The introduction of lithium therapy mainly used in curing some psychiatric diseases responsible for numerous undesirable side effects on different organs in humans. The present study explores the beneficial effect of sobatum, a purified compound of Solanum trilobatum, on lithium carbonate (Li$_2$CO$_3$)-induced multiple organ toxicity in rats. Li$_2$CO$_3$ (150 mg/kg body weight) was administered orally in drinking water for a period of 30 days to induce toxicity in rats. Li$_2$CO$_3$ could induce lipid peroxidation to a significant extent that was accompanied by marked reduction in reduced glutathione, SOD, CAT, GST, GPX activities, and parallel decline in ATP in tissues. Toxicity resulted in abnormal elevation of lipids such as cholesterol, triglycerides, phospholipids, and fatty acids in liver tissues. Treatment with sobatum affords substantial protection in liver and heart by altering all the parameters to near normal levels that were further confirmed by histological examination. Sobatum prevents Li$_2$CO$_3$-induced oxidative damage of DNA by reducing DNA fragmentation indicating its block on cell death. However, these results demonstrated that sobatum has the ability to suppress the drug-induced toxicity.

Key words: Antioxidants, lithium toxicity, oxidative damage, sobatum, Solanum trilobatum

DOI: 10.4103/0975-1483.62217

The introduction of lithium therapy mainly used in curing some psychiatric diseases responsible for numerous undesirable side effects on different organs in humans. The present study explores the beneficial effect of sobatum, a purified compound of Solanum trilobatum, on lithium carbonate (Li$_2$CO$_3$)-induced multiple organ toxicity in rats. Li$_2$CO$_3$ (150 mg/kg body weight) was administered orally in drinking water for a period of 30 days to induce toxicity in rats. Li$_2$CO$_3$ could induce lipid peroxidation to a significant extent that was accompanied by marked reduction in reduced glutathione, SOD, CAT, GST, GPX activities, and parallel decline in ATP in tissues. Toxicity resulted in abnormal elevation of lipids such as cholesterol, triglycerides, phospholipids, and fatty acids in liver tissues. Treatment with sobatum affords substantial protection in liver and heart by altering all the parameters to near normal levels that were further confirmed by histological examination. Sobatum prevents Li$_2$CO$_3$-induced oxidative damage of DNA by reducing DNA fragmentation indicating its block on cell death. However, these results demonstrated that sobatum has the ability to suppress the drug-induced toxicity.

Key words: Antioxidants, lithium toxicity, oxidative damage, sobatum, Solanum trilobatum

DOI: 10.4103/0975-1483.62217

The introduction of lithium therapy mainly used in curing some psychiatric diseases responsible for numerous undesirable side effects on different organs in humans. The present study explores the beneficial effect of sobatum, a purified compound of Solanum trilobatum, on lithium carbonate (Li$_2$CO$_3$)-induced multiple organ toxicity in rats. Li$_2$CO$_3$ (150 mg/kg body weight) was administered orally in drinking water for a period of 30 days to induce toxicity in rats. Li$_2$CO$_3$ could induce lipid peroxidation to a significant extent that was accompanied by marked reduction in reduced glutathione, SOD, CAT, GST, GPX activities, and parallel decline in ATP in tissues. Toxicity resulted in abnormal elevation of lipids such as cholesterol, triglycerides, phospholipids, and fatty acids in liver tissues. Treatment with sobatum affords substantial protection in liver and heart by altering all the parameters to near normal levels that were further confirmed by histological examination. Sobatum prevents Li$_2$CO$_3$-induced oxidative damage of DNA by reducing DNA fragmentation indicating its block on cell death. However, these results demonstrated that sobatum has the ability to suppress the drug-induced toxicity.

Key words: Antioxidants, lithium toxicity, oxidative damage, sobatum, Solanum trilobatum

DOI: 10.4103/0975-1483.62217
and produce no toxicities. Sobatum also possesses anti-inflammatory and free radical scavenging activities by inhibiting superoxide radical production. These points made us interested to do research on sobatum against lithium-induced toxicity. However, no studies have been performed to study whether sobatum have beneficial effects on lithium carbonate (Li$_2$CO$_3$)-induced toxicity in experimental models of organ injury, which may have implications in managing humans with accidental exposures to such compounds.

