Indian J Physiol Pharmacol 2014; 58(4) : 416–422

Original Article

Ellagic acid modulates sodium valproate induced reproductive toxicity in male Wistar rats

Chandrashekaran Girish[1]*, Oommen Shweta[2], Vishnu Raj[3], Sadasivam Balakrishnan[4] and Renu G'boy Varghese[5]

Department of Pharmacology[1],
JIPMER, Puducherry

Departments of Pharmacology[2,3],
Pondicherry Institute of Medical Sciences, Puducherry

Department of Pharmacology[4],
All India Institute of Medical Sciences,
Bhopal, Madhya Pradesh

Abstract

This study evaluated the protective effect of ellagic acid on sodium valproate-induced sperm abnormalities in male Wistar rats. A total of 30 rats were grouped into five groups, each having 6 animals. Vehicle, sodium valproate (400 mg/kg) and ellagic acid (10, 25, 50 mg/kg) were given orally from day 1 to day 7, and ellagic acid was continued for 3 more days. On day fourteen, animals were sacrificed and the different parameters were recorded. There was a significant decrease in the sperm count and sperm motility after the exposure to sodium valproate. The percentage of abnormal sperms increased in a dose-dependent manner. The histopathological examination revealed that sodium valproate had caused degeneration and desquamation of germinal cells in the epithelium and also showed a decrease in the Johnsen's scoring. Ellagic acid provided partial protection at the doses of 10 and 25 mg/kg and complete protection at 50 mg/kg, against sodium valproate induced testicular and spermatozoal damage.

Introduction

Epilepsy is a chronic neurological disease, characterized by repeated seizures (convulsions) over time. Seizures are episodes of disturbed brain activity that makes changes in attention or behavior and these demands for continuous treatment with an antiepileptic drug (1). Sodium valproate is a broad spectrum antiepileptic and is considered to have diverse mechanism of actions attributing to its safety and efficacy. It is also used in other conditions such as bipolar disorder, migraine prophylaxis, schizophrenia and major depression (2). However, recent research on animal models have revealed that sodium valproate causes reversible changes in sperm motility, sperm morphology, sperm count and histoarchitecture of the testes (3, 4).

Ellagic acid is a flavonoid compound found in numerous fruits and vegetables. Ellagic acid has been

***Corresponding author :**

Chandrashekaran Girish, Department of Pharmacology, Jawaharlal Institute of Postgraduate Medical Education and Research (JIPMER), Dhanwanthri Nagar, Puducherry – 605 006, India, E-mail: girish.c@jipmer.edu.in, Ph. No.: (0413) 2296239, Fax No.: 91-413–2272067

(Received on Sept. 9, 2012)

receiving large attention because of its wide array of biological properties, such as antioxidant, free radical scavenging, chemo-preventive and anti-apoptotic actions (5). Recent studies on rats with ellagic acid suggested its protective effects against reproductive toxicity induced by various anticancer drugs such as cisplatin and cyclophosphamide (6, 7). The purpose of the present study was to evaluate the protective effect of ellagic acid against sodium valproate induced reproductive toxicity in male Wistar rats.

Materials and Methods

Animals

Male Wistar rats (2-3 months old) weighing 200-250 g were obtained from the central animal house and were kept under standard laboratory conditions with food and water *ad* libitum under a 12:12 hour light/ dark cycle. The study protocol was approved by the Institutional Animal Ethics Committee (Approval no: CPCSEA/p.no.3/ 30.03.2012) and the procedures in this study were performed according to the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), India.

Drugs/Chemicals

Sodium valproate and ellagic acid (Sigma Chemicals Co, USA) was purchased from commercial suppliers. NaCl, KCl, $Na_2HPO_4.2H_2O$, KH_2PO_4 were of analytical grade and were purchased locally. Sodium valproate was dissolved in saline whereas ellagic acid was made as a suspension in saline and was administered by oral gavage.

Experimental design

The rats were randomly divided into five groups, containing six animals each. The control group was given saline orally. Another group received sodium valproate (400 mg/kg, oral) for 7 days and served as toxic control. The remaining three groups received ellagic acid orally at doses of 10, 25 and 50 mg/kg respectively along with sodium valproate for 7 days and ellagic acid was continued for 3 more days. On 14^{th} day, the rats were sacrificed under ether anesthesia. Laprotomy was conducted and the right epididymis was dissected out for sperm counts, sperm motility and sperm morphology. The right testis was removed, weighed and processed for histopathological analyses.

The dose selection of sodium valproate and ellagic acid was based on previous reports (4,8,6). The minimum effective dose of ellagic acid that produced a protective effect against other toxic drugs was found to be 10 mg/kg (8, 6). In a pilot study, we observed that at 10 mg/kg, ellagic acid showed a partial protection against sodium valproate induced reproductive toxicity. Therefore, we decided to increase the doses in a logarithmic manner, i.e. 25 and 50 mg/kg, in order to find any dose response relationship.

