Impact of antidepressant drug clomipramine on hepato-renal functions, lipid peroxidation, micronucleus frequencies and DNA damage using an alkaline comet assay

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ABSTRACT
Clomipramine is a tricyclic antidepressant commonly used to treat anxiety related behavioral disorders in human and animals. The current study was performed to assess the effects of different therapeutic doses of clomipramine hydrochloride supplementation on hepato-renal functions, lipid peroxidation, frequencies of micronucleated polychromatic erythrocytes in bone marrow, DNA damage in peripheral blood lymphocytes using the comet assay and effects of withdrawal of the drug for 4 weeks on these parameters. Forty two Swiss albino male mice were divided into seven equal groups. The first group served as a control, while groups 2, 4 and 6 were orally treated with low (75 mg/kg of body weight), medium ((100 mg/kg of body weight), and high doses (250 mg/kg of body weight), respectively of clomipramine hydrochloride daily for 30 days. However, groups 3, 5 and 7 served as low, medium and high withdrawal groups for 4 weeks. Different therapeutic doses of clomipramine hydrochloride resulted in a significant increase in the levels of serum ALT, AST, BUN and creatinine compared to control. Also, plasma malondialdehyde levels showed were significant increase in mice treated by different therapeutic doses of clomipramine hydrochloride compared to control group. In contrast, the administration of clomipramine hydrochloride in three therapeutic doses for 30 days caused a significant increase in the frequency of micronuclei in polychromatic erythrocytes of mice bone marrow, this increase reached more than ten times the value determined before the treatment and directly proportional to the dose. The level of basal endogenous DNA damage measured as the mean of percentage of DNA in the tail of lymphocytes in mice treated by low, medium and high therapeutic doses of clomipramine hydrochloride were significantly higher than in controls. However, in the recovery period (4weeks), a significant amelioration in all studied parameters was observed. Results indicated that, clomipramine is a hepato-renal toxic drug and induces a significant amount of DNA damage and in-vivo genotoxic drug.

KEYWORDS: Antidepressant; Clomipramine; Kidney function test; Liver function test; Oxidative stress; Mouse bone marrow; DNA damage; Comet assay
INTRODUCTION

Depression is a common disorder which may affect 1 out of 10 individuals during their life-time, giving rise to serious health and socio-economic problems (Andrews and Nemeroff, 1994). The disease can be caused by various endogenous and exogenous factors, and it is characterized by irritability, insomnia, fatigue, psychomotor and concentration alterations in addition to a suicidal tendency (Wise et al., 2007). The extent of clinical damage as well as its socio-economic importance has favored the use of a number of drugs such as the tricyclic antidepressants (Nalecz-Jawecki, 2008).

Tricyclic antidepressants (TCAs) have been prescribed along time for the treatment of depression. Moreover, the indications for the use of TCAs are associated with anxiety, eating disorders and chronic pain syndromes (El-Demerdash and Mohamadin, 2004). The mechanism by which TCAs exert their antidepressant effects is not clearly established until now. However, the capacity of TCAs in blocking the reuptake of centrally active neurotransmitters is generally accepted as their primary mode of action (Potter et al., 1998). Indeed, TCAs, as well as serotonergic receptors, can also connect to several other receptors, such as histamine (H1E H2), adrenergic (alpha-1 and alpha-2), GABA-A, and muscarinic receptors (Rudorfer et al., 1994).