Considering above information, the present study aims to investigate the effect of sobatum on toxicity caused by Li$_2$CO$_3$ in liver, heart, and kidney tissues of experimental rats through evaluating lipid peroxidative content, hepatic lipid levels, antioxidant enzymes, adenosine triphosphate (ATP) levels, apoptotic cell death, and histological changes.

**MATERIALS AND METHODS**

**Chemicals**

All chemicals and reagents were of analytical grade or of highest purity, commercially available. Lithium carbonate, glutathione, disodium phenyl phosphate, and bovine serum albumin were purchased from Sigma Chemical Co., St Louis, MO, USA. EDTA, DNAase, and proteinase K were purchased from British Drug House Pvt. Ltd Glaxo Division, Mumbai, India.

**Plant materials and extraction of sobatum**

The whole plant of *Solanum trilobatum* was collected in and around Chennai, India, and authenticated in the Department of Pharmacognosy, Captain Srinivasa Murthi Drug Research Institute for Ayurveda, Chennai, India. A voucher specimen has been deposited in the same institute (No: 1785). The plant samples were shade-dried and powdered using an electric blending machine. For the preparation of the extract, 100 g of powdered plant material was subjected to extraction with 90% petroleum ether in a Soxhlet apparatus up to four cycles. After filtration through Whatman filter paper no. 40, the filtrate was dried using a lyophilizer.

The petroleum ether extract of *S. trilobatum* was subjected to chromatography using Spherisorb-ODS2 25 cm × 4.6 cm, 5 cm, C$_{18}$ silica column using water and petroleum ether/ethyl acetate (75:25) as a solvent at a flow rate of 1 ml/min. These fractions were concentrated and crystallized from solvent giving only one pure crystalline compound that was identified and compared with authentic sample. For oral administration, the sobatum compound was dissolved in 0.5 ml of 10% dimethyl sulphoxide (DMSO) immediately before use.

**Animals**

Adult male albino rats of Wistar strain weighing about 150-200 g were purchased from Fredrick Institute, Padapai, India. The animals were housed at 27 ± 2°C temperature, 55% humidity, and a 12 h-light/dark cycle. They were fed with standard laboratory chow (Hindustan Lever Foods, Bangalore, India) and provided with water *ad libitum*. Experimental protocols were approved by our Institute ethical committee, which follows the guidelines of Institutional Animals Ethics Committee (IAEC) (360/01/a/CPSEA).

**Experimental design**

Animals were divided into four groups each containing six animals. Group I animals served as control who received the vehicle (0.5 ml of 10% DMSO) alone orally. Group II animals constituted the toxicity group, who received Li$_2$CO$_3$ (150 mg/kg body weight) orally in drinking water for a period of 30 days. Group III animals received Li$_2$CO$_3$ (150 mg/kg body weight) orally in drinking water for a period of 30 days and treated simultaneously with sobatum orally at doses (30 mg/kg body weight/day) dissolved in 0.5 ml of 10% DMSO. Group IV rats received sobatum orally at doses (30 mg/kg body weight/day) dissolved in 0.5 ml of 10% DMSO.

After 24 h of sobatum last dose treatment, animals were sacrificed by cervical decapitation under pentobarbitone sodium (60 mg/kg) anesthesia and blood was collected without an anticoagulant to separate serum for biochemical investigation. The liver and heart organ were dissected out immediately and washed in ice-cold saline. The tissues were then homogenized in ice-cold Tris-HCl buffer (0.1 M, pH 7.4) and 10% homogenate was obtained and used for the following biochemical investigations.

