arch. 2002; 445, 437-443.
19. Huang SH. Rheumatology. Basics of therapy CMAJ. 2000; 163(4): 417-423.
20. Hu J, Xu X, Yang J, Wu G, Sun C, Lv QA. Antihypertensive Effect of Taurine in Rat Advances in Experimental Medicine and Biology, 643, Taurine 7, Biomedical and Life Sciences, Springer New York, Part I 2009; pages 75-84.
21. Jayatilleke E, and Shaw S. A high-performance liquid chromatographic assay for reduced and oxidized glutathione in biological samples. Anal. Biochem. 1993; 214(2): 452-457.
22. Karatepe M. Simultaneous Determination of ascorbic acid and free malonialdehyde in human serum by HPLC-UV. LCGC NORTH AMERIKA 2004; 22(4): 362-365.
23. Kondo Y, Himeno S, Satoh M, Naganuma A, Nishimura T, Imura N. Citrate enhances the protective effect of orally administered bismuth subnitrate against the nephrotoxicity of cis-diamminedichloroplatinum. Cancer Chemother. Pharmacol., 2004; 53:33-38.
24. Kou R, Greif D, Michel T. Dephosphor-ylation of endoththelal nitric-oxide synthase by vascular endothelial growth factor. Implications for the vascular responses to cyclosporine A. J. Biol. Chem. 2002; 277, 29669-29673.
25. Kozlowaska I, Rozaniski J, Ciechanowski K. Neurotoxicity of cyclosporine. Wiad. Lek. 2006; 59(7-8):516-520.
26. Louzada PR, Lima AC, Mendonca-Silva DL, Noel F De Mello FG Ferreira ST. Taurine prevent the neurotoxicity of beta amyloid and glutamate receptor agonists: activation of GABA receptors and possible implications for Alzheimer's disease and other neurological disorders. FASEB-J. 2004; 18(3):511-518.
27. Luo XD, Liu QF, Ning J, Fan ZP, Xu D, Wei YQ. A clinical analysis of sever cyclosporine A-related neurotoxicity after allogenic hema-tobiotic stem cell transplantation. Zhonghua

Nei. Ke. Za. Zhi. 2008; 47(1):40- 43.
28. Mignat, C. Clinically significant drug interactions with new immunosuppressive agents. Drug Saf., 1997; 16(4): 267-78.
29. Nadia MS., Suzanne FI. El-Sisi, Salwa MT. Evaluation of the role of fluoroquinones co-administered with analgesic drugs on CNS in albino rats. J. of drug Res. Egypt. 2004; 25(1-2): 92-101.
30. Noach LA, Eekhof JL, Bour LJ, Posthumus_Meyjes FE, Tytgat GN, Ongerboer De Visser BW. Bismuth salts and neurotoxicity. A randomized, single-blind and control study. Hum. Exp. Toxicol. 1995; 14 (4): 349-355.
31. Pagel P, Blome J, Uwe WH. High performance liquid chromatographic separation and measurement of various biogenic compounds possibly involved in the pathomechanism of Parkinson's disease. J. Chromatography B. 2000; 746: 297-304.
32. Raza S, Ullah K, Ahmed P, Satti TM, Kamal MK, Chaudhry QU. Cyclosporine induced neurotoxicity in a stem transplant recipient. J. Pak. Med. Assoc. 2007; 57(12): 611-613.
33. Ross JF, Switzer RC, Poston MR, Awhorn GT. Distribution of bismuth in the brain after intraperitoneal dosing of bismuth subnitrate in mice: implication for routs of entry of xenobiotic metals into the brain. Brain Res. 1996; 725 (2): 137-54.
34. Rossi S. Australian Medicines Handbook Adelaide: Australian Medicines Handbook; 2006. IBN 0-9757919-2-3.
35. Salwa MT, Nadia MS, Suzanne FI, El-Sisi Study of drug interaction of antacid and some Analgesic drugs. J. Drug Res. Egypt 2002; 24, (1-2):125-131.
36. Sercova N, Donohoe P, Gottschalk S. et al. Comparison of the effects of cyclosporine A on the metabolism of perfused rat brain slices during normoxia and hypoxia. J. Cerb. Blood Flow Metab. 2002; 22, 342-352.
37. Sercova N, Jacobsen W, Niemann CU, Litt L, Benet LZ, Leibfritz D. Christian U. Sisolimus, but not the structurally related RAD (everolimus) enhances the negative effects of cyclosporine on mitochondrial metabolism in rat brain. Br. J. Pharmacol. 2001; 133, 875-885.
38. Sercova N.J, Christians U, Benet LZ. Biochemical mechanisms of cyclosporine nephrotoxicity Molecular Intervention 2004; 4: 97-107.
39. Serkova N, Husen B, Berry GJ. Jacobsen W, Benet LZ, Morris RE Christians U. Tissue distribution and clinical monitoring of the novel macrolide immunosuppressant SDZ-RAD and its Metabolites in monkey lung transplant recipients: Interaction with cyclosporine. J. Pharmacol. Exp. Ther. 2000; 294, 323-332.
40. Serkova N, Litt L, James TL, Sadee W, Leibfritz D, Benet LZ, Christian U. Evaluation of individual and combined neurotoxicity of immunosuppressant cyclosporine and sirolimus by in vitro multinuclear NMR spectroscopy. J. Pharmacol. Exp. Ther. 1999; 289, 800-806.
41. Shah AK. Cyclosporine A neurotoxicity among bone marrow transplant recipient. Clin. Neuropharmacol. 1999; 22, 67-73.
42. Slagle, M. A.. Featured CME Topic: Pain Management. 2001; Southern Med. J. 94, 771-774.
43. Solomon D, Avorn J, Glynn R, Mogun H, Schneweiss S. Cardiovascular outcomes in new users of coxbs and nonsteriodal anti-inflammatory drugs: high risk subgroups and time course. Arthritis Rheum. 2006; 54(5): 1378-1389.
44. Sun H, Li H, Harvey I, Sadler PJ. Interaction of Bismuth complexes with metallothionin (II). J. Biol. Chem. 1999; 274(41): 29094-29101.
45. Trinder P. Determination of blood glucose in blood using glucose oxidase, an alternative oxygen acceptor. Ann. Clin. Biochem., 1969; 6:24-28.
46. Whelton A. Nephrotoxicity of nonesteriodal anti-inflammatory drugs: Physiologic foundation and clinical implications Am J. Med. 1999; 106 (5B): 13S-24S.
47. Wijdiks EF.M. Neurotoxicity of immune-suppressive drugs. Liver Transpl. 2001; 7, 937-942.
48. Zhang YW. Long. and Shi SY. Determination of the adenosine triphosphate in myocardial tissue by ion-pair reversed phase high performance liquid chromate-graphy. Se Pu. 2000; 18(4):322-324.

3/9/2009