

Lactulose and Donepezil Ameliorate Thioacetamide-Induced Hepatic Encephalopathy in Rats

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ABSTRACT: Hepatic encephalopathy (HE) is neuropsychological complication that is common in patients with acute or chronic liver disease as well as in porto-systemic shunting of blood flow. The pathophysiology of this disease is quite complex, as it involves overproduction and reduced metabolism of various neurotoxins, particularly ammonia. Thioacetamide (TAA)-induced HE is a reliable model of HE in which rats were given thioacetamide (TAA) 200mg/kg orally for 2 consecutive days. The TAA group showed lower motor activity than the normal group by using open field and forced swimming tests. Oxidative stress conditions were manifested by free radical production, lipid peroxidation, reduced glutathione and nitric oxide contents. Alterations in the metabolism of monoamine neurotransmitters have been proposed to be involved in the development of the HE associated with experimental and human liver failure. Pretreatment with lactulose or donepezil could counteract these effects. The protective effect of both lactulose and donepezil can be attributed to their antioxidant and neuromodulatory potential.

Keywords: Hepatic encephalopathy; Thioacetamide; Lactulose; Donepezil; Oxidative Stress; Neurotransmitters.

INTRODUCTION:

Acute liver failure (ALF) is a rare but life-threatening consequence of an abrupt loss of hepatic function in a patient with no previous history of liver disease. Hepatic encephalopathy (HE), in particular, is associated with serious neurological complications, characterized by severe cognitive and psychiatric disturbances ranging from alteration of consciousness to coma (Ferenci *et al.*, 2002). Ammonia has been thought to play a major role in the pathogenesis of the neurological complications of HE, but recent studies strongly suggest that inflammation, acts alone or in concert with ammonia (Butterworth, 2011).

The diminution of cerebral function that occurs in human patients with liver disease and hyperammonaemia is mirrored by lower cerebral metabolic rates of glucose and oxygen (Hawkins and Mans, 1989). Portacaval shunting in rats, a common model of chronic liver dysfunction, also results in hyperammonaemia and a substantial decrease in the cerebral metabolic rates of glucose (Mans *et al.*, 1986). Ammonia is suspected to be responsible for causing cerebral dysfunction (Butterworth *et al.*, 1987).

Thioacetamide (TAA) is a thiono-sulphur-containing compound, that when administered in one dose leads to acute toxic liver injury characterized by centrilobular necrosis with subsequent regenerative response (Chen *et al.*, 2008). Chronic use of TAA causes hepatic cirrhosis and liver tumours (Natarajan *et al.*, 2006).

Thioacetamide-intermediates and reactive oxygen species can covalently bind to biologically important molecules and increase lipid peroxidation, and deplete glutathione (Sanz *et al.*, 2002). Both necrosis and apoptosis appear in the process of cell death after TAA application (Amodio *et al.*, 2004).

Lactulose passes unchanged into the colon where it is hydrolysed by bacterial action to organic acids, principally acetate and lactate. Proposed mechanisms of action are lowering colonic pH, thereby decreasing the production of ammonia by bacteria (Vince *et al.*, 1973) and the absorption of non-ionised ammonia (Castell and Moore, 1971). In addition lactulose serves as substrate to increase the incorporation of ammonia into bacterial protein (Vince *et al.*, 1978); and decreases the intestinal transit time available for production and absorption of ammonia because of its cathartic effect (Agostini *et al.*, 1972). Lactulose is effective for secondary prophylaxis of HE in patients with cirrhosis (Agrawal *et al.*, 2012).

Donepezil could improve cognitive dysfunction in a case of delayed encephalopathy (Song *et al.*, 2011). It is a specific non-competitive reversible inhibitor of acetylcholinesterase (AChE) (Rogers and Friedhoff, 1996). Donepezil exhibits a relatively high degree of selectivity for neuronal AChE (Dickinson, 1996; Rogers and Friedhoff, 1996); at the present time, donepezil is the leading compound for AD treatment in the world (Sugimoto *et al.*, 2002). As compared with other AChEIs, donepezil exhibits the best pharmacological profile in terms of cognitive improvement (Giacobini, 2006).

Thus it seemed interesting to investigate the possible protective potential of both lactulose and donepezil in TAA-induced HE in rats.

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MATERIALS AND METHODS:

Animals:

Adult male Sprague-Dawley rats weighing 200-210 g were used. They were obtained from the breeding colony maintained at the animal house of the National Organization for Drug Control and Research (NODCAR, Cairo, Egypt). Animals were allowed free access to standard diet and tap water ad libitum. The animals were housed at room temperature.

Selection of the doses of the used test drugs was based on the published literatures as follows:

1- Thioacetamide (200 mg/kg); p.o (Jia and Zhang, 2005).

2- Lactulose (8 ml/kg); p.o (Jia and Zhang, 2005).

3- Donepezil hydrochloride (10 mg/kg); p.o (Yamaguchi *et al.*, 2001).

The rats were randomly allocated into four groups.

Each group consisted of 12 rats. The animals were treated according to the following scheme:

Group 1: animals received saline for 7 consecutive days, p.o., and served as normal group.

Group 2: animals received saline for 7 consecutive days, p.o., and served as control HE group.

Group 3: animals received lactulose (8 ml/kg; p.o) for 7 consecutive days.

Group 4: animals received donepezil (10 mg/kg; p.o) for 7 consecutive days.

In all groups except the 1st group (normal control group) HE was induced by administration of TAA (200mg/kg, p.o) on 6th and 7th days of treatment that served as normal group.