**Determination of lipid peroxidation and reduced glutathione levels**

LPO was measured in hepatic homogenates according to the method of Okhawa *et al.* based on the formation of thiobarbituric acid reactive substances (TBARS) and expressed as the extent of malondialdehyde (MDA) production. Glutathione (GSH) levels were quantified by the method of Moron *et al.*(1979). The tissue homogenate was treated
with 1 ml of 5% TCA in 1 mM EDTA and centrifuged at 2000 g for 10 min. After that 1 ml of the filtrate was mixed with 5 ml of 0.1 M phosphate buffer (pH 8.0), and 0.4 ml of 5,5’-dithiobis (2-nitrobenzoic acid). The absorbance of the solutions was estimated at 412 nm against blank. The level of ATP in the heart tissue was determined by the method of Ryder.[104]

**Estimation of antioxidant enzymes**

The activity of superoxide dismutase (SOD) was measured, using an assay based on the ability of SOD to inhibit the autoxidation of pyrogallol by 50%. One unit of enzyme is defined as the amount of enzyme that causes half maximal inhibition of pyrogallol autooxidation/mg protein/min.[17] The assay of catalase was performed by following the method of Aebi. One unit of enzyme activity is defined as the amount of enzyme required to decomposed 1 mmol of H₂O₂/mg protein/min.[18] The activity of glutathione peroxidase (GPx) was measured using a coupled enzyme assay as described by Lawrence and Burk.[19]

**Estimation of lipid profile**

Lipid was extracted from liver tissues by the method of Folch et al. using the chloroform-methanol mixture (2:1 v/v).[20] The total cholesterol, free cholesterol, free fatty acids (FFA), triglyceride (TG), phospholipids (PL) after perchloric acid digestion have been determined in liver tissues of sobatum-treated rats.[21-25]

**Histopathology**

The heart tissue samples were preserved in 10% neutral buffered formalin for 24 h, processed for routine paraffin block preparation, sections of 5µm thick were cut and stained with hematoxylin and eosin. These were examined under the light microscope for histological changes in tissues.

**Studies on DNA damage**

The kidney tissue was used for the isolation of DNA by homogenizing in PBS-E (50 mM sodium phosphate buffer containing 0.9% saline and 20 mM EDTA, pH 8) and suspended in 2 ml of PBS-E containing 0.5 mg/ml of collagenase. The suspension was incubated at 37°C for 1 h with stirring, followed by the addition of pronase E (1 mg ml), and further incubated for 15 min at 37°C. It was then centrifuged at 1000 rpm for 5 min. The pellet was dispersed and incubated with 2 ml of a lysis buffer containing 50 mM Tris-HCl¹, pH 8, 20 mM EDTA, 10 mM NaCl and 1% w/v SDS for 15 min. It was centrifuged again at 14,000 X g for 15 min, and DNA was isolated from the lysate by a phenol-chloroform extraction procedure.[20] DNA was dissolved in 10 mM Tris-HCl⁻, pH 8, containing 1 mM EDTA by gentle shaking at 65°C. Residual contaminating RNA was removed by incubating the DNA solution with 1 µg/ml DNase-free RNase at 37°C for 1 h followed by 0.1 mg ml proteinase K for 3 h. Phenol-chloroform extraction was repeated to obtain purified DNA that was dissolved in 10 mM Tris-HCl² buffer, pH 8, containing 1 mM EDTA. To study DNA fragmentation, DNA was loaded on to a 1.5% agarose gel. In addition to compare the test samples with the standard antioxidant 1 µg of catalase was used and electrophoresis was carried out at 100 V for 1.5 h in TBE (90 mM Tris borate, 2 mM EDTA, pH 8) buffer and DNA was visualized by UV exposure after staining with ethidium bromide.

**Statistical analysis**

Data are expressed as mean ± SD. The results were computed statistically (SPSS software package, version 11.0) using one-way analysis of variance. Post hoc testing was performed for inter-group comparison using the Dunnett’s ‘t’ test. P < 0.001, P < 0.01, P < 0.05 were considered to be statistically significant.

**RESULTS**

Compared with controls, lithium-toxicated rats were accompanied by increased TBARS production and decreased antioxidant defense enzyme activities, suggesting oxidative stress in liver tissue [Table 1]. Administration of sobatum led to a marked reduction in the level of TBARS oxidative stress in liver tissue [Table 1]. Administration of sobatum-alone-treated rats, no significant change in enzyme activities could be observed, as compared to lithium-treated rats. On the other hand, in sobatum-alone-treated rats, no significant change in enzyme activities could be observed, as compared to that of normal control.

Li₂CO₃ caused a significant increase in free cholesterol, total cholesterol, triglycerides, phospholipids, and fatty acid content as compared to the corresponding control group.

