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Research Article
Protective Effect of L-cysteine Against Sodium Valproate-induced Oxidant Injury in Testis of Rats

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Abstract

Background and Objective: Sodium Valproate (SV) is a medicine that is used to treat epilepsy and to prevent headache. In this study, L-cysteine (LC) was used to decrease the stress and biochemical variations induced by SV treatment. The present study investigated the defensive actions of LC vs, SV that induced testicular impairment. **Materials and Methods:** The rats were split into six groups (n = 10) as following: 1st control animals were saline-treated, 2nd and 3rd groups were administrated two doses of SV (100 and 500 mg kg^{-1} b.wt.) presenting low and high doses, respectively, 4th group was treated with 100 mg kg^{-1} of LC, in addition 5th and 6th groups were treated with SV-LD+LC and SV-HD+LC, respectively. The experiment was run for 30 successive days. Weights of the testis, serum testosterone, testicular oxidative/anti-oxidant capacity and histopathological damage scores of testis were recorded. **Results:** The SV group had significantly increased the tissue oxidative stress markers and significantly declined all antioxidant enzymes activities as compared to the control group. When the animals treated with combination of LC and any dose of SV, levels of oxidative parameters significantly declined, as well as the anti-oxidant significantly elevated compared to the SV-LD and SV-HD groups. The Johnsen's testicular score values showed improvement when LC was co-treated with the SV. **Conclusion:** Current results indicated that LC had partial protective effects against SV-induced testis damage at the biochemical and histopathological levels that could be due to the enhanced tissue anti-oxidant capacity.

Key words: L-cysteine, testis, serum testosterone, oxidative parameters, anti-oxidant, histopathological damage scores

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

L-cysteine (LC) is one of the important exogenous antioxidants that shelter against oxidative impairment. This effect could be related to the LC itself (inactivated the free radicals) or to the induction of reduced glutathione (GSH) formation[1]. Ahmed *et al.*[2] evaluated some anti-oxidants activity of LC and they found that it antagonizes cisplatin (CP) induced testicular injury of rats. It also reduced the lipid peroxidation (LPO) and enhanced the antioxidant levels of the rats treated with CP[3].

The epileptics require suitable treatment to prevent mortality[4]. Sodium Valproate (SV) is one of the anti-epileptic drugs that have a diverse mechanism of actions which were attributing to its toxicity and efficacy. It was also used in bipolar disorder, migraine prophylaxis, schizophrenia and depression[5]. Acid SV is the famous drug that can control all types of seizures incorporated with the idiopathic epilepsies[6].

Hamza *et al.*[7] found that LC administration inhibited liver injury and improved the redox state of rats due to SV-toxicity as well as improved the nephrotoxicity induced by SV[8]. Moreover, the protective effect of LC in brain tissue of rat was mediated through attenuation of oxidative stress, suggesting a therapeutic role for LC in the side effect of epilepsy, when treated with SV[9].

Animal models had revealed that SV causes reversible changes in sperm motility and count in addition, the histoarchitecture of the testis[10,11]. The histopathological examination showed that SV (400 mg kg^{-1}) had caused degeneration of germinal cells and decrease in the Johnsen's scoring after 14 days of exposure[12].

Spermatogenesis process occurs by the action of many hormones including Follicle-stimulating Hormone (FSH), which is secreted by the pituitary gland to stimulate the Sertoli cells. Luteinizing Hormone (LH) secreted from Leydig cell, stimulates testosterone synthesis. Therefore, these hormones were used as markers of testicular activity in males[13]. Henriksen *et al.*[14] reported that rats have shown a decline in testosterone levels caused by losing the germ cell.

There is no data available on the effects of LC against LPO, $NO^{\cdot}$ and OH^{-} as well as the histological construction of the rat testis tested with SV. It is commonly known that LC prevents neurotoxicological damages as well as the neoprene and hepatotoxicity. So, the study was concerned to describe the injurious effects of SV on the testis weight and oxidative damage. The mechanisms of this protective effect against testicular toxicity may be due to the enhanced tissue anti-oxidant capacity. Thus, this study was full of clinical importance as it focuses on the testicular damage in case of treatment with SV and the role of LC that could reduce the testis damage. Therefore, the aim of the existing study was to research the possible protective importance of LC in the testis toxicity of male rats induced by SV.