To avoid hypoglycemia and electrolyte imbalance, all rats were given a supportive therapy (25 ml/kg, s.c) which consisted of a solution containing 10% glucose solution mixed with lactate Ringer (1:1) every 12 hours after the first injection of TAA (Gammal *et al.*, 1990; Chu *et al.*, 2001).

At the end of the experiment (7th day) and after 4 hours from the second dose of TAA. Each group was subdivided into two subgroups:

At the 1st subgroup animals were subjected to the open field behavioral test (Cunha and Masur, 1978) to record latency period, ambulation, grooming and rearing frequencies. While the animals of the 2nd subgroup were subjected to the forced swimming behavioral test (Porsolt *et al.*, 1978) for recording Immobility time and Struggling time.

Blood was withdrawn via the retro-orbital plexus of each animal using a capillary tube, in order to measure blood ammonia (Huizenga *et al.*, 1994), serum albumin (Burtis and Ashwood, 1999), total protein (Gornal *et al.*, 1949) levels and estimate serum alanine aminotransferase (ALT) (Burtis *et al.*, 1999), aspartate aminotransferase (AST) (Burtis *et al.*, 1999), γ -glutamyl transferase (GGT) (Szasz, 1969), and alkaline phosphatase (ALP) (Belfield and Goldberg, 1971) activity.

Rats were then sacrificed by decapitation; brain and liver were carefully isolated, blotted and chilled at -80°C till used for estimation of oxidative stress biomarkers: malondialdehyde (MDA) (Deniz *et al.*, 1997; Buege and Aust, 1978), reduced glutathione (GSH) (Beutler *et al.*, 1963) and nitric oxide (NO)

(Montgomery and Dymock, 1961) contents in both tissues. Furthermore, the activity of acetylcholinesterase (CHE) (Weber, 1966) and the content of brain neurotransmitters: serotonin (5-HT), dopamine (DA), noradrenaline (NA) (Ciarlone, 1978) and γ -aminobutyric acid (GABA) (Sutton and Simmonds, 1974) were estimated.

Statistical Analysis:

All values were presented as arithmetic mean with their standard error. Data were assessed by analysis of variance (ANOVA) followed by Tukey-Kramer's multiple comparison test. The value was considered significant when $P < 0.05$.

RESULTS:

Open field behavioral test: (Fig.1)

The latency time and grooming frequency of normal rats were 1.17 ± 0.17 sec & 2.17 ± 0.48 sec respectively. Administration of TAA increased both latency time & grooming frequency to be 314% & 208% respectively as compared to normal rats. Mean while ambulation & rearing frequencies of normal rats were 14.0 ± 0.73 & 14.5 ± 1.09 respectively. Administration of TAA reduced ambulation & rearing frequencies to be 24% & 23% respectively of that in normal group.

Pretreatment with lactulose (8ml/kg, p.o.) reduced both latency time & grooming frequency and increased both ambulation & rearing frequencies by 50%, 48%, 145% and 145% respectively of TAA-control group.

Pretreatment with donepezil (10mg/kg, p.o.) reduced latency time and increased both ambulation and rearing frequencies by 68%, 255% and 255% respectively of that in TAA-control group.

Forced swimming behavioral test: (Fig.2)

The immobility and struggling times (sec.) of normal rats were 138.83 ± 2.98 and 161.17 ± 2.98 respectively. Administration of TAA increased the immobility time to be 185.35% and decreased struggling time to be 26.5% as compared with that of normal rats. Administration of lactulose (8ml/kg, p.o.) or donepezil (10mg/kg, p.o.) daily for 7 days starting 5 days before TAA administration decreased the immobility time by 27%, and 29% respectively and increased the struggling time by 169% and 177% respectively as compared with TAA-treated rats.

Biochemical parameters:

Serum ammonia level ($\mu\text{mol/ml}$): (Table 1)

The ammonia (NH_3) level of normal rats was 6.41 ± 0.69 . TAA administration increased the NH_3 level to be 815% as compared with that of normal rats. Both lactulose & donepezil reduced serum NH_3 level by 37% and 20% respectively of that in TAA-treated rats.

Serum albumin level (g/dL): (Table 1)

The albumin level of normal rats was 4.90 ± 0.05 . TAA administration decreased the albumin level to be 77% of that in normal rats. Oral administration of donepezil increased the albumin level by 11% of that in TAA-treated rats.

Serum total protein level (g/dL): (Table 1)

The total protein level of normal rats was 7.70 ± 0.16 . TAA administration decreased the total protein level to

be 79% of that in normal rats. Lactulose and donepezil increased the total protein level by 25% and 18% respectively as compared with TAA-treated rats.

Serum γ - glutamyl transferase (GGT) activity (U/L): (Table 1)

The γ - glutamyl transferase (GGT) activity of normal rats was 1.70 ± 0.15 . Administration of TAA increased the GGT activity to be 301% as compared with that of normal rats. Both lactulose and donepezil decreased the GGT activity by 41% and 44% respectively as of that in TAA-treated rats.

Serum aminotransferases activity (U/L): (Table 1)

The aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities of normal rats were 22.00 ± 1.41 & 17.17 ± 0.70 respectively. Administration of TAA increased both AST and ALT activities to be 337% and 508% respectively as of that in normal rats. Oral administration of lactulose and donepezil showed no effect on AST and ALT activities as compared with TAA-treated rats.