Clomipramine belongs to a class of TCAs and is widely used for the treatment of depression and obsessive compulsive disorder mainly by inhibiting the reuptake of serotonin and nor-epinephrine in the brain (Trimble, 1990). It has been shown that the chronic administration of clomipramine, at doses relatively low, evokes benefits on depression (Srikumar et al., 2006; Bhagya et al., 2008) and reduces the behavioral deficits and cholinergic dysfunction induced by stress in rats (D’Aquila et al.,2000). However, the species and sex of animals as well as type, duration and severity of stress can modify the response induced by clomipramine (Consoli et al., 2005). Literature data have also reported that high doses of clomipramine are toxic to experimental animals (Calegari et al., 2007). The adverse effects induced by clomipramine administration, due to the blockade of serotonin reuptake, include difficulties in learning and memory as well as an increase in the consumption of sweet foods, which are characterized as initial symptoms of depression and anxiety (Ely et al., 1997). Regarding the mechanism of toxicity, in vitro studies have evidenced that the exposure to different classes of TCAS drugs increases the production of reactive oxidative stress (ROS) (El-Demerdash and Mohamadin, 2004).

Drug-induced hepatotoxicity is a rare but obviously serious and life-threatening side effect of the antidepressant agents. Tricyclic antidepressant drugs are known to cause hepatotoxicity, but few cases were reported lately (DeSanty and Amabile, 2007). Tricyclic drugs might be more prone to causing such damage than are the newer antidepressant agents (Lucena et al., 2003). In one case, acute hepatitis developed with administration of the tricyclic amineptine and recurred after taking clomipramine (Larrey et al., 1986). Amineptine was withdrawn from the market due to hepatotoxicity. Prolonged cholestatic hepatitis developed in one person on imipramine (Horst et al., 1980). Iprindole, another older tricyclic drug, is also associated with injury to the liver (Richards, 1970). Reversible acute renal failure due to acute interstitial nephritis, probably induced by the use of clomipramine hydrochloride, which is commonly administered for the treatment of psychosis, neurosis, or panic disorder (Onish et al., 2007).

Micronuclei represent chromosomal fragments or whole chromosomes abnormally segregated during cell division resulting from genetic damage of numerous xenobiotics; their quantification in most international batteries is recommended to determine genotoxicity, mainly because they are easy to identify (Madrigal-Bujaidar et al., 2008). The comet assay is useful for detecting DNA strand
breaks induced by oxidative stress (Re et al., 1997). We designed the present study to determine whether a daily administration of different therapeutic doses of clomipramine drug in mice for 30 days produces hepato-renal toxicity, an increase in the rate of micronucleated polychromatic erythrocytes (MNPE), also to verify if such treatment may induce bone marrow cytotoxicity, detecting DNA strand breaks by comet assay and effect of withdrawal of the drug for 4 weeks on these parameters.

**MATERIALS AND METHODS**

**Chemicals**

Clomipramine hydrochloride purchased from Novartis Pharmaceutical Company (Egypt). All other chemicals were analytical reagent grade and chemicals required for all biochemical assays were obtained from Sigma–Aldrich Chemicals Co (St. Louis, MO, USA), and Merck (Darmstadt, Germany).

**Animals and treatment**

The animal care and handling was done according to the guidelines set by the World Health Organization, Geneva, Switzerland and according to approval from the committee for animals care at the National Research Centre, Egypt. Forty two Swiss albino male mice 2-3 months old and initial body weight of 25–30 gm were obtained from the animal house of National Research Center, Cairo, Egypt. Animals were acclimatized for one week before dosing and maintained under standard conditions of temperature, humidity and light. The animals were given standard food (a standard rodent diet, type 4RF21 GLP, Mucedola, Italy) and water ad libitum. The human therapeutic doses of the tested drug was converted to mice therapeutic equivalent doses using the dose-conversion table of Paget and Barnes, 1964. Animals were divided into control and different experimental groups, each comprising six animals as shown in (Table 1).

