Protective Effect of Curcumin Against Hepatic Toxicity Induced by Lithium Carbonate (Li$_2$Co$_3$)

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Abstract: The current work was done on four groups of rats (10 for each) to detect the hepato-protective characters of curcumin on the toxicity induced by lithium carbonate. The oral administration of Li$_2$Co$_3$ for one-month lead to noticeable decrease in the SOD, CAT and GSH while the increase in the GPx and some histological changes in the portal tissues including hydropic degeneration and thickening in the wall of the veins with numerous vacuolar degenerations were detected in most of the liver cells. Haemorrhagic, edematous blood vessels were noticed in the portal tissues. Focal areas of lymphocytic infiltration were located around the congested blood vessels and spread between the liver hepatic cords. Noticeable significant elevation of total chromosomal structure aberrations (a centaromeric, dicentric, break, fragment, deletion, sticky, end to end and ring) and total chromosomal numerical aberrations (hypoploidy, hyperploidy and polyploidy). While the pre-treatment with curcumin lead to improvement of the hepatic architecture and biochemical parameters. Also diminish of all chromosomal structure aberrations and all chromosomal numerical aberrations were noticed after treatment with curcumin. Curcumin can prevent the hazard effect of lithium carbonate on liver tissue.

Keywords: Curcumin, Hepatic Toxicity, Lithium Carbonate, Cytogenetic

Introduction

Li$_2$Co$_3$ is considered monovalent cation which is used as therapeutic agent on wide range all over the world. It commonly can be valuable in dealing with depression and mental disorders (Markowitz et al., 2000; Niethammer and Ford, 2007).

It also used for management and control of suicide (Cerqueira et al., 2008). Although the Li$_2$Co$_3$ is used for the control of psychiatric disorder, it also has harmful effect and may lead to toxicity in different organs and may cause death (Kumarguru et al., 2013).

Some important studies showed that up use of Li$_2$Co$_3$ as medication lead to non-curable alterations in the brain, heart and liver and this may lead to direct death in several months. (Delva and Hawken, 2001).

Lithium compound are widely used as medication in different forms although its dangerous side effects on the general health (Kielczykowska et al., 2006).

The effect of Li$_2$Co$_3$ on the kinetics of the antioxidant enzymes was studies in blood and tissues. It has no significant changes on glutathione peroxidase (GPx) and superoxide dismutase (SOD) and also on malonyldialdehyde concentration in blood. It causes diminishes in the kinetics of the enzymes in tissues, highly detected in liver (SOD) and kidney (GPx and SOD).

The Li$_2$Co$_3$ had no detectable effect on lipid peroxidation in tissues (Kielczykowska et al., 2006). It also had detectable reduction in reduced glutathione, SOD, CAT, GSH, GPx activities, and parallel diminish in ATP in rat tissues. The total lipids, and fatty acids in hepatic tissues were elevated in abnormal manner due to lithium carbonate toxicity.

Curcumin is now days used as important element in the structure of compound or single medication for the control of inflammatory disorders especially in hepatic tissues, since it has powerful effects on the body immunity and is a powerful inhibitor of different reactive oxygen-generating enzymes (Araujo and Leon 2001; Chainani, 2003).
Materials and Methods

Drugs and Chemicals

Li₂Co₃ (Prianil CR): Commercial drugs widely used in Egypt as tablets. Each tablet contains 400 mg of Li₂Co₃. Produced by the Nile Company (Cairo, Egypt). The recommended dose was dissolved in 20 ml distilled water.

Curcumin: The curcumin powder was used in the current work which was extracted from the turmeric roots by acetone solvent according to (Tonnesen, 2002)

Experimental Protocol

Animal experiments were done following guidelines set by Ethical Committee of Benha University. The rats were divided into 4 groups, each of 10 rats:

I. Group: Rats were given distilled water and normal saline orally by intra-gastric gavage for (30 days)

II. Group: Rats were given curcumin orally at doses (150 mg/kg body weight/day) dissolved in 0.5 ml of 10% DMSO according to (ElMackawy and Sharaf 2006)

III. Group III: Rats were given (150 mg/kg b.wt/ day.) of Li₂Co₃ orally (Vijaimohan et al., 2010) which was dissolved in distilled water for 30 days and were sacrificed after 24 hrs of last dose. This dose was toxic for rats.