Serum alkaline phosphatase (ALP) activity (IU/L): (Table 1)

The alkaline phosphatase (ALP) activity of normal rats was 95.15 ± 3.44 . Administration of TAA increased ALP activity to be 276% as compared with that of normal rats. Oral administration of lactulose and donepezil decreased ALP activity by 29% and 16% respectively as compared with TAA-treated rats.

Oxidative stress biomarkers in liver and brain tissues:

Malondialdehyde (MDA) content (nmol/g wet tissue): (Fig.3)

The liver and brain malondialdehyde (MDA) content of normal rats were 52.80 ± 2.83 and 126.27 ± 2.79 (nmol/g wet tissue) respectively. Administration of TAA increased both liver and brain MDA content to be 227% & 150% respectively as of that in normal rats. Pretreatment with lactulose and donepezil decreased both liver and brain MDA content by 23%, 43%, 29% and 21% respectively as of that in TAA-treated rats.

Reduced glutathione (GSH) content (mg/g wet tissue): (Fig.3)

The liver and brain reduced glutathione (GSH) content of normal rats were 764.73 ± 16.04 and 183.55 ± 2.46 (mg/g wet tissue) respectively. TAA decreased both liver and brain GSH content to be 60% & 52% as of that in normal rats. Pretreatment with lactulose and donepezil increased both liver and brain GSH content by 20%, 14%, 88% and 128% respectively as of that in TAA-treated rats.

Nitric oxide (NO) content ($\mu\text{mol/g}$ wet tissue): (Fig.3)

The liver and brain nitric oxide (NO) content of normal rats were 80.30 ± 6.04 and 94.47 ± 10.06 ($\mu\text{mol/g}$ wet tissue) respectively. TAA increased both liver and brain NO content to be 247% and 289% respectively as of that in normal rats. Pretreatment with lactulose and donepezil decreased both liver and brain NO content by 39%, 39.5%, 57% and 66% respectively as of that in TAA-treated rats.

Neurochemical parameters:

Brain serotonin (5-HT) content ($\mu\text{g/g}$ wet tissue): (Table 2)

The brain serotonin (5-HT) content of normal rats was 0.53 ± 0.01 ($\mu\text{g/g}$ wet tissue). TAA administration increased the 5-HT content to be 173% as of that in normal rats. Both lactulose and donepezil decreased the 5-HT content by 34% and 28% respectively as of that in TAA-treated rats.

Brain dopamine (DA) content ($\mu\text{g/g}$ wet tissue): (Table 2)

The brain dopamine (DA) content of normal rats was 6.93 ± 0.12 ($\mu\text{g/g}$ wet tissue). TAA decreased the DA content to be 77% as of that in normal rats. Both lactulose and donepezil increased the DA content by 15% and 16% respectively as of that in TAA-treated rats.

Brain noradrenaline (NA) content ($\mu\text{g/g}$ wet tissue): (Table 2)

The brain noradrenaline (NA) content of normal rats was 1.08 ± 0.04 ($\mu\text{g/g}$ wet tissue). TAA decreased the NA content to be 68% as of that in normal rats. Pretreatment with lactulose increased the NA content by 23.5% as of that in TAA-treated rats.

Brain γ -aminobutyric acid (GABA) content ($\mu\text{g/g}$ wet tissue): (Table 2)

The brain γ -aminobutyric acid (GABA) content of normal rats was 707.27 ± 12.30 ($\mu\text{g/g}$ wet tissue). TAA increased the GABA content to be 136% as compared with that of normal rats. Pretreatment with lactulose and donepezil decreased the GABA content by 19% and 26% respectively as compared with TAA-treated rats.

Brain cholinesterase (CHE) activity (U/g wet tissue): (Table 2)

The brain cholinesterase (CHE) activity of normal rats was 8308.75 ± 632.01 (U/g wet tissue). TAA increased the CHE activity to be 193% as of that in normal rats. Both lactulose and donepezil decreased the CHE activity by 26% and 30% rats.

DISCUSSION:

In the current study, oral administration of thioacetamide (TAA) in a dose of (200mg/kg/day) for 2 consecutive days significantly prolonged the latency period, in the open field test (OFT) accompanied with a significant reduction in both ambulation and rearing frequencies and increased grooming frequency. Furthermore, TAA-treated rats showed increased immobility time and decreased struggling time in the forced swimming test. Behavioral changes are in accordance with previous studies that found HE is associated with disturbances in spontaneous and evoked motor functions (Rao *et al.*, 1994; Borkowska *et al.*, 1997). Increased passive behavior responses in the forced swimming test such as immobility and decreased active behaviors like swimming or struggling, are thought to be a clear indication of depressive like symptoms (Andrade *et al.*, 2007). In addition, rats receiving consecutive injections of TAA had apparently lower motor activity (Chu *et al.*, 2001). The motor symptoms of HE are a consequence of basal ganglia dysfunction (Weissenborn and Kolbe, 1998). Another study suggested that psychomotor activity slowing in liver disease is due to alterations affecting

the neuronal circuits between basal ganglia and prefrontal cortex (Amodio *et al.*, 2004). This network includes basal ganglia, motor thalamus and cerebral cortex. The basal ganglia produce signals that go to the thalamus which sends signals to the cortex to modulate movement execution. The signals originated in the thalamus are modulated by substantia nigra pars reticulata (SNr), which sends inhibitory projections to the ventro-medial thalamus (VMT) (Oertel and Mugnaini, 1984).

In addition to behavioral alterations elevated blood ammonia level was also observed. This was in harmony with previous studies that showed increased blood ammonia, which is a catabolic product of protein and nitrogenous compounds, is characteristic in fulminant hepatic failure (FHF) (Avni *et al.*, 2003; Sathyaikumar *et al.*, 2007a). Hyperammonemia is caused by reduced hepatic synthesis of urea and glutamate by which the normal liver removes ammonia from the portal blood (Clemmesen *et al.*, 2001; Clemmesen, 2002).