**Table 1.** Details of the control and experimental groups used.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Control group</strong></td>
<td>Received distilled water orally by gavages once daily for 30 days</td>
</tr>
<tr>
<td><strong>Low therapeutic dose group</strong></td>
<td>Received clomipramine hydrochloride (75mg/kg ) orally by gavages daily for 30 days</td>
</tr>
<tr>
<td><strong>Low dose, recovery group</strong></td>
<td>Received clomipramine hydrochloride (75mg/kg) orally by gavages daily for 30 days followed by 4 weeks without treatment.</td>
</tr>
<tr>
<td><strong>Medium therapeutic dose group</strong></td>
<td>Received clomipramine hydrochloride (100mg/kg ) orally by gavages daily for 30 days</td>
</tr>
<tr>
<td><strong>Medium dose, recovery group</strong></td>
<td>Received clomipramine hydrochloride (100mg/kg) orally by gavages daily for 30 days followed by 4 weeks without treatment.</td>
</tr>
<tr>
<td><strong>High therapeutic dose group</strong></td>
<td>Received clomipramine hydrochloride (250mg/kg ) orally by gavages daily for 30 days</td>
</tr>
<tr>
<td><strong>High dose, recovery group</strong></td>
<td>Received clomipramine hydrochloride (250mg/kg) orally by gavages daily for 30 days followed by 4 weeks without treatment.</td>
</tr>
</tbody>
</table>
At the end of the treatment, blood samples were collected from the anaesthetized mice, by direct puncture of the right ventricle and from the retro-orbital vein plexus, in sterile separator tubes and centrifuged at 1300xg for 15 min. Serum was separated and collected by Pasteur pipette into a dry clean tube for the following biochemical tests: ALT (alanine aminotransferase) according to Bergmeyer et al., (1985), AST (aspartate aminotransferase) according to Klauke et al., (1988), blood urea nitrogen according to Tietz, (1995) and serum levels of creatinine according to Mazzachi et al., (2000). All samples were assayed using the commercial kit which obtained from Roche Diagnostics. Plasma malondialdehyde (MDA) levels were determined as described by Yoshioka et al. (1979).

**Micronucleus test**

Bone marrow slides were prepared according to the method described by Krishna and Hayashi (2000). The bone marrow was washed with 1 ml of fetal calf serum and then smeared on clean slides. The slides were left to air dry and then fixed in methanol for 5 minutes, followed by staining in May-Grunwald- Giemsa for 5 minutes then washed in distilled water and mounted. For each animal, 2000 polychromatic erythrocytes (PCEs) were examined for the presence of micronuclei.

**Comet assay**

The comet assay was performed under alkaline conditions essentially according to the procedure of Singh et al., (1988) with modification (Klaude et al., 1996) as previously described (Blasjak et al., 2003). A freshly prepared suspension of cells in 0.75% low melting point agarose (Sigma Chemicals) dissolved in phosphate buffered saline (PBS; Sigma Chemicals) was cast onto microscope slides per coated with 0.5% normal melting agarose. The cells were then lysed for 1 h at 4 °C in a buffer consisting of 2.5M NaCl, 100mM EDTA, 1% Triton X-100, 10mM Tris, and pH 10. After the lyses, DNA was allowed to unwind for 40 min in electrophoretic solution consisting of 300mM NaOH, 1mM EDTA, pH > 13. Electrophoresis was conducted at 4 °C for 30 min at electric field strength 0.73 V/cm (30 mA). The slides were then neutralized with 0.4M Tris, pH 7.5, stained with 2 μg/ml (Ethidium Bromide; Sigma Chemicals) and covered with cover slips. The slides were examined at 200x magnification in fluorescence microscope (Nikon, Tokyo, Japan) to a COHU 4910 video camera (Cohu, Inc., San Diego, CA, USA) equipped with a UV filter block consist an excitation filter (359 nm) and barrier filter (461 nm) and connected to a personal computer-based image analysis system, Lucia-Comet v. 4.51 (Laboratory Imaging, Praha, Czech Republic). Fifty images were randomly selected from each sample and the comet tail DNA was measured. Two parallel tests with aliquots of the same sample of cells were performed for a total of 100 cells. Each experiment was repeated two times. Percentage of DNA in the tail (% tail DNA) was analyzed. It is positively correlated with the level of DNA breakage or/and alkali labile sites in the cell and is negatively correlated with the level of DNA cross links (Ashby et al., 1995; Tice et al., 2003). The mean value of the % tail DNA in a particular sample was taken as an index of DNA damage in this sample. Because our measurement system was not calibrated, tail length and tail moment were presented in arbitrary units. In the DNA repair study, the difference between the absolute value of the extent of DNA damage in repair incubation at 120 and 0 min, normalized to the initial value of DNA damage, was taken as an index of the efficacy of DNA repair.