**Table 1: Effect of sobatum on the changes of liver antioxidant defense enzyme activities in Li₂CO₃ administered rats**

| Groups | SOD | CAT | GPx | TBARS | GSH |
|--------|-----|-----|-----|-------|-----|
| I      | 8.61±0.45 | 4.81±0.19 | 0.02±0.001 | 0.56±0.04 | 5.98±0.03 |
| II     | 6.51±0.32 | 2.98±0.15 | 0.05±0.003 | 1.02±0.09 | 3.99±0.02 |
| III    | 8.17±0.35 | 4.01±0.23 | 0.04±0.002 | 0.70±0.05 | 4.82±0.03 |
| IV     | 8.64±0.41 | 4.83±0.31 | 0.02±0.001 | 0.54±0.04 | 6.01±0.05 |

Results are expressed as mean± SD for groups of six animals each; SOD: Superoxide dismutase; CAT: Catalase; GPx: Glutathione peroxidase; U/mg protein. TBARS nmol/mg protein; GSH-reduced glutathione - µg of GSH/mg protein. Values are expressed as significant difference at *P <0.001 as compared with control group; significant difference at †P <0.001 as compared with Li₂CO₃ alone administered rats.
Sobatum treatment showed a marked protection against this enhanced lipid levels in the liver tissue of Group II rats [Figure 1]. No significant differences were observed between normal and sobatum-treated rats. Lipid levels and other biochemical parameters were not altered upon sobatum treatment.

Figure 2 shows the level of myocardial ATP content in normal and experimental groups of rats. Significant reduction in the level of ATP content in the heart tissue of lithium-induced rats as compared to that of Group I rats. The administration of sobatum maintained the level of ATP at near normalcy in Group III rats as compared to that of Group II rats, reflecting its ability to maintain the function of the heart mitochondria at near normal status.

Microscopic examination of heart tissue of the Group I control rats showed normal myocardial fibers and muscle bundles with normal architecture. Heart tissue of Group II rats showed separation of myocardial fibers with inflammatory mononuclear collections, edema, and myocardial necrosis. Sobatum-alone-treated Group IV rats showed normal myocardial fibers with no pathological changes. Myocardial section of sobatum-treated Group III rats showed slightly separated myocardial fibers with small focus of inflammatory mononuclear collections with the absence of necrotic damage [Figure 3].

Agarose gel electrophoresis of kidney tissue DNA, treated with or without sobatum in Li₂CO₃-treated rats is shown in Figure 4. Control rat and sobatum-treated rat showed the absence of DNA fragmentation in kidney tissue, which was observed in Lane 3 and Lane 4. In Lane 1, the toxic effects of Li₂CO₃ were reflected in a significantly higher rate of apoptosis in kidney tissue of Group II rats so no DNA band was observed. However, in Lane 2, Li₂CO₃-induced DNA fragmentation was markedly reduced by treatment with sobatum suggesting its role to prevent cell death. Sobatum provides significant protection against DNA damage in comparison with the already known antioxidant, catalase (Lane 5) suggesting its antiapoptotic role during oxidative damage.

DISCUSSION

Lithium toxicity represents a state of increased oxidative stress, which is mainly based on the evidence of increased lipid peroxidation (LPO), or by indirect evidence of reduced antioxidant reserve, such as SOD and catalase enzymes, in animal models.[27] Sobatum have been reported to play a beneficial role in the reduction of free radical production.[11]

In our present study, the administration of sobatum exerted beneficial effects against lithium toxicity followed by the fall in lipid peroxidation products. During physiological states, SOD metabolizes superoxide anion (O₂⁻), producing hydrogen peroxide (H₂O₂), which can react with iron to generate highly reactant hydroxyl radicals via the Fenton reaction. CAT is the most important peroxidase in detoxifying excess hydrogen peroxide to prevent hydroxyl production. Thus an increase in SOD or CAT levels per se does not necessarily indicate increased oxidative stress, whereas an imbalance between SOD and CAT activities could lead to an excessive generation of free radicals.[28]