TAA-induced liver injury was evidenced by increased serum transaminases (AST & ALT), alkaline phosphatase (ALP) and γ -glutamyl transferase (GGT) activities accompanied with decrease in albumin and total protein level. These results were in accordance with previous studies (Osada *et al.*, 1986; Matsushashi *et al.*, 2005; Huang *et al.*, 2007). Measurements of serum aminotransferases are sensitive tests of hepatocyte injury and their plasma levels are sensitive indicators of liver necrosis (Kirchain and Gill, 2002). These enzymes were shown to be stored in hepatocytes and released when hepatocytes are acutely damaged. Enhanced activities of these two enzymes might be related to the damage of the liver tissue (Shimamoto *et al.*, 2000).

TAA-induced HE was association with alteration in oxidative stress biomarkers in both the liver and brain, which was manifested as increased malondialdehyde (MDA), nitric oxide (NO) contents accompanied with decreased glutathione (GSH) content. Similar results have been reported in previous studies (Reddy *et al.*, 2004; Tunez *et al.*, 2005; Sathyaikumar *et al.*, 2007b).

Oxidative stress reported in the brain during HE is an evolving concept in the pathogenesis of this syndrome and in ammonia neurotoxicity (Kosenko *et al.*, 1999). Brain is more prone to oxidative stress because of its low levels of antioxidant enzymes (Brannan *et al.*, 1981), high iron content and low repair mechanisms thereby making it more vulnerable to ROS (Kowaltowski and Vercesi, 1999). Higher levels of eNOS may be due to increased numbers of eNOS expressing infiltrating macrophage in FHF. In cirrhosis and in FHF iNOS expression was induced significantly on hepatocytes and kupffer cells/macrophage (Leifeld *et al.*, 2002). Furthermore, ammonia toxicity stimulates nitric oxide synthase (Garcia-Moreno *et al.*, 2005).

In the present study, TAA altered the level of brain neurotransmitters, where brain serotonin (5-HT) and γ -aminobutyric acid (GABA) contents were increased accompanied with a decrease in brain dopamine (DA) and noradrenaline (NA) contents.

In addition, the content of 5-HT precursor, tryptophan and its metabolites 5-HIAA were increased in brains of rats with HE and were significantly correlated to hyperammonia which bypass the liver and accumulate in the brain, where NH_3 is detoxified by amidation to glutamine the accumulation of glutamine leads to increased activity of the brain neutral amino acid transport system resulting in a significant increase in brain concentration of precursor amino acids for biogenic amines (Colombo *et al.*, 1996; Lozeva *et al.*, 2004).

On the other hand, decreased brain dopamine may be secondary to increased dopamine turnover. Altered dopaminergic transmission may be due to manganese (Mn) accumulation in the brain reported to occur in cirrhotic patients (Montes *et al.*, 2001). Meanwhile, reduced NA level may be attributed to decreased transporter sites leading to depletion of intracellular stores of NA in brain. In addition NH_3 accumulation in brain has a reserpine-like effect thus reducing the release of NA (Michalak *et al.*, 2001).

Increased GABA level in plasma enhanced blood-brain barrier permeability, increased GABA concentration and enhanced GABA-ergic activity are considered to be associated with the development of HE in the CNS (Jones, 2000). Previous study showed that rats with hepatic encephalopathy, mRNA expression levels of GABA-A receptor subunits $\alpha 1$, $\beta 1$, $\gamma 2$ increase significantly in basal nuclei, substantia nigra and hippocampi, suggesting that this change may contribute to the pathogenesis of HE (Li *et al.*, 2005).

Enhanced acetylcholinesterase (CHE) activity was observed in TAA-treated rats as compared to the normal group leading to diminished cholinergic transmission. In previous studies, thioacetamide administration alters of acetylcholinesterase activity in brain limbic system regions, which plays a role in attention and memory (Mendez *et al.*, 2011).

Administration of lactulose improved the altered rats' behavior induced by TAA; the improvement was manifested using OFT and FST. Lactulose may improve cognitive functions by reducing ammonia level which is the key factor in the pathogenesis of overt HE in patients with cirrhosis (Prasad *et al.*, 2007).

Furthermore, lactulose significantly improved the altered liver function manifested by lowering GGT and ALP activities as well as albumin, total protein and NH_3 levels.

Lactulose is a nonabsorbable disaccharide, and its mechanism of action is not precisely known. A recent meta-analysis of lactulose in minimal HE (MHE) showed that lactulose prevented the progression to overt HE, reduced blood ammonia levels, and improved health-related quality of life (Alfawaz and Aljumah, 2012).

Moreover lactulose administration improved oxidative status in both liver and brain such as (MDA, GSH, NO) as compared with TAA-treated rats. lactulose can produce considerable amount of hydrogen, as a unique antioxidant. They propose that lactulose is an indirect antioxidant that mobilizes endogenous hydrogen

production which in turn can reduce oxidative stress (Chen *et al.*, 2011).

Furthermore, lactulose improved the altered neurotransmitters such as (5-HT, DA, NA and GABA) as compared with TAA-treated rats. This action may be due to its potency in lowering NH₃ level.

In the present experiments, administration of donepezil showed a significant improvement of the altered rats' behavior induced by TAA; the improvement was manifested using OFT and FST.