**Statistical analysis**

Data were expressed as means ± S.E. and analyzed statistically using Student’s t-test. Results were considered to be statistically significant when P < 0.05.
RESULTS

At low therapeutic dose of clomipramine hydrochloride (Table 2), there was a significant increase in the levels of serum ALT (P<0.01), BUN (P<0.001) and creatinine (P<0.05). While, there was insignificant increase in the levels of AST in comparison to control. In contrast, treatment of mice by median and high therapeutic doses of clomipramine hydrochloride resulted in significant increase in the levels of serum ALT (P<0.001), AST (P<0.05), BUN and creatinine (P<0.001) compared to control. However, in the recovery period (4weeks), the levels of serum ALT, AST, BUN and creatinine were significant decreased (P<0.05 in low and medium doses, P<0.001 in high dose) in withdrawal groups compared to clomipramine hydrochloride treated groups.

Table 2. Effect of low, medium and high therapeutic doses of clomipramine on serum alanine aminotransferase, aspartate aminotransferase, blood urea nitrogen and serum creatinine levels.

<table>
<thead>
<tr>
<th>Groups</th>
<th>ALT (U/ml)</th>
<th>AST (U/ml)</th>
<th>BUN (mg/dl)</th>
<th>Serum creatinine (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control group</td>
<td>50.8±1.72</td>
<td>36.4±0.8</td>
<td>36.6±1.48</td>
<td>0.666±0.012</td>
</tr>
<tr>
<td>Low therapeutic dose group</td>
<td>64.4±2.94b**</td>
<td>37.8±0.748 b NS</td>
<td>45.2±0.748b***</td>
<td>0.708±0.0116 b*</td>
</tr>
<tr>
<td>Low dose, recovery group</td>
<td>56±1.4 c*</td>
<td>36.6±1.02 c NS</td>
<td>37.6±1.019 c***</td>
<td>0.686±0.01 c NS</td>
</tr>
<tr>
<td>Medium therapeutic dose group</td>
<td>74.2±1.66b***</td>
<td>39±0.632b*</td>
<td>68.6±1.625 b***</td>
<td>0.736±0.0224 b*</td>
</tr>
<tr>
<td>Medium dose, recovery group</td>
<td>68.8±1.166c*</td>
<td>36.2±0.748 c*</td>
<td>58.2±2.13 c**</td>
<td>0.674±0.01 c*</td>
</tr>
<tr>
<td>High therapeutic dose group</td>
<td>77.4±1.2b***</td>
<td>40.2±1.17b*</td>
<td>77.8±1.166 b***</td>
<td>0.746±0.01 b***</td>
</tr>
<tr>
<td>High dose, recovery group</td>
<td>61.8±1.6 c***</td>
<td>36.8±0.75 c*</td>
<td>66.8±1.4 c***</td>
<td>0.696±0.01 c**</td>
</tr>
</tbody>
</table>

Data are presented as mean ± SE of the six animals.

* P<0.05, **P<0.01 and ***P<0.001

(b) Significantly different from control group.
(c) Significantly different from clomipramine hydrochloride treated group.

Plasma malondialdehyde levels were significantly increased (P<0.001) in mice treated by different therapeutic doses of clomipramine hydrochloride compared to the control group. While, in the recovery period (4weeks), the plasma malondialdehyde levels were significantly decreased (P<0.05 in low & P<0.001 in medium and high doses) in withdrawal groups compared to clomipramine hydrochloride treated groups (Table 3).