Sperm count and motility

The epididymal sperm suspension was prepared in 1 ml of phosphate buffered saline (PBS) at pH 7.2. An aliquot from the suspension (1 ml) was diluted in a ratio of 1:40 with PBS. A sample of the diluted suspension was charged into a hemocytometer. The total sperm count in eight squares (except the central erythrocyte area) of one mm^2 each was determined and multiplied by 5×10^4 to get the total count (4). Then the same eight squares were also examined for motile sperms and the percentage of motile sperms were recorded (9).

Sperm morphology assay

A fine epididymal sperm suspension was made and stained with 0.2 ml of 1% aqueous eosin. About one drop of stained suspension was placed on a clean slide and was dried. Slides were examined for abnormalities in five hundred sperms per animal and were classified into normal and abnormal sperms. Further, the abnormal sperms were designated under head abnormalities and tail abnormalities. The head abnormalities were further sub-grouped as amorphous, hookless and banana shaped and the tail abnormalities as coiled/folded and broken (4, 9).

Histopathology of testis

The testis was removed and fixed in 10% formalin for 24 hours and processed for paraffin embedding. Then five micron thick paraffin sections were made and stained with hematoxylin and eosin. The sections were analyzed for the presence or absence of vacuoles, gaps and abnormal cells (9). Johnsen's testicular score was performed for control and treatment groups. All cross-sectioned tubules were evaluated systematically, and a score between 1 (very poor) and 10 (excellent) was given to each tubule according to Johnsen's criteria (10). Twenty-five tubules were evaluated for each animal (9).

Statistical analysis

Data were expressed as mean±S.D. The comparison between different groups was done by using one way ANOVA followed by post hoc Tukey-HSD test and nonparametric data were analyzed by Kruskal-Wallis test followed by Dunn's multiple comparison test. A p value<0.05 was considered as statistically significant.

Results

Body weight and testis weight

Table I shows the changes in body weights and testis weights after receiving different treatments. The body weight of rats in the sodium valproate treated group was significantly reduced as compared to the control group ($p<0.05$). Though not statistically significant, there was a dose dependent increase in the body weight of rats which received sodium valproate and ellagic acid at 10, 25 and 50 mg/kg. At the dose 50 mg/kg, the bodyweight of rats was almost restored to that of normal control group.

The weight of the testis in the toxic group (sodium valproate, 400 mg/kg) was significantly reduced compared to the control group ($p<0.001$) and there was a significant increase in the weights of the testes in the groups received ellagic acid (10 and 50 mg/kg) as compared to the toxic group.

Sperm count

As shown in (Fig. 1a), compared to the normal control group, there was a statistically significant decrease in the sperm count in the sodium valproate treated group ($p<0.01$). A dose dependent increase in the sperm count was observed in the group treated with different doses of ellagic acid (10, 25 and 50 mg/kg) compared to the toxic group. The maximum protective effect being observed for the group treated with ellagic acid at 50 mg/kg ($p<0.05$).

Sperm morphology

Saline treated rats showed about 4% of total abnormal sperms with 2.32% of sperm head abnormalities and 1.68% of tail abnormalities (Fig. 1b). The rats exposed to sodium valproate showed teratozoospermia i.e., a greater degree of sperm abnormalities when compared to the normal control group ($p<0.001$). The head abnormalities accounted for about 18% whereas tail abnormalities were about 10%. A decrease in the sperm abnormalities was observed in all the three groups treated with different doses of ellagic acid compared to the toxic group ($p<0.001$). The rats which received ellagic acid 10, 25 and 50 mg/kg, percentage of head abnormalities were reduced to 4.4, 5.16 and 2.84 respectively and percentage of tail abnormalities to 1.12, 2.12 and 1.08 respectively.

TABLE I: Effect of sodium valproate and ellagic acid on different parameters in rats.

Parameters	*Control*	*S.V. (400 mg/kg)*	*S.V+EA (10 mg/kg)*	*S.V+EA (25 mg/kg)*	*S.V+EA (50 mg/kg)*
Final body weight (g)	223±31.14	135±5.0*	153±35.9	179±46.2	193±53.22
Testis (g)	1.03±0.093	0.604±0.159**	0.915±0.119#	0.8436±0.165	1.005±0.139##
Johnsen's score	9.4±0.55	5.2±0.447**	5.8±0.707*	6.2±0.8367	8.2±0.837#

Data are expressed as mean±SD. *$p<0.05$, **$p<0.001$, when compared to control group. #$p<0.05$, ##$p<0.01$, when compared to sodium valproate treated group. (SV, sodium valproate; EA, ellagic acid).