Donepezil has shown to improve attention/psychomotor (Salloway *et al.*, 2004), executive (Saykin *et al.*, 2004) and language domains (Petersen, 2005). The previous study found that the antidepressant-like and anti-amnesic effects of donepezil involve an interaction with the σ_1 receptor in brain (Maurice *et al.*, 2006).

Moreover, donepezil increased GSH and reduced the elevated levels of both MDA and NO. This is in accordance with the results of previous study who found that donepezil suppressed oxidative stress (Saxena *et al.*, 2008).

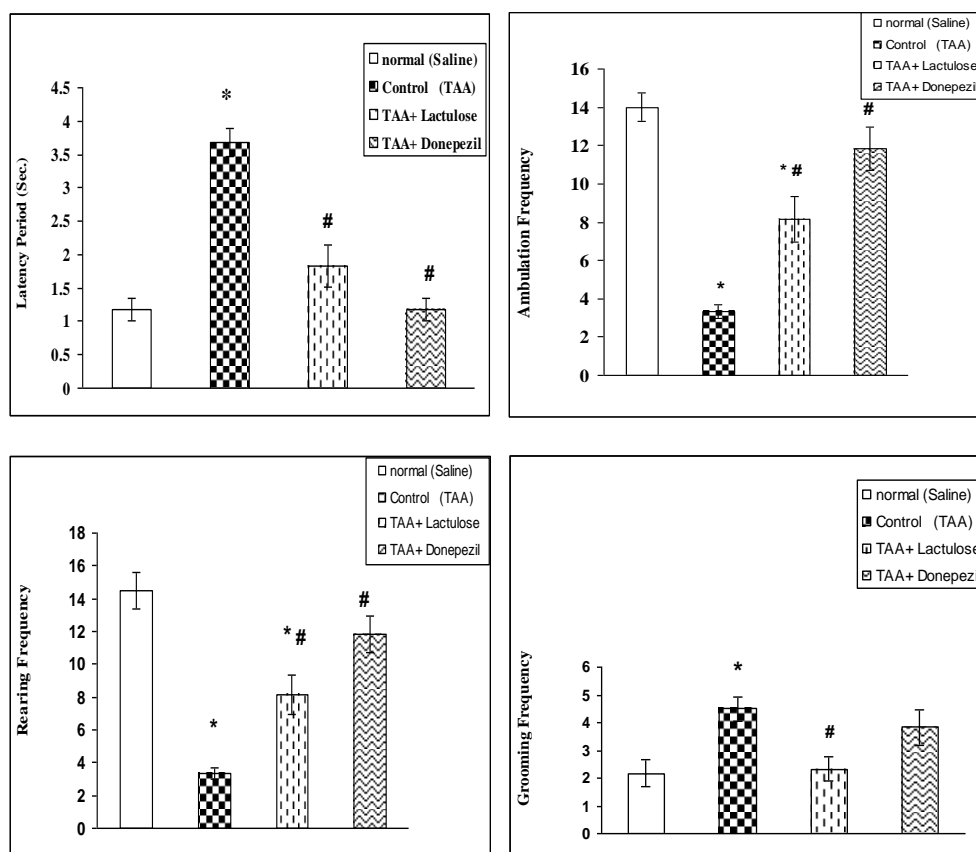
In the present investigation, there were marked

alteration in neurotransmitters such as (5-HT, DA, GABA) after donepezil treatment as compared with TAA-treated rats. A marked elevation of ACh after oral administration of donepezil was associated with a significantly increased release of dopamine (DA) in the medial prefrontal cortex (mPFC) or hippocampus (Liang and Tang, 2006).

In donepezil-treated group, rats showed a significant decrease in acetylcholinesterase activity (CHE) as compared to TAA-treated group. It has been indicated that donepezil administered orally has a potent and selective effect on the central cholinergic system, increasing significantly and dose-dependently the extracellular acetylcholine concentration in the rat cerebral cortex due to inhibited acetylcholinesterase activity (Dimitrova and Getova-Spassova, 2006).

Conclusion: One can conclude that treatment for one week with lactulose (8ml/kg) or donepezil (10mg/kg) restored motor activities and improved depression mode, liver enzymes activities and oxidative stress disturbance that are characteristics of TAA-treated rats. respectively as of that in TAA-treated rats.

Figure (1): Effect of Lactulose and Donepezil on the Latency Period, Ambulation, Rearing, and Grooming Frequencies in Thioacetamide (TAA)-induced Hepatic Encephalopathy in Rats Using the Open Field Test.



lactulose (8 ml/kg) and donepezil (10 mg/kg) were orally administered for 7 consecutive days. TAA (200 mg/kg, p.o) was administered on the 6th & 7th days of drugs administration to induce HE. Open field test was performed 4 hours after the second dose of TAA. Another group of animals was orally administered saline for 7 consecutive days and served as normal group.

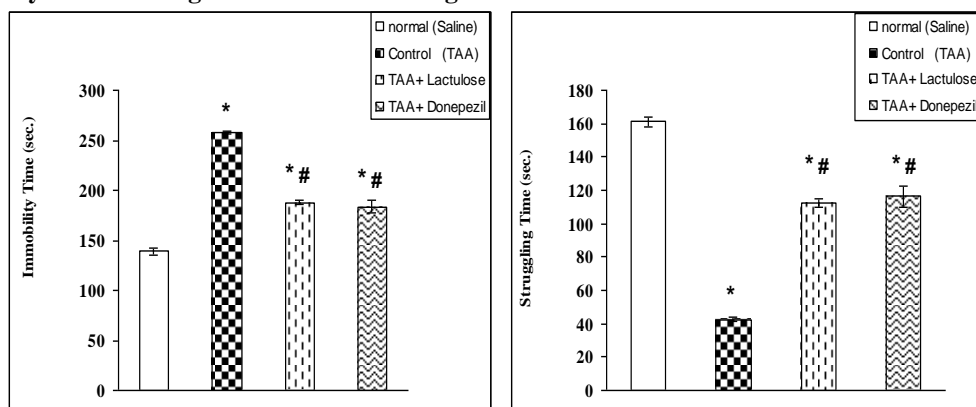
Each bar represents the mean \pm SE of n = 6.