MATERIALS AND METHODS

Chemicals: All chemicals were of high analytical grade and obtained from Sigma Chemical Co., St. Louis, Mo., USA.

Experimental animals and design: The Wistar rats weighing 200±5 g were brought from Faculty of Veterinary Medicine, Zagazig University. They were housed under standard laboratory conditions with 12/12 light and dark cycle, with suitable temperature and humidity. The European Community Directive (86/609/EEC) on animal care guidelines has been followed for the Care and Use of Laboratory Animals 8th Edition. Animal experiments received approval from the Ethical Committee of the Pharmacy Faculty of Zagazig University (No. P22/2/2015). The experiment was carried out during July-September, 2015.

The rats were allocated to one group served as control and five-treated groups of 10 animals each. All the rats were orally medicated by gastric tube for 30 successive days. The 1st control group received saline, the 2nd and 3rd groups were given the lower and the higher doses of SV (SV-LD, SV-HD; 100 and 500 mg kg^{-1}, respectively) according to Khan *et al.*[6], 4th group was treated with 100 mg kg^{-1} L-cysteine[2], 5th and 6th groups were given SV-LD+LC and SV-HD+LC as the above doses, respectively. The higher dose of SV was selected as described before by Leclair-Visonneau *et al.*[15] depending on the clinical experiment.

Organo-somatic index of the testis: The body weight of each rat was recorded in the beginning and on the determinative of the experiment. Then, the rats were sacrificed and testis was removed to weight them. From these values, the Organo-Somatic Index (OSI) of testis was calculated using the equation cited by Chirumari and Reddy[16].

Preparation of testis homogenates: The testis was homogenized in 10 mL cold buffer per gram tissue using a Potter-Elvehjem homogenizer in 10 volumes of buffer[17]. The homogenate was centrifuged at 10,000 Xg or 2500 Xg for 20 min at 4°C for estimating antioxidant enzymes assays and MDA level, respectively. The supernatant was preserved in a deep freeze until being used for various assays.

Determination of oxidative biomarkers: The LPO was measured as malondialdehyde (MDA) as described by Ohkawa *et al.*[18]. 1,1,3,3-tetra-ethoxy propane as standard was used to determine MDA levels which expressed as μM g^{-1} tissue. Nitric oxide ($NO^{\bullet}$) reacts with oxygen to produce stable products (nitrate and nitrite) that had been estimated using Griess reagent[19].

Determination of hydroxyl radical scavenging activity of L-cysteine: The evaluation of hydroxyl radical capacity of LC was measured according to Halliwell *et al.*[20]. The percentage inhibition was calculated using the equation:

$$\text{Inhibition (\%)} = \left(\frac{A_0 - A_1}{A_0}\right) \times 100$$

where, A_0 was the absorbance of the control reaction and A_1 was the absorbance in presence of the sample and reference.

Determination of enzymatic anti-oxidant biomarkers: The superoxide dismutase (SOD) activity was expressed as U mg^{-1} protein according to the method of Marklund and Marklund[21]. Catalase (CAT) activity was presented as mM mg^{-1} protein according to Aebi[22]. Glutathione peroxidase (GPx) activity was evaluated by the Hafeman *et al.*[23]. The activity of GPx was expressed in terms of mM GSH consumed/min/g wet weight tissue. Glutathione reductase (GR_X) activity was measured by Couri and Abdel-Rahman[24]. The unit of GRx activity was defined as an increase in the log (GSH) of 0.001 per min.

Determination of non-enzymatic anti-oxidant: Total anti-oxidant capacity was evaluated using ferric reducing anti-oxidant power assay[25].

Determination the testosterone hormone level: Plasma testosterone levels were measured by electrochemiluminescence by automata (Elecsys, Roche Diagnostics)[26].

Histological evaluation: A part of the testis was fixed in 10% neutral buffered formalin and embedded in paraffin. The paraffin blocks were sectioned into slices in 5 μ thickness, mounted onto slides and stained with hematoxylin and eosin as described[27]. The histological evaluations, general characters of tissues, the progressive degeneration of the tubule, proceeding to the loss of spermatogonia and number of Sertoli cells were examined semi quantitatively under a light microscope. The data in each group were reported as Means±SE based on the sum of the score histological criteria of spermatogenesis[28].