The antioxidative defense system like SOD, CAT, and GPx showed lower activities in liver during toxicity condition. Treatment of the lithium-induced animals with sobatum restored the altered activities of SOD, CAT, and GPx. This study suggests that possible mechanism of this protective
activity against lithium toxicity may be due to free radical-scavenging and antioxidant activities.\textsuperscript{[9]}

Lithium-induced elevation in lipid levels could be due to increase in biosynthesis and decrease in its utilization. \(\text{Li}_2\text{CO}_3\) induces free radicals, which may cause cellular cholesterol accumulation, by increasing cholesterol biosynthesis and its esterification. The significant increase in the rate of TG, PL, and FFA synthesis by the liver is a well-known risk of toxicity caused by \(\text{Li}_2\text{CO}_3\).\textsuperscript{[29]} Abnormal activities of lipid-metabolizing enzymes contributed to these hyperlipid changes in liver caused by \(\text{Li}_2\text{CO}_3\). \textit{S. trilobatum} is a hypolipidemic agent, which reduces the rise in total cholesterol, free cholesterol, TG, FFA, and PL.\textsuperscript{[30]} In addition, antioxidants such as sobatum could be beneficial in preventing the increase in lipidemic status, suggesting that it would prevent the toxicity of \(\text{Li}_2\text{CO}_3\) on lipid metabolism.

In the heart, ATP is synthesized mainly in the mitochondria through oxidative phosphorylation and transported to
Involvement of ROS, oxidative damage of DNA, and DNA fragmentation has also been evident in apoptotic cell death in renal injury.[32] Cytotoxic effects of Li$_2$CO$_3$ that affect the liver tissue are manifested by the disturbances of NO, a key mediator of signaling events linked to apoptotic cell death.[33] Sobatum prevents Li$_2$CO$_3$-induced DNA fragmentation, suggesting its antiapoptotic role to block cell death during damage. Sobatum could have a unique capacity to block this oxidative damage similar to that shown by H$_2$O$_2$ scavenger, catalase, indicating its potent antioxidant role to protect DNA from the attack of ROS.

**CONCLUSION**

The results of the present study indicate that administration of sobatum attenuates lithium-induced organ toxicity in rats through counteraction of free radicals by its antioxidant property, antiapoptotic effect by blocking DNA fragmentation and attenuation of toxic changes in vital organs.