Statistical analysis was carried out by one way ANOVA followed by Tukey-Kramer multiple comparison test.

* Significantly different from normal group at p<0.05.

Significantly different from control (TAA) group at p<0.05.

Figure (2): Effect of Lactulose and Donepezil on the Behavior of Thioacetamide (TAA)-induced Hepatic Encephalopathy in Rats Using the Forced Swimming Test.



lactulose (8 ml/kg) and donepezil (10 mg/kg) were orally administered for 7 consecutive days. TAA (200 mg/kg, p.o) was administered on the 6th & 7th days of drugs administration to induce HE. Forced swimming test was performed 4 hours after the second dose of TAA. Another group of animals was orally administered saline for 7 consecutive days and served as normal group. Each bar represents the mean \pm SE of n = 6-10.

Statistical analysis was carried out by one way ANOVA followed by Tukey-Kramer multiple comparison test.

* Significantly different from normal group at p<0.05.

Significantly different from control (TAA) group at p<0.05.

Table (1): Effect of Lactulose and Donepezil on Blood Ammonia (NH₃) Level and Liver Function Measurements of Thioacetamide (TAA)-induced Hepatic Encephalopathy Rats.

Groups Parameters	Normal (Saline)	HE TAA (200 mg/ kg)		
		Saline	Lactulose (8 ml/ kg)	Donepezil (10mg/kg)
Ammonia (NH ₃) Level (μ mol/ml)	6.41 \pm 0.69	52.24* \pm 2.98	32.70*# \pm 0.72	41.78*# \pm 1.94
Albumin Level (g/dl)	4.90 \pm 0.05	3.77* \pm 0.08	4.19* \pm 0.12	4.20*# \pm 0.14
Total Protein Level (g/dl)	7.70 \pm 0.16	6.08* \pm 0.08	7.61# \pm 0.27	7.19# \pm 0.19
γ - glutamyl transferase (GGT) Activity (U/L)	1.70 \pm 0.15	5.11* \pm 0.53	3.03# \pm 0.46	2.86# \pm 0.44
Aspartate aminotransferase (AST) Activity (U/L)	22.00 \pm 1.41	74.17* \pm 3.22	72.50* \pm 1.31	70.83* \pm 2.52
Alanine aminotransferase (ALT) Activity (U/L)	17.17 \pm 0.70	87.17* \pm 1.25	81.33* \pm 0.42	85.17* \pm 1.33
Alkaline Phosphatase (ALP) Activity (IU/L)	95.15 \pm 3.44	262.23* \pm 1.58	185.74*# \pm 2.22	220.12*# \pm 5.01

Lactulose (8 ml/kg) and donepezil (10 mg/kg) were orally administered for 7 consecutive days. TAA (200 mg/kg, p.o) was administered on the 6th & 7th days of drugs administration to induce HE. Another group of animals was orally administered saline for 7 consecutive days and served as normal group. On the 7th day, animals were sacrificed 4 hours after the second dose of TAA, blood was collected to be used for estimation of blood NH₃, serum albumin, total protein levels, serum GGT, AST, ALT and ALP activities.