With respect to the frequency of micronucleated polychromatic erythrocytes (MNPE), present results in the control group showed a homogeneous low amount of damaged cells all through the assay. In contrast,
the administration of clomipramine hydrochloride in three therapeutic doses for 30 days caused a significant increase in the frequency of micronuclei in polychromatic erythrocytes of mice bone marrow, this increase reached more than ten times the value determined before the treatment and directly proportional to the dose. However in the recovery period (4 weeks), the micronuclei values were significant (p<0.01) decreased in different withdrawal groups compared to clomipramine hydrochloride treated groups (Table 4).

**Table 3.** Effect of low, medium and high therapeutic doses of clomipramine on plasma malondialdehyde levels.

<table>
<thead>
<tr>
<th>Groups</th>
<th>MDA (nmol/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control group</td>
<td>41.0±1.0</td>
</tr>
<tr>
<td>Low therapeutic dose group</td>
<td>58.0±2.73b***</td>
</tr>
<tr>
<td>Low dose, recovery group</td>
<td>47.6 ± 2.5c*</td>
</tr>
<tr>
<td>Medium therapeutic dose group</td>
<td>87.6±2.50b***</td>
</tr>
<tr>
<td>Medium dose, recovery group</td>
<td>69.8±1.44c***</td>
</tr>
<tr>
<td>High therapeutic dose group</td>
<td>129.0±1.94b***</td>
</tr>
<tr>
<td>High dose, recovery group</td>
<td>97.8±1.7c***</td>
</tr>
</tbody>
</table>

Data are presented as mean ± SE of the six animals.

* P<0.05, ***P<0.001

(b) Significantly different from control group.

(c) Significantly different from clomipramine hydrochloride treated group.

**DISCUSSION**

Medication in most cases may produce secondary health effects of variable degree, which in some cases may be a serious human health hazard. This potential damage is of particular concern with respect to compounds used for long periods and/or during pregnancy. The antidepressants studied are medications that may be continuously consumed for 6 months or longer, with a possible repetition of the treatment (Frommer et al., 1987). In the present study, treatment of mice by different therapeutic doses of clomipramine hydrochloride resulted in a significant increase in the levels of serum ALT, AST, BUN and creatinine compared to control. On the other hand these parameters were significantly decreased by withdrawal of the drug for 4 weeks. These results were in agreement with Atmaca, (2011) who reported, a female patient has developed acute severe hepatotoxicity associated with increased level of aspartate and alanine aminotransferases following treatment with...
clomipramine. Bethesda, (2009) observed that, elevations in serum ALT and AST concentrations exceeding 3 times the upper limit of normal were reported in approximately 1 and 3%, respectively, of patients receiving clomipramine. In most cases, these elevations in hepatic enzyme concentrations were not associated with other clinical findings suggestive of hepatic injury, and jaundice was not observed.

Most antidepressant agents have the potential to produce idiopathic liver injury. There is no way to prevent idiopathic drug-induced liver injury, but the severity of the reaction may be minimized with prompt recognition and early withdrawal of the agent. The clinician must be careful to provide ongoing therapy of the underlying depressive disorder and be aware of possible drug discontinuation syndromes should potential hepatotoxicity be suspected (DeSanty and Amabile, 2007). Lucena et al., (2003) stated that, antidepressant-induced hepatotoxicity was ranged from transient increases in liver enzymes to fulminate liver failure.

Mohammadi et al., (2003) revealed that, both clomipramine and fluoxetine caused an increase in the density of SGPT and SGOT of liver function in obsessive-compulsive of Iranian children and adolescents after eight weeks of treatment. Wiersma et al., (2000) observed that, clomipramine-induced allergic hepatitis with massive eosinophilia and elevated hepatic transaminases. Alderman et al. (1993) reported a case at 67-year-old man who developed concurrent severe agranulocytosis and hepatitis as a result of treatment with clomipramine. Therefore, individual genetic factors may be playing an important role. Meanwhile it should also be taken into consideration that there may be cross- hepatotoxicity between tricycle antidepressants (Larrey et al., 1986).