Statistical analysis: All analyses were performed using SPSS statistical version 20 (SPSS Inc., USA). Data were presented as mean values ±SE (n = 10). To assess significant differences among treated-groups, statistical analysis was performed using two-way analysis of variance (ANOVA). The criterion for statistical significance was set at $p \leq 0.05$.

RESULTS

Organo-somatic index and biochemical parameters: The OSI of the testis in the SV group was significantly declined as compared to the control rats, according to the dose by 41.2 and 57.1% for SV-LD and SV-HD, respectively (Fig. 1). There was a significant rise in OSI of the testis of the groups received LC with different doses of SV by 1.2- and 1.7-fold for SV-LD+LC and SV-HD+LC, respectively.

The levels of testosterone in control and LC groups were approximately the same. The testosterone level diminished only in rats that were treated with SV-HD. The LC did not improve the level of testosterone when given with SV-LD and SV-HD as compared with SV groups (Fig. 2).

The testicular oxidative parameters of NO and LPO levels were significantly elevated by SV in a dose-dependent manner

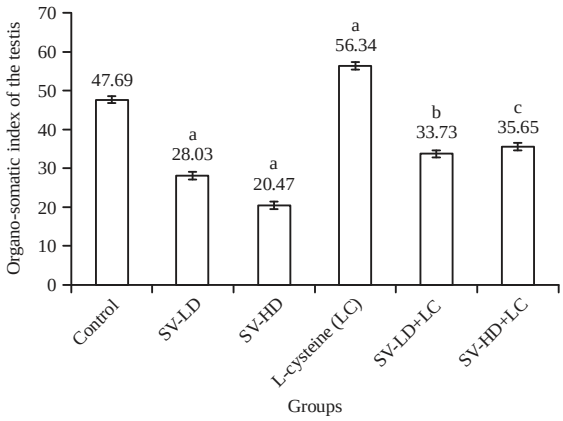

Fig. 1: Effect of sodium valproate and L-cysteine separately or in combination with the organo-somatic index of the testis tissue of rats

Values were expressed as Mean±SE, (n = 10), [a]Significant difference as compared to the control group, [b]Significant difference as compared to SV-LD, [c]Significant difference as compared to SV-HD

$$\text{Organo-somatic index} = \frac{\text{Weight (g) of the testis}}{\text{Day 30 total body weight (g)}} \times 100$$

after 30 days of treatment (Fig. 3). The LC declined all the oxidative parameters as compared to control rats, especially the LPO level which was significantly decreased. The decline in intratesticular oxidative levels was noticed in LC and SV combined groups as compared to its related group of SV (low or high dose). There were no significant alternations in the NO levels.

L-cysteine -OH radical activity was evaluated and showed that the concentration 100 µg mL^{-1} had high -OH free radical activity that confirmed the LC anti-oxidant activity (Fig. 4).

Data in Table 1 revealed that there was no remarkable change in SOD, CAT, GPx, GRx and TAC of control rats and those treated with LC. In contrast, the reduction in all the previous mentioned anti-oxidant enzymes activities and TAC level were recorded in rats treated with SV-LD or SV-HD for 30 days. Administration of the LC with different doses of SV resulted in a significant elevation of anti-oxidant enzyme activity and improved the TAC content as compared to its related group of SV.

Histopathology evaluation: The histological appearance of the testicular tissues of the control group was normal (Fig. 5a). Control group showed normal cells of spermatogenic, spermatocytes and rounded spermatids attached in the layers of seminiferous tubules. Also, LC-treated rats had normal seminiferous tubules with normal spermatogenesis (Fig. 5b). SV-LD induced histopathological variations in the testis such as necrosis, germ cell degeneration, desquamation, edema and congestion in addition to degeneration and atrophy of seminiferous tubules (Fig. 5c). It was observed that there was a marked increase in necrotic and degenerative changes in germinal cells of rats that received SV-HD (Fig. 5d). The improvement was also observed in SV-LD and LC treated groups where there was partial restoration of germinal cells in addition, seminiferous tubules lined by several layers of spermatogenic cells up to sperm formation but surrounded by mild edematous stroma (Fig. 5e). The well-defined germinal cells with the reduction in necrosis, edema and congestion were found in the animals that received SV-HD and LC together (Fig. 5f).