IV. Group IV: Rats were given curcumin (150 mg/kg b.wt./day) orally 1 hour before the dose of Li₂Co₃ as group III daily for 30 days

After the end of the experiment, the hepatic tissue was collected and kept in 10% neutral buffered formalin for histological preparations according to (Bancroft and Gamble, 2008). The hepatic tissue sample was kept for biochemical analysis. The bone marrow of the femurs bone was prepared for cytogenetic materials.

Genotoxic Studies (Chromosomal Aberration)

According to (Alder, 1984) all rats were prepared for IP injection by colchicine 4 mg/kg bwt. After 2 hours of injection rats were scarified, then dissect femurs bone and flush bone marrow by normal saline in centrifuge tube which centrifuged at 1000 rpm for 300 sec, then decant supernatant. Add sufficient amount of hypotonic potassium chloride (KCL 0.56% solution) at 37°C to each tube and kept in the incubator in degree similar to room temperature for 10 min then centrifuged and decant the supernatant.

The sediment was fixed by methanol (cold): acetic acid glacial (3:1), the fixation was repeated twice time with 10 minutes interval, the last fixation for 30 minutes at 4°C then centrifuged and decant supernatant. Pellets were re-putted in fresh fixative solution. Dropping 2-3 drops of the content by paster pipette on clean cold glass slides in a distance more than 50cm length and placed on wormer slid for drying then stained with 5% Geimsa stain according to the appropriate time and washed by distilled water, left to dry. These slides were examined by oil immersion microscope lens to determine structural and numerical aberration for each rat according to (Ogo et al., 1996).

Determination of Catalase (CAT) Activity

CAT activity (in U/g tissue) was assessed liver homogenate by the method of (Aebi, 1984).

Determination of Glutathione

Total glutathione level was measured enzymatically in the liver tissue by a slightly modified method of (Mangino et al., 1991).

GSH (reduced glutathione) levels were quantified by the method of (Moron et al., 1979). The absorbance of the solutions were estimated at 412 nm against blank. The level of ATP in the heart tissue was determined by the method of (Ryder, 1985).

Estimation of Antioxidant Enzymes (SOD)

The activity of superoxide dismutase (SOD) was measured according to (Marklund and Marklund, 1974).

Statistical Analysis

The data were analyzed by Bartlett’s test for equal variance and by Gaussian-shaped distribution for normality using the Kolmogorov–Smirnoff goodness-of-fit test. As the data passed the normality test (p > 0.001), group means were compared with the ANOVA with post hoc testing using Tukey-Kramer Multiple Comparisons Test. All results were expressed as means ± SEM and the significance were defined as p < 0.001 for all tests.

Results

The antioxidant activities of the liver were decreased in the lithium carbonate administered group compared to the control as shown in (Table 1). Pretreatment with curcumin lead to improve in the antioxidant defense mechanism enzyme in compare to the third group. While in the second group (Curcumin) no significant changes in the enzyme activity in compare to the first group (Control).
Table 1: Antioxidants Enzymes activities in liver tissue

<table>
<thead>
<tr>
<th></th>
<th>SOD</th>
<th>CAT</th>
<th>GSH</th>
<th>GPx</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>7.51± 0.36</td>
<td>3.81±0.19</td>
<td>4.99±0.02</td>
<td>0.02±0.002</td>
</tr>
<tr>
<td>Curcumin</td>
<td>7.64± 0.41</td>
<td>3.83±0.31</td>
<td>5.01±0.04</td>
<td>0.02±0.001</td>
</tr>
<tr>
<td>Lithium</td>
<td>5.51± 0.32*</td>
<td>2.98±0.15*</td>
<td>2.99±0.02*</td>
<td>0.05±0.004</td>
</tr>
<tr>
<td>Lithium+ curcumin</td>
<td>7.17± 0.35a</td>
<td>3.01±0.23a</td>
<td>3.79±0.04a</td>
<td>0.03±0.001a</td>
</tr>
</tbody>
</table>