Table 4. Effect of low, medium and high therapeutic doses of clomipramine on frequency of micro nucleated polychromatic erythrocytes in mice bone marrow.

<table>
<thead>
<tr>
<th>Groups</th>
<th>No. of animals</th>
<th>No. of polychromatic erythrocytes</th>
<th>MN-PCE/2000 in bone marrow</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control group</td>
<td>6</td>
<td>2000</td>
<td>0.40 ± 0.54</td>
</tr>
<tr>
<td>Low therapeutic dose group</td>
<td>6</td>
<td>2000</td>
<td>4.6 ± 0.54 b***</td>
</tr>
<tr>
<td>Low dose, recovery group</td>
<td>6</td>
<td>2000</td>
<td>2.2 ± 0.83 c*</td>
</tr>
<tr>
<td>Medium therapeutic dose group</td>
<td>6</td>
<td>2000</td>
<td>19.2 ± 1.09 b***</td>
</tr>
<tr>
<td>Medium dose, recovery group</td>
<td>6</td>
<td>2000</td>
<td>11.0 ± 1.41 c**</td>
</tr>
<tr>
<td>High therapeutic dose group</td>
<td>6</td>
<td>2000</td>
<td>28.2 ± 2.16 b***</td>
</tr>
<tr>
<td>High dose, recovery group</td>
<td>6</td>
<td>2000</td>
<td>17.8 ± 1.78 c**</td>
</tr>
</tbody>
</table>

Data are presented as mean ± SE of the six animals.

* P<0.05, **P<0.01 and ***P<0.001
(b) Significantly different from control group.
(c) Significantly different from clomipramine hydrochloride treated group.
Table 5. Endogenous DNA damage measured as the mean comet tail DNA of peripheral blood lymphocytes of mice treated by low, medium and high therapeutic doses of clomipramine.

<table>
<thead>
<tr>
<th>Groups</th>
<th>No. of animals</th>
<th>DNA damage (%DNA in tail) by Comet assay</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control group</td>
<td>6</td>
<td>1.8 ± 0.748</td>
</tr>
<tr>
<td>Low therapeutic dose group</td>
<td>6</td>
<td>5.0 ± 0.624 b*</td>
</tr>
<tr>
<td>Low dose, recovery group</td>
<td>6</td>
<td>2.6 ± 0.489 c*</td>
</tr>
<tr>
<td>Medium therapeutic dose group</td>
<td>6</td>
<td>8.2 ± 0.448 b***</td>
</tr>
<tr>
<td>Medium dose, recovery group</td>
<td>6</td>
<td>6.6 ± 0.49 c*</td>
</tr>
<tr>
<td>High therapeutic dose group</td>
<td>6</td>
<td>13.6 ± 1.02 b***</td>
</tr>
<tr>
<td>High dose, recovery group</td>
<td>6</td>
<td>10.2 ± 0.4 c*</td>
</tr>
</tbody>
</table>

Data are presented as mean ± SE of the six animals.

* P<0.05, ***P<0.001

(b) Significantly different from control group.

(c) Significantly different from clomipramine hydrochloride treated group.

The mechanism of clomipramine causes serum aminotransferase elevation is not known. It undergoes extensive hepatic metabolism, a possible cause of liver injury is production of a toxic intermediate of metabolism that may be directly toxic or may induce a hypersensitivity reaction. A minority of TCA-induced hepatotoxic reactions appeared to be immune-mediated as manifested by significant extrahepatic symptoms, eosinophilia, and eosinophilic infiltration of the liver (Anderson and Henrikson, 1978). Also apart from hepatotoxicity, TCAs have serious cardiovascular toxicity (Dawson, 2004), which may be exacerbated under disturbances of intrahepatic flow. Some tricyclic drugs caused an increase in perfusion pressure accompanied by a coincidental decrease in oxygen uptake. The most potent drug among them, clomipramine, was selected as a representative to characterize their action on hepatic microcirculation (Matsuda et al., 2004).