Histological Activity Index (HAI) was evaluated using the degree of microscopic lesions in testis tissue as the effect of SV and LC separately or in combination (Fig. 6). A decrement in scoring was noticed in the SV exposed group depend on the

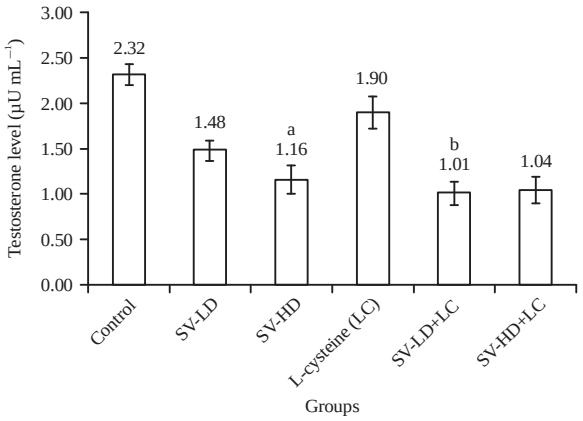

Fig. 2: Effect of sodium valproate and L-cysteine separately or in combination, on testosterone levels of the testis tissue of rats

Values were expressed as Mean±SE, (n = 10), [a]Significant difference as compared to the control group, [b]Significant difference as compared to its related group of SV-LD

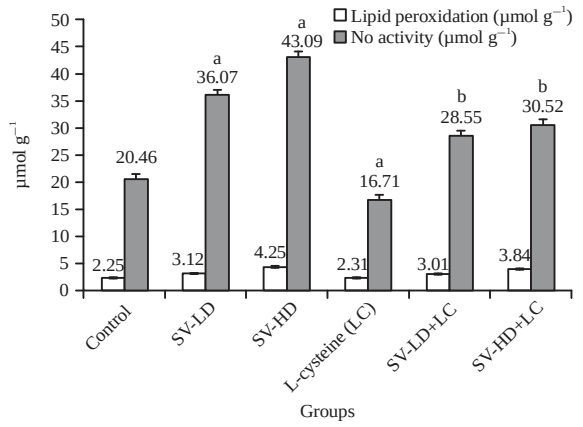

Fig. 3: Effect of sodium valproate and L-cysteine separately or in combination on the levels of lipid peroxidation and nitric oxide of the testis tissue of rats

Values were expressed as Mean±SE, n = 10, [a]Significant difference as compared to the control group, [b]Significant difference as compared to its related group of SV-LD

Table 1: Effect of sodium valproate and L-cysteine separately or in combination on enzymatic and non-enzymatic antioxidant of the testis of male rats

Parameters	Control	SV-LD	SV-HD	L-cysteine (LC)	SV-LD + LC	SV-HD + LC
SOD (U g^{-1})	75.47±1.58	62.97±1.20[a]	51.28±2.69[a]	79.27±0.71	65.59±1.60[b]	61.18±1.17[c]
CAT (U g^{-1})	40.13±0.83	27.93±0.67[a]	27.24±0.74[a]	41.65±1.72	32.63±1.03[b]	31.63±0.95[c]
GPx (U g^{-1})	12.11±0.65	7.86±0.76[a]	6.97±0.32[a]	11.40±0.30	9.05±0.20[b]	9.17±0.38[c]
GRx (U g^{-1})	9.44±0.34	6.17±0.23[a]	5.93±0.56[a]	10.02±0.55	7.76±0.20[b]	8.11±0.31[c]
TAC (%)	94.00±0.71	84.00±1.30[a]	75.20±1.59[a]	93.40±1.21	85.60±1.03	77.20±1.93[d]

Values were expressed as Mean±SE, (n = 10), Rats treated with low and high doses of SV (100 and 500 mg kg^{-1}, respectively), [a]Significant difference as compared to the control group, [b]Significant difference as compared to SV-LD, [c]Significant difference as compared to SV-HD, [d]Significant difference as compared to SV-LD+LC