SOD: Superoxide dismutase (u/g); CAT: Catalase (u/g); GSH-reduced glutathione - mg of GSH/mg protein. GPx- Glutathione peroxidase: U/mg.

These values were appeared as significant difference at *P ≤0.001 as compared with first control group; significant difference at aP ≤0.001 as compared with second group (lithium carbonate group)

Fig. 1 and 2: Photomicrograph of the liver tissue showing normal structure of the hepatic tissue; central vein (C) and hepatic cords (h) H&E

Fig. 3, 4 and 5: The third group had numerous histopathological changes as minutes vacuoles (v) hydropic degeneration (hy) thick wall blood vessels (arrow), Focal areas of lymphocytic infiltration (arrow), Some blood vessels showed edema and thickening their walls (o) H&E
Histopathological Findings

The liver of the control group consisted of hepatic cords with central vein. The hepatocytes were consisted of polygonal cells with prominent nuclei which had basophilic stain and acidophilic stained cytoplasm (Fig. 1). The liver tissue of the second group had well developed hepatic cords (Fig. 2). The hepatocytes of the lithium carbonate administered group were filled with minutes vacuoles as hydropic degeneration and thickening in the wall of the veins with numerous vacuolar degenerations were noticed in most of the hepatocytes. congested blood vessels (Fig. 3). Focal areas of lymphocytic infiltration were located around the congested blood vessels and spread between the hepatic cords (Fig. 4). Hepatic blood vessels showed edema and thickening their walls (Fig. 5). The liver of the treated group showed regeneration in the cells of the hepatic cords and some hepatocytes still suffered from vacuolar degeneration (Fig. 6).

Genotoxic (Chromosomal Aberrations) Studies

Prominent significant elevation of total chromosomal structure aberrations (a centromeric, dicentric, break, fragment, deletion, sticky, end to end and ring) and total chromosomal numerical aberrations (hypoploidy, hyperploid and polyploid) in group III (Lithium Carbonate only) in relation to the control one, group II (Curcumin only) and group IV treated with (Lithium Carbonate and Curcumin). While prominent significant decrease of total chromosomal structure aberrations and total chromosomal numerical aberrations in group II (Curcumin only) and group IV treated with (Lithium Carbonate and Curcumin) than group III (Lithium Carbonate only) (Figs. 7, 8, 9, 10, 10a, 11 and 12).

Fig. 6: The treated group showed improvement in the architecture of the hepatic tissue although some hepatocytes showed vacuolar degeneration (v) H&E. Scale bar.50

Fig. 7: Meta phase spread shown structural chromosomal aberration in rats treated with Lithium (arrow) end to end aberration
**Fig. 8:** Meta phase spread shown structural chromosomal aberration in rats treated with Lithium ring aberration

**Fig. 9:** Meta phase spread shown structural chromosomal aberration in rats treated with Lithium (fragment and) deletion aberration

**Fig. 7:** Meta phase spread shown structural chromosomal aberration in rats treated with Lithium (arrow) end to end aberration
Fig. 8: Meta phase spread shown structural chromosomal aberration in rats treated with Lithium ring aberration.

Fig. 9: Meta phase spread shown structural chromosomal aberration in rats treated with Lithium (fragment and) deletion aberration.