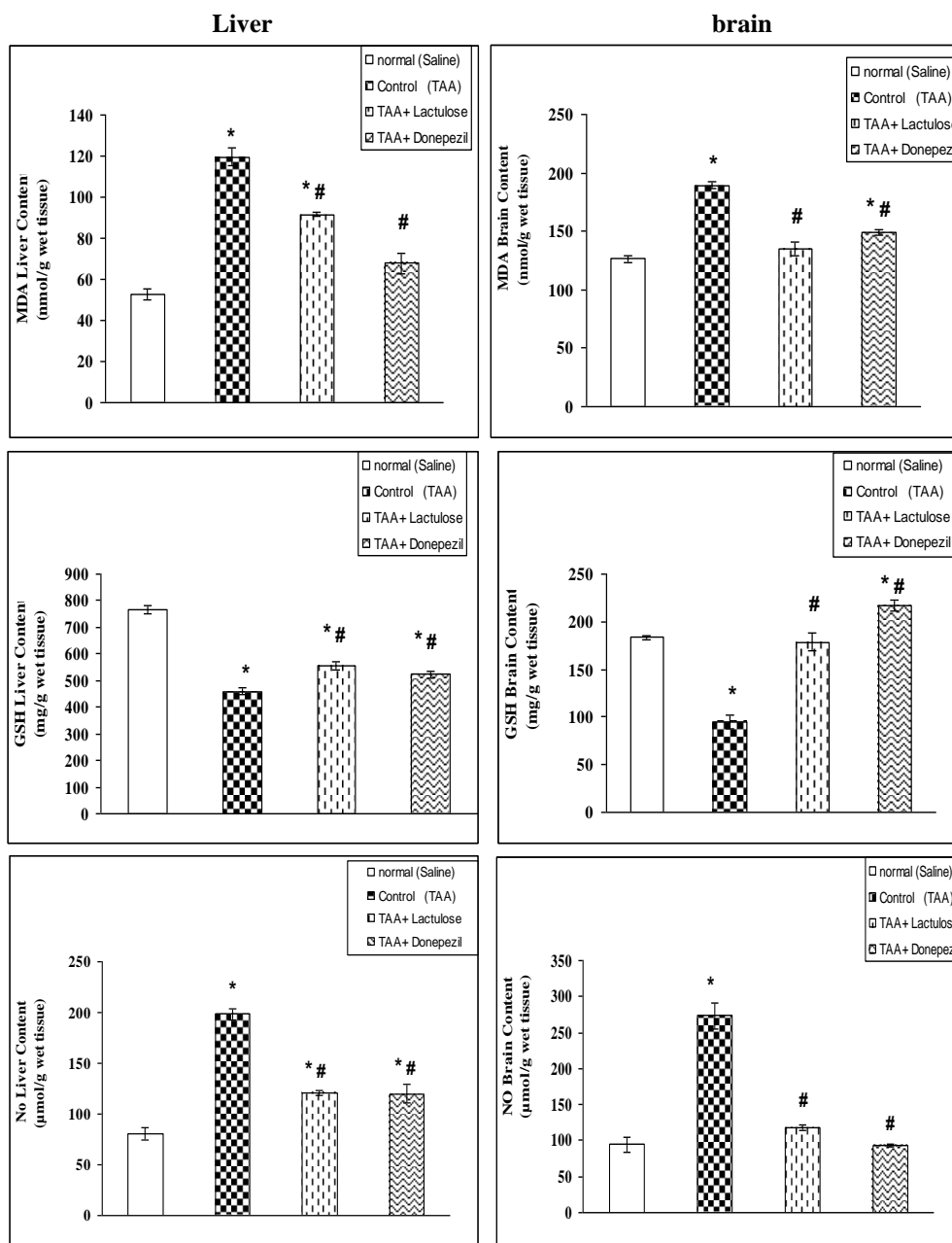
Each value represents means \pm standard error.

Statistical analysis was carried out by one way ANOVA followed by Tukey-Kramer multiple comparison test.

*Significantly different from normal group at p<0.05.

#Significantly different from control (TAA) group at p<0.05.

Figure (3): Effect of Lactulose and Donepezil on Liver and Brain Malondialdehyde (MDA), Reduced Glutathione (GSH) and Nitric Oxide (NO) Contents of Thioacetamide (TAA)-induced Hepatic Encephalopathy Rats.



lactulose (8 ml/kg) and donepezil (10 mg/kg) were orally administered for 7 consecutive days. TAA (200 mg/kg, p.o) was administered on the 6th & 7th days of drugs administration to induce HE. Another group of animals was orally administered saline for 7 consecutive days and served as normal group. On the 7th day, animals were sacrificed 4 hours after the second dose of TAA; livers and brains were collected to be used for estimation of MDA, GSH and NO contents.

Each bar represents the mean \pm SE of n = 6-10.

Statistical analysis was carried out by one way ANOVA followed by Tukey-Kramer multiple comparison test.

* Significantly different from normal group at p<0.05.

Significantly different from control (TAA) group at p<0.05.

Table (2): Effect of Lactulose and Donepezil on Brain Serotonin (5-HT), Dopamine (DA), Noradrenaline (NA), γ -aminobutyric acid (GABA) Contents and Cholinesterase (CHE) Activity of Thioacetamide (TAA)-induced Hepatic Encephalopathy Rats.

Parameters	Normal (Saline)	HE TAA (200 mg/ kg)		
		Saline	Lactulose (8 ml/ kg)	Donepezil (10mg/kg)
Serotonin (5-HT) Content (μg/g wet tissue)	0.53 \pm 0.01	0.92* \pm 0.03	0.61*# \pm 0.01	0.67*# \pm 0.01
Dopamine (DA) Content (μg/g wet tissue)	6.93 \pm 0.12	5.31* \pm 0.14	6.08*# \pm 0.21	6.14*# \pm 0.04
Noradrenaline (NA) Content (μg/g wet tissue)	1.08 \pm 0.04	0.73* \pm 0.02	0.90*# \pm 0.05	0.88* \pm 0.02
γ-aminobutyric acid (GABA) Content (μg/g wet tissue)	707.27 \pm 12.30	958.75* \pm 11.48	774.58*# \pm 22.08	711.38# \pm 8.12
Cholinesterase (CHE) Activity (U/g wet tissue)	8308.75 \pm 632.01	16031.00* \pm 1088.54	11827.75# \pm 667.31	11241.25# \pm 1415.91

lactulose (8 ml/kg) and donepezil (10 mg/kg) were orally administered for 7 consecutive days. TAA (200 mg/kg, p.o) was administered on the 6th & 7th days of drugs administration to induce HE. Another group of animals was orally administered saline for 7 consecutive days and served as normal group. On the 7th day, animals were sacrificed 4 hours after the second dose of TAA, and brains were collected to be used for estimation of 5-HT, DA, NA, GABA contents and CHE activity.

Each value represents means \pm standard error.

Statistical analysis was carried out by one way ANOVA followed by Tukey-Kramer multiple comparison test.

*Significantly different from normal group at $p < 0.05$.

#Significantly different from control (TAA) group at $p < 0.05$.