**REFERENCES**

1. Yip KK, Yeung WT. Lithium overdose causing non-convulsive status epilepticus-the importance of lithium levels and the electroencephalography in diagnosis. Hong Kong Med J 2007;13:471-4
2. Warick LH. Lithium poisoning report of a case with neurologic, cardiac and hepatic sequelae. West J Med 1979;130:259-63.
3. Pilcher HR. Drug research: The ups and downs of lithium. Nature 2003;425:118-20.
4. Nadkarni KM. Indian Medical Material, vol.1, 3rd (Eds.), Bombay: Popular Prakashan Pvt. Ltd; 1976. p. 1153-4.
5. Subramani J, Josekutty PC, Mehta AR, Bhatt PN. Solasodine levels in Solanum trilobatum Linn. Indian J Exp Biol 1989;27:189.
6. Shahjahan M, Sabitha KE, Mallika J, Shyamala Devi CS. Effect of sobatum in carbon tetrachloride induced hepatotoxicity in rats. Indian J Med Res 2004;120:294-8.
7. Swappalatha P, Kannabiran K. Antimicrobial activity and phytochemicals of Solanum trilobatum Linn. African J Biotech 2006;5:2402-4.
8. Emmanuel S, Ignacimuthu S, Perumalsamy R, Amalraj T. Antiinflammatory activity of Solanum trilobatum. Fitoterapia 2006;77:611-2.
9. Mohanan PV, Devi KS. Toxictological evaluation of sobatum. Cancer Lett 1998;127:135-40.
10. Mohanan PV, Devi KS. Effect of sobatum on tumour development and chemically induced carcinogenesis. Cancer Lett 1997;12:219-23.
11. Mohanan PV, Rathinam K, Devi KS. Effect of sobatum on ultraviolet-induced damage and superoxide production. Indian J Pharmacol 1997;29:129-31.
12. Oktem F, Ozguner F, Sulak O, Sögar S, Akturk O, Yilmaz HR, et al. Lithium-induced renal toxicity in rats: Protection by a novel antioxidant caffeic acid phenethyl ester. Mol Cell Biochem 2005;277:109-15.
13. Vaji M, Mallika J, Subramaniyam S. Attenuation of brain damage with sobatum in rat brain neuronal cells. Proceedings of International Conference on Genetic and Molecular Diagnosis in Modern Medicine; Hyderabad: 2008. p. 7-9.
14. Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxide in animal tissues by thiobarbituric acid reaction. Anal Biochem 1979;95:351-7.
15. Moron MS, Deptierre JW, Mannervik B. Levels of glutathione, glutathione reductase reductase and glutathione S-transferase activities in rat lung and liver. Biochem Biophys Aeta 1979;582:67-72.
16. Ryder JM. Determination of adenosine triphosphate and its breakdown products in fish muscle by high performance liquid chromatography. J Agri Food Chem 1985;33:678-80.
17. Marklund S, Marklund G. Involvement of the superoxide anion radical in the autooxidation of pyrogallol and a convenient assay for superoxide dismutase. Eur J Biochem 1974;47:469-74.
18. Abe H, Wyss SR, Scherz B, Skvaril F. Heterogeneity of erythrocyte catalase II. Isolation and characterization of normal and variant erythrocyte catalase and their subunits. Eur J Biochem 1974:48:137-45.
19. Lawrence RA, Burkh RF. Glutathione peroxidase activity in selenium-deficient rat liver. Biochem Biophys Res Comm 1976;71:952-8.
20. Folch J, Lees M, Sloane Stanley GH. A simple method for the isolation and purification of total lipids from animal tissues. J Biol Chem 1957;226:497-501.
21. Parekh AC, Jung DH. Cholesterol determination with ferric acetate-uranyl acetate and sulphuric acid-ferrous sulphate reagents. Anal Chem 1970;42:1423-7.
22. Leftfer HH, McDougall CH. Determination of cholesterol in serum by means of improved techniques. Am J Clin Path 1963;39:311-3.
23. Rice RW. Triglycerides (neutral fat) in serum. In: Roederick P, Mac- Donald RP, editor. Standard methods of clinical chemistry. New York: Academic Press; 1970. p. 215-22.
24. Fiske CV, Subbarow Y. Colorimetric determination of phosphorous. J Biol Chem 1925;66:375-400.
25. Hron WT, Menahan LA. A sensitive method for the determination of free fatty acids in plasma. J Lipid Res 1981;22:377-9.
26. Misko TP, Moore WM, Kasten TP, Nickols GA, Corbett JA, Tilton RG, et al. Selective inhibition of the inductive nitric oxide synthase by aminoguanidine. Eur J Pharmaco 1998;33:119-22.
27. Tandon A, Dhawan DK, Nagpal JP. Effect of lithium on hepatic lipid peroxidation and antioxidative enzymes under different dietary protein regimens. J Appl Toxicol 1998;18:87-90.
28. Andrades M, Ritter C, Moreira JC, Dal-Pizzol F. Oxidative parameters differences during non-lethal and lethal sepsis development. J Surg Res 2005;125:68-78.
29. Sharma SD, Iqbal M. Lithium induced toxicity in rats: A hematological, biochemical and histopathological study. Biol Pharm Bull 2005;28:834-7.
30. Pillaik CK, Siddha Vaidya Padhartha Guna Vilakam (Moolavarkam), Materia Medica,. India: Ratna Naicker and Sons; 1973. p. 428-9.
31. Lithium carbonate, Eskalith, Lithane, Lithobid Pharmacology - http://www. HealthyPlace.com.html [homepage on the Internet].
32. Kim SH, Kim YS, Kang SS, Bae K, Hong TM, Lee SM. Anti-apoptotic and hepatoprotective effects of gomisin A on fulminating hepatic failure induced by D-galactosamine and lipopolysaccharide in mice. J Pharmaco Sci 2008;106:225-33.
33. Tajes M, Gutierrez-Cuesta J, Folch J, Ferrer I, Caballero B, Smith MA, et al. Lithium treatment decreases activities of kinases in a murine model of senescence. J Neuropathol Exp Neurol 2008;67:12-3.

Source of Support: Nil, Conflict of Interest: None declared.