Fig. 10 and 10a: Meta phase spread shown structural chromosomal aberration in rats treated with Lithium (A) a centromeric attenuation and (S) sticky aberrations.
Our findings showed the use of lithium carbonate as medication lead to hepatic toxicity and the liver tissues showed numerous histopathological alterations. These findings were augmented by the results of (Bhat et al., 2014), Shah et al., 1995; Sharief et al., 2011). While the liver of patients had oral dose of lithium carbonate showed increase level of serum aminotransferases, with minimal hepatic hazard effects. The histopathology findings showed no hepatic injury or other alterations including jaundice (McKnelly et al., 1970).

The oxidative stress was initiated after lithium toxicity which direct lead to increase lipid peroxidation. SOD and catalase enzymes characterized by decreased their levels in the tissue (Tandon et al., 1998).

The curcumin, because it has potent antiinflammatory (Bereswill et al., 2010), antioxidant properties (Al-Jassabi et al., 2012), antimicrobial activity (Tajakhsh et al., 2008), antiviral activity (Kutluay et al., 2008), anticarcinogenic (Das and Vinayak, 2012) and anti-diabetic (Aziz et al., 2013) effects, so it used for the cure of hepatic disorders including injury due to toxicity (Altenburg et al., 2001; Chattopadhyay et al., 2004).

Both of carbon tetrachloride and aflatoxin B caused intensive damage to the hepatic tissue (Morsy et al., 2012; El-Agamy, 2010 respectively). So, it was discovered that the curcumin had protective effect on liver tissues (Jobin et al., 1999). Also the curcumin has silymarin like action (Girish, 2009) which lead to prevention the hepatic injury (Li et al., 2013) via inhibition of MAO-A enzyme in hepatic tissue (Yu et al., 2002).

Curcumin is considered one of the powerful scavenger of (ROS) reactive oxygen species including superoxide anion radicals and hydroxyl radicals. Curcumin played an fundamental role in inhibition of blood (Erythrocyte) lipid peroxidation during the process of inflammation (Borra et al., 2013). Also, it prevents the creation of free radicles which has harmful role in myocardial ischemia and paraquat
induced lung injury in rats (Manikandan et al., 2004; Venkatesan, 2000). Also, curcumin protected blood, liver, and erythrocyte in response to diazinon-toxicity in Wistar rats (Messarah et al., 2013).

Regarding to the effect of lithium carbonate on the chromosomes revealed prominent increase of total chromosomal structure aberrations and total chromosomal numerical aberrations in group III in comparison with control one, group II and group IV. Our studies are in confirmation with those of (Rafael et al., 1976) where in the chromosome breakdown caused by lithium carbonate was dose dependent. Srivastava et al. (1986) recorded that chromosomal damages caused by Lithium Carbonate was due to breaks and fragmentations of DNA. Moreover, that Lithium could have several ways of acting on DNA as Li binds selectively to DNA (Kuznetsov et al., 1971) as it competes with Mg2+-leading to impairment on DNA synthesis (Becker and Tyobeka, 1990) and DNA repair.

While in group II and group IV than group III our result showed that significant decrease of total chromosomal structure aberrations and total chromosomal numerical aberrations which agree with Treshiana et al., 1998; Petr et al., 2006), where they explain the anti-mutagenic effect of curcumin against different mutagenic agents, as they found administration of antioxidants inhibited the breakdown of DNA strand induced by lithium Carbonate toxicity.

**Conclusion**

The lithium carbonate was toxic to the hepatocytes when used as medication for depression but the addition of curcumin as replacement medication can ameliorate the hazard effect of lithium carbonate.

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**Author’s Contributions**

**Gehan Youssef**: Contributed in proposal writing, final report, performance of scientific comments and publishing the paper.

**Ahlam Hamouda**: Contribute in preparing the extract, review articles and interpretation the cytogenetics

**Rabab Elzoghby**: Contributed in proposal writing, drugs preparation and final report.

**Fatma Elgendy**: Contributed in design of the experiment, performance of the scientific comments and interpretation the cytogenetics.

**Shimaa Attwa**: Contributed in biochemical analysis, final report and performance of static analysis.

**Conflict of Interests**

The authors declare that there is no conflict of interests regarding the publication of this paper.

**References**