Onishi et al., (2007) described a 41-year old man with obsessive-compulsive neurosis who developed acute renal failure due to acute interstitial nephritis during 6 weeks of treatment with clomipramine hydrochloride. After the withdrawal of clomipramine hydrochloride and treatment with prednisolone, his urinary output improved, along with improvement of his renal function. Karimi et al., (2011) reported that, the levels of urea and creatinine were increased in the male guinea pigs that received antidepressant fluoxetine.

Concerning plasma malondialdehyde levels, there were significant increase in mice treated by different therapeutic doses of clomipramine hydrochloride compared to the control group. While drug recovery period for 4 weeks resulted in a significant decrease of plasma malondialdehyde levels in all treated groups. These results were in-accordance with El-Deemerdes and Mohamadin, (2004) who revealed clomipramine-induced a significant increase in myocardial lipid peroxides level (133%) of the control value. Balk et al., (2010) reported that chronic clomipramine treatment caused an increase of the lipid peroxidation in brain striatum. Post et al., (2000) found that, the exposure to TCAs drugs led to
increase generation of reactive oxygen species (ROS) and a concomitant reduction of intracellular glutathione level as well as an induction of DNA fragmentation. Also, Xia et al. (1996, 1998) found in two serial studies that TCAs, mainly clomipramine, can induce apoptosis in lymphocytes, a finding that reflect the possible generation of free radicals. Abdel-Salam et al., (2011) observed that, the higher dose of imipramine which markedly enhanced lipid peroxidation (MDA) in brain of mice. Bautista-Ferrufino et al., (2011) stated that, amitriptyline treatment induces oxidative stress in liver, lung, kidney, brain, heart, skeletal muscle, and serum of mice in a dose-dependent manner. Cordero et al., (2010) revealed that amitriptyline produces the highest cellular damage, inducing high levels of ROS followed by irreversible serious mitochondrial damage. Viola et al., (2000) found that, free radical scavengers like butylated hydroxanisole and glutathione significantly reduced lipid peroxidation induced by TCAs. According to these results, liver and kidney seems to be the organs more predisposed to clomipramine oxidative toxicity.

MN frequencies have been considered to be a reliable index for detecting chromosome breakages and loss (Lajmanovich et al., 2005). MN are small chromatin bodies that appear in the cytoplasm by the condensation of acentric chromosome fragments or by whole chromosomes (Martinez et al., 2005), often induced by clastogenic substances or spindle-poison in dividing cells such as bone marrow (Fenech, 2000). In the present study, the administration of clomipramine hydrochloride in three therapeutic doses for 30 days caused a significant increase in the number of micronucleated polychromatic erythrocytes, this increase reached more than ten times the value determined before the treatment and directly proportional to the dose. While in the recovery period (4 weeks), the micronuclei values were significantly decreased compared to clomipramine hydrochloride treated groups.

These results were in-agreement with Madrigal-Bujaidar et al., (2008) who showed a significant increase in both micronucleated polychromatic erythrocytes (MNPE) and micronucleated normochromatic erythrocytes (MNNE) induced by Imipramine & desipramine from the first week of administration. At the fourth week, imipramine increased three times the control level, while the effect of desipramine was about seven times such level. We also observed a recovery in the following 4 weeks, when the chemical administration was suspended. Wyatt and Pittman, (2006) revealed that, induction of micronuclei by desipramine was higher than the one observed with Methyl methanesulfonate (MMS), a direct monofunctional alkylating agent that produces various DNA alterations and used as a positive control agent.