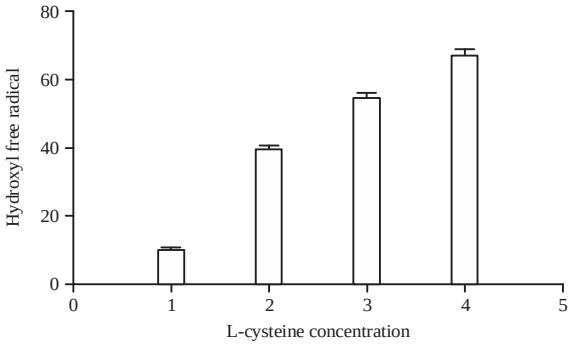

Fig. 4: Free hydroxyl radical activity ($^{-}$OH) of L-cysteine was presented as μM g^{-1}

dose as compared to the normal animals. An improvement in score was noted in the rats that were given LC with SV, thus showing its protective action.

DISCUSSION

This study was designed to assess the effects of LC intake on SV-induced testis toxicity of male rats after exposure to two different doses. According to the literature, this is the first study that evaluates the defensive effects of LC against testicular damage induced by SV in experimental animals. The reason to select

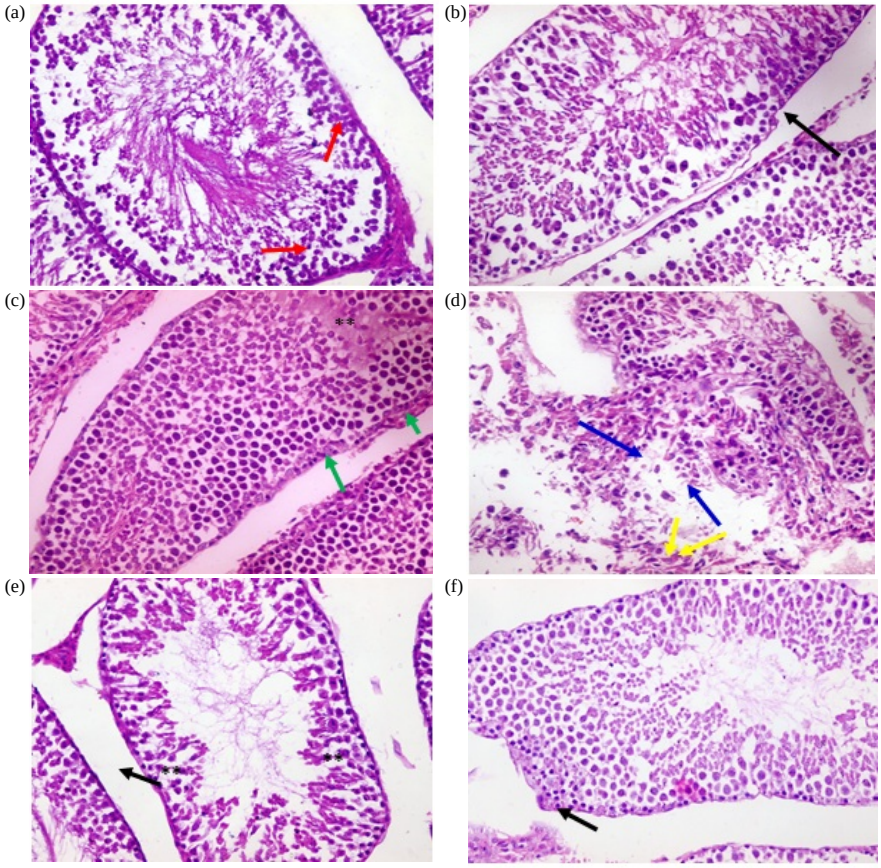

Fig. 5(a-f): Histopathological slides of the testis stained with haematoxylin and eosin in (a) Control groups of rat showing normal seminiferous tubules lined by several layers of spermatogenic cells (Red arrow) up to sperm formation (400X), (b) Cross section of rat testis treated with L-cysteine (400 mg kg^{-1}) showing normal seminiferous tubules (Black arrow) with normal spermatogenesis (400X), (c) Sodium valproate (100 mg kg^{-1}) treated rats showing distended seminiferous tubules (Blue arrow) by 1 and 2 spermatogenic cells with few spermatids and number of sperms (**). i.e., maturation arrest (400X), (d) Sodium valproate (500 mg kg^{-1}) showing necrosis and ruptured tubules (Blue arrow) with infiltration by macrophages (Black arrow) and plasma cells (400X), (e) Low dose of SV and L-cysteine showing partial restoration of germinal cells as well as seminiferous tubules lined by several layers of spermatogenic cells (**) up to sperm formation but surrounded by mild edematous stroma (Black arrow) (400X) and (f) High dose of SV and L-cysteine showing reduction in necrosis, edema and congestion of testicular tissue with seminiferous tubules surrounded by edematous stroma (Black arrow) containing small groups of Leydig cells (400X)