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معادلة اللاكتيلوز و دونبيلز للاعتلال الدماغي الكبدي المحث بثيواستاميد في الجرذان

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** قسم الادوية والسموم بالهيئة القومية للرقابة والبحوث الدوائية

يعتبر الإعتلال الدماغي الكبدي من المضاعفات العصبية النفسية لأمراض الكبد. وتشير العديد من الدراسات إلى أن زيادة الأمونيا في الدم هو المسبب الرئيسي لحدوث الاعتلال الدماغي الكبدي كما أن هناك دراسات سابقة أظهرت تغيراً في محتوى بعض الموصلات العصبية وحدوث إجهاد تأكسدي في الدماغ في المصابين بهذا المرض.

ويمكن تلخيص نتائج التجارب كالتالي :

- تم اختيار جرعة ثيواستاميد (200 مجم/كجم/ يوم) وذلك بإعطائها عن طريق الفم لمدة يومين متعاقبين لإحداث الاعتلال الدماغي الكبدي في الجرذان.

- تم اختيار الجرعات التالية من الأدوية المستعملة كالتالي:

لاكتيلوز (8 مل / كجم/ يوم)

دونبيلز (10 مجم/كجم/ يوم)

لاعطائها للجرذان يوميا عن طريق الفم لمدة 7 أيام.

- التجارب السلوكية :

تم إجراء هذه التجارب بعد 4 ساعات من الجرعة الثانية لمادة ثيواستاميد.

- ترتب على إعطاء مادة ثيواستاميد زيادة كل من المدة السابقة للحركة وعدد مرات لعق الجسم ونقص كل من عدد المربعات المقطوعة سيراً وعدد مرات الشب في اختبار الميدان المفتوح بالإضافة الى زيادة في وقت السكون ونقص في وقت المقاومة في اختبار السباحة.

- أدت المعالجة بكل من لاکتيلوز و دونبيلز إلى نقص المدة السابقة للحركة في اختبار الميدان المفتوح وأدى لاکتيلوز إلى نقص عدد مرات لعق الجسم في هذا الاختبار بالإضافة إلى المعالجة بكل من لاکتيلوز و دونبيلز التي ترتب عليها زيادة في عدد المربعات المقطوعة سيراً وعدد مرات الشب في هذا الاختبار.

- أدى استعمال كل لاکتيلوز و دونبيلز إلى نقص في وقت السكون بالإضافة إلى زيادة في وقت المقاومة في اختبار السباحة.

- التجارب الكيميائية الحيوية :

ترتب على إعطاء مادة ثيواستاميد ارتفاع مستوى الأمونيا في الدم ونقص في مستوى الألبومين والبروتين الكلى في مصل الدم بالإضافة الى زيادة في نشاط إنزيمات أسبرتات أمينو ترانسفيريز و ألانين أمينو ترانسفيريز وفوسفوتيز القلوي وجاما جلوتاميل ترانسفيريز في مصل الدم.

- أدت المعالجة بكل من لاکتيلوز و دونبيلز إلى نقص مستوى الأمونيا في الدم وزيادة مستوى البروتين الكلى.

- أدت المعالجة بدونبيلز إلى زيادة مستوى الألبومين في مصل الدم.

- أدت المعالجة بكل من لاکتيلوز و دونبيلز إلى نقص في نشاط إنزيم فوسفوتيز القلوي في مصل الدم.

- أدت المعالجة بكل من لاکتيلوز و دونبيلز إلى تحسن في نشاط إنزيم جاما جلوتاميل ترانسفيريز في مصل الدم.

- ترتب على إعطاء مادة ثيواستاميد إحداث إجهاد تأكسدي في كل من الكبد والمخ ظهر في صورة زيادة في محتوى الكبد والمخ من مادتي مالون داي الديهايد و أكسيد النيتروجين ونقص في محتوى مادة جلوتاثيون في كل من الكبد والمخ.

- أدت المعالجة بكل من لاکتيلوز و دونبيلز إلى مناهضة تأثير مادة ثيواستاميد وكان الدليل على ذلك هو نقص في محتوى مالون داي الديهايد وأكسيد النيتروجين في كل من الكبد والمخ مع زيادة في محتوى المخ من مادة جلوتاثيون بالإضافة الى تحسن في محتوى الكبد من مادة جلوتاثيون بعد المعالجة بكل من لاکتيلوز و دونبيلز.

- الموصلات العصبية في المخ :

- ترتب على إعطاء مادة ثيواستاميد زيادة محتوى المخ في كل من سيروتونين و حمض جاما أمينو بيوتيرك أسيد ونشاط انزيم اسيتيل كولين استيريز ونقص في محتوى المخ في كل من نورادرينالين ودوبامين.

- أدت المعالجة بكل من لاکتيلوز و دونبيلز إلى تحسن في محتوى المخ في كل من سيروتونين و حمض جاما أمينو بيوتيرك.

- أدى استخدام لاکتيلوز الى زيادة محتوى المخ من نورادرينالين.

- أدت المعالجة بكل من لاکتيلوز و دونبيلز إلى تحسن في محتوى المخ من دوبامين.

- أدت المعالجة بكل من لاکتيلوز و دونبيلز إلى تحسن في نشاط انزيم اسيتيل كولين استيريز في المخ.

يستنتج من هذا البحث ما يلي:

أحدثت مادة ثيواستاميد تلفاً في الكبد عن طريق إحداث إجهاد تأكسدي وأضعفت قدرته على التخلص من الأمونيا، كما أدت إلى زيادة أنشطة إنزيمات الكبد.

يمكن إستنتاج أن لاکتيلوز و دونبيلز لهم تأثير وقائي للكبد ويمكن أن يكون لهم تأثير وقائي للمخ.