Therapeutic drugs may produce genotoxic damage by a direct interaction with DNA or after their metabolic transformation (Farber, 1987). The genotoxic effect induced by the antidepressants may be related with their potential to increase the number of reactive oxygen species (Xia et al., 1999; Slamon et al., 2001). The metabolism of tricyclic compounds is very complex and it involves N-desamination first to a secondary amine, then to a primary amine, in addition to N-oxidation and ring hydroxylation. Moreover, direct conjugation leads to a quaternary ammonium-linked glucuronide (Nalecz-Jawecki, 2008). During these processes, various reactive intermediates, can form and they produce adducts on the DNA (Isobe et al., 2005). Metabolism of clomipramine can form various reactive intermediates such as N-desmethylclomipramine and corresponding 8-hydroxy-metabolites that may produce adducts on the DNA (Masabuchi et al., 2007). The chemical structure of the antidepressants includes two potentially dangerous components related with mutagenic and carcinogenic events, and particularly with the formation of SCE’s: one of these components is the aromatic ring, and the other the nitro group (Weinstein, 1988).
Clomipramine may be transformed into nitroso compounds causes genotoxic damage (Brambilla and Martelli, 2007).

So much more care should be taken when we used antidepressant drugs that cause an excess of micronuclei, which have been related with the development of cancer, particularly when the involved agent acts in successive cell generations. This may be related with their mechanism of action which seems to involve some activity of their metabolites (Murgia et al., 2008; Saran et al., 2008). Epidemiologic surveys have detected a correlation between the consumption of tricyclic antidepressants and the increase in the proportion of breast or prostate cancer (Sharpe et al., 2002).

The percentage of DNA in the tail (tail intensity) has been shown to be proportional to the frequency of DNA strand-breaks (Olive et al., 1990). In the present study, the mean of percentage of DNA in the tail of the lymphocytes in mice treated with low, medium and high therapeutic doses of clomipramine hydrochloride were significantly higher than in controls. However, in the recovery period (4weeks), the endogenous DNA damage values were significantly decreased. These results were in-accordance with Korobkova et al., (2011) who found both clomipramine and desipramine degraded DNA in the presence of HRP/H2O2 by agarose gel electrophoresis.

On the other hand, Brambilla et al. (2009) demonstrated that, Clomipramine gave positive result(s) in genotoxicity assays but tested negative in carcinogenicity assays. Draz et al., (2009) concluded that, fluoxetine, sertraline and clomipramine may induce DNA damage with fluoxetine produced the most, sertraline produced the intermediate and clomipramine produced the least damage. DNA fragmentation has been observed under clomipramine treatment of human peripheral lymphocytes (Hansson et al., 1997) and human leukemic cells (Xia et al., 1999). Clomipramine was shown to be genotoxic to wing cells of Drosophila melanogaster (van Schaik and Graf, 1993). Supporting the present study, a study on assessment of DNA damage in C6 glioma cells after antidepressant treatment using an alkaline comet assay conclude that there were increases in DNA damage with increasing concentrations of antidepressants in C6 cells (Slamon et al., 2001). Dundaroz et al., (2002) observed that, statistically higher DNA damage by comet assay in lymphocytes of the enuretic children who were taking imipramine than control group. DNA damage can occur as a result of direct radical-mediated nuclear damage, or indirectly from the consequences of numerous other sites of cellular damage. It is a relatively common event in the life of a cell and may lead to mutation, cancer, and cellular or organismic death. DNA damage induces several cellular responses that enable the cell either to eliminate or cope with the damage or to activate a programmed cell death process, presumably to eliminate cells with potentially catastrophic mutations (Sancar et al., 2004).

**SUMMARY**

The results obtained from this study demonstrate that oxidative damage plays a major role in clomipramine induced hepatorenal impairments. The drug also caused a significant increase in the frequency of micronuclei in polychromatic erythrocytes of mice bone marrow and DNA damage; also a recovery observed in the following 4 weeks after the drug administration was suspended. However, considering that cumulative mutations may seriously affect stability of the organism, present results suggest that the chronic use of the tested antidepressant poses a risk to human health. Clomipramine should be used with caution in patients with known hepatic and renal disease, and periodic monitoring of hepatic enzyme concentrations and kidney function tests in such patients is essential. Therefore, it may be pertinent for regulatory agencies to update the genotoxic information on the drugs.
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