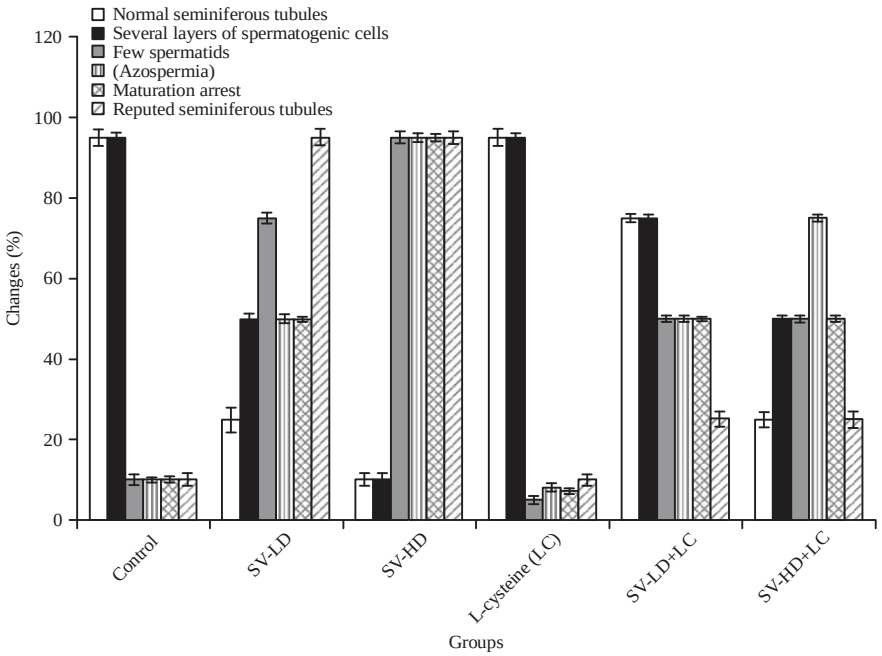

Fig. 6: Histological Activity Index (HAI) was assessed as percentage based on the degree of microscopic lesions in testis tissue as the effect of sodium valproate and L-cysteine separately or in combination on this basis - no lesions (0-10%), ---+ lesions found in 1-3 rats (25%), ++-- lesions found in 4-6 rats (50%), +++- lesions found in 7-8 rats (75%), ++++ lesions found in 9-10 rats at least (95%) (n = 10)

the high dose of SV was to mimic the accidental exposure or increasing the time of exposure to the low dose.

The results established that the testosterone level had significantly declined in SV groups when compared with control. This observation is parallel to previous report by Bauer *et al.*[29] and Vijay *et al.*[30] who found that valproate acts directly on the testis, to inhibit testosterone synthesis by inhibiting the effect of the Leydig cells. Moreover, Soliman and Abla[31] had reported that there was a decline in the levels of testosterone, FSH and LH after exposure to SV that diminished by part the germ cell number. The intensity of damage occurred by SV was partially prevented by LC that may be influencing testosterone synthesis.

In conformity with the obtained results, SV affected the reproductive capacity in general as it is effective at the biochemical and cellular levels of the testis. It is acceptable to suggested that valproate could not be the first anti-epileptic drug of consideration for the patient[32].

It was found that there was a correlation between the histological and oxidative status induced by SV. The testis is the major target organ for oxidative stress that could be due to its high content of polyunsaturated lipids membrane[33]. In the present study, SV-treatment induced LPO as indicated by a significant elevation in TBARS level that causes irreparable cell damage in testis tissue by interfering with plasma membrane lipids.

The results showed that the concentrations of LPO and NO were significantly elevated, which were incorporated with a reduction in anti-oxidant enzymes (SOD, CAT, GPx and GRx) activities in SV-treated groups. However, there was a significant decline in LPO values in SV+LC treated-group when compared with SV-treated rats. Similarly, Vidya and Subramanian[34] found that SV-treatment was found to raise the levels of MDA and hydroperoxides, in addition, the decline in the enzymatic anti-oxidants and level of glutathione (GSH) of rats. In the erythrocytes of children with epilepsy treated with SV, the activities of anti-oxidant enzymes were insignificantly lower, whereas the MDA concentration was insignificantly higher[35].

The data presented were in parallel with Zhang *et al.*[36] who found the MDA levels in neutrophils of SV-treated patients were higher, while the activities of SOD and CAT were significantly declined than the control groups. Thus, the testicular damage that was induced in rats could be attributed to the oxidative stress provoked by SV.

The LC has antioxidant characters because of its ability to go through the redox reactions. Cysteine can be formed in the human body under normal physiological conditions if there was enough quantity of methionine. Cysteine's anti-oxidant properties were found in the tripeptide GSH[37]. Therefore, one of the important aims of the current research was to highlight the role of LC in reducing oxidative stress and in case of

treatment with SV was to increase the GSH content of the cell. This appeared clearly in SV-LD and SV-HD treated-groups where GPx and GRx activities decreased in a dose-dependent manner. These results proved that LC had anti-oxidant activity due to its mechanism of action. The cysteine could be offered the role of increasing the beneficial antioxidant GSH, which was diminished in oxidative stress status. The LC could be used as antioxidant but it was the first time, according to our knowledge to be used with SV.

Previous study by Hamza *et al.*[7], El-Shenawy and Hamza[8] and Jain *et al.*[38] showed that LC was a strong anti-oxidant compound and protects liver, kidney and brain tissues against the oxidative stress. Droge[39] revealed that LC treatment intercepts oxidative stress and thus the obtained result was committed by our conclusion. This effect may be directly concomitant to the LC itself [1] whereas, the conservation of free sulfhydryl groups was appraised crucial to the biological functions of many proteins[39].

The histopathological discovery showed that SV-treatment caused major structural changes in testis compared to the control rats. However, it was determined that LC treatment alleviated the toxic effects of SV on testis when given together with SV. Girish *et al.*[12] found that the testis was extremely sensitive to the side effect of SV.

Moreover, histopathological observation in the testis of rats treated with SV revealed vascular changes in the form of congestion and interstitial edema and degenerative alternation with the presence of multinucleate spermatid giant cells. Similarly, Cansu[10], Bairy *et al.*[11] and Girish *et al.*[12] reported that SV-treatment caused necrosis, atrophy in the seminiferous tubules, multinucleated giant cell formation, interstitial edema with congestion, a reduction in the germinal cell count and impaired spermatogenesis. A decline in Johnsen's testicular score was also realized in the histological observation as an effect of SV treatment. The histo-architecture of the testis and Johnsen's scores were partially-restored to nearly normal by LC treatment with SV.

The SV can disrupt the cellular mechanisms in different ways that can cause toxicity. It may induce the formation of LPO, NO and OH^{-}, which were chemical mechanisms capable of changing the structure and function of the testis. The antioxidant properties of LC may play a critical role in preventing the toxicity of drugs, especially that of epilepsy. The mechanism elaborated in the protection of LC against SV induced testicular toxicity was unknown but our obtained results confirmed that the anti-oxidant activities of LC that were carried out by performing ^{-}OH radical activity, confirmed its anti-oxidant activity, that may contribute by way or another in the protection of testis tissues from oxidative stress induced by SV treatment either in the lower or the higher dose.

CONCLUSION

Sub-chronic exposure to two doses of SV altered the anti-oxidant enzymatic activities and induced oxidative damage in addition to the histopathological alterations of the testis in a dose-dependent manner. The co-administration of SV with LC showed the beneficial effect on all the previous parameters. The histoarchitecture of the testis was also partially-restored by LC confirming its protective effect against SV. This action of LC may be closely related to its anti-oxidant capacities and its ability to scavenge free radicals and thus protect testicular tissues from oxidative damage of SV.

SIGNIFICANCE STATEMENT

This study discovered the protective effect of L-cysteine against the toxicity of sodium valproate.

The study can be beneficial for declining the side effect of sodium valproate on the testes using L-cysteine.

This study will help the researchers to uncover the crucial areas of sodium valproate toxicity that many researchers were not able to explore.

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