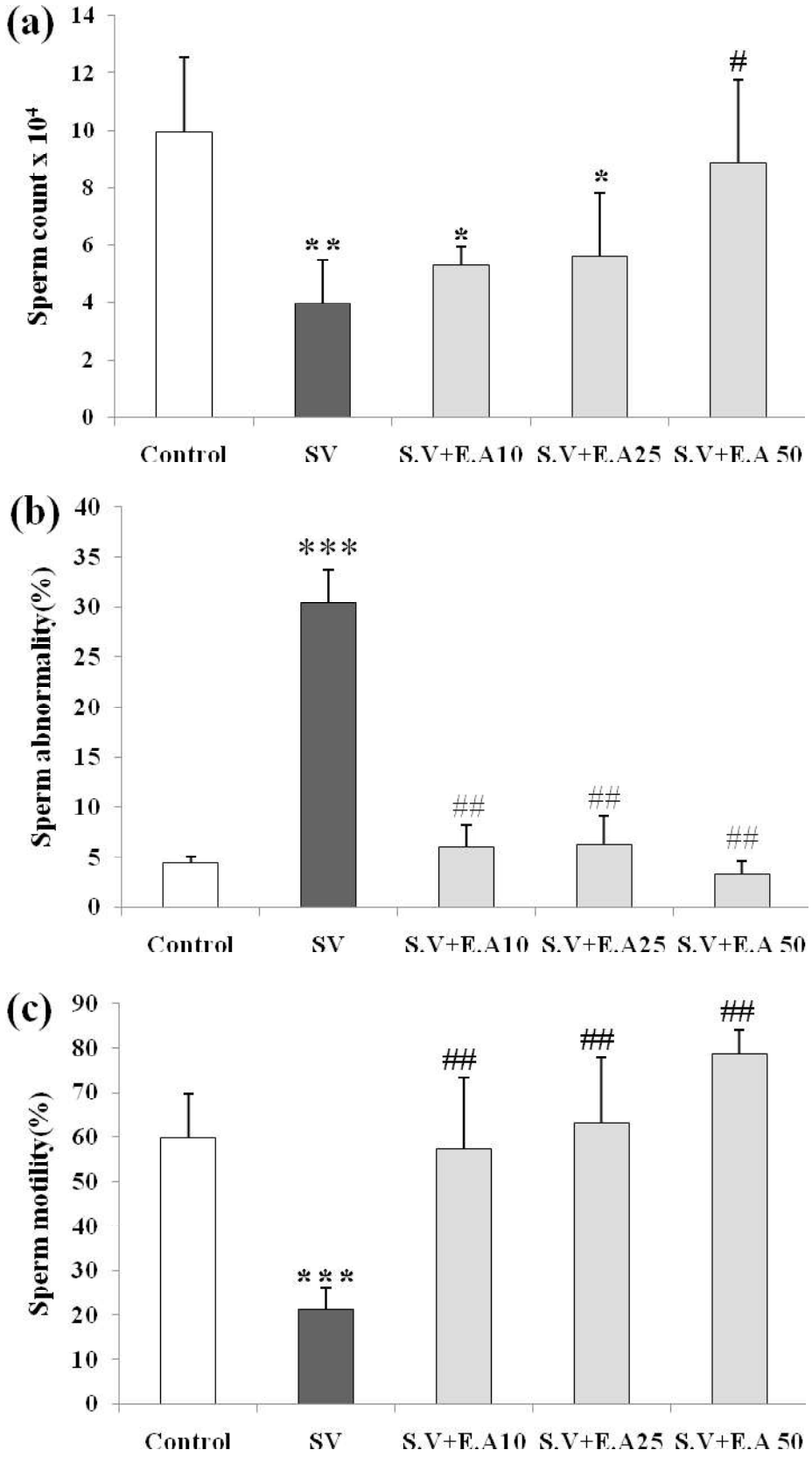

Fig. 1: Effect of sodium valproate (S.V) and ellagic acid (E.A) on (a) sperm count (b) sperm abnormality and (c) sperm motility. Data are mean±S.D. *P<0.05, **P<0.01 and ***P<0.001 compared to control group and # P<0.05 and ##P<0.001 compared to sodium valproate group by One way ANOVA followed by Tukey-HSD test.

Sperm motility

As shown in (Fig. 1c), a statistically significant asthenozoospermia was observed in the sodium valproate treated group ($p<0.001$) and a significant increase in the sperm motility was noted in the groups treated with ellagic acid (10, 25 and 50 mg/kg) when compared to the toxic group ($p<0.001$).

Histopathology

The histological appearance of the testicular tissues of the control group was normal in appearance (Fig. 2a). Sodium valproate 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. 2b). It was observed that there was a marked decrease in necrotic and degenerative changes in germinal cells of rats that received ellagic acid along with sodium valproate (Fig. 2c&d). A reduction in Johnsen's scoring was observed in the sodium valproate exposed group compared to the normal animals ($p<0.001$; Table I). An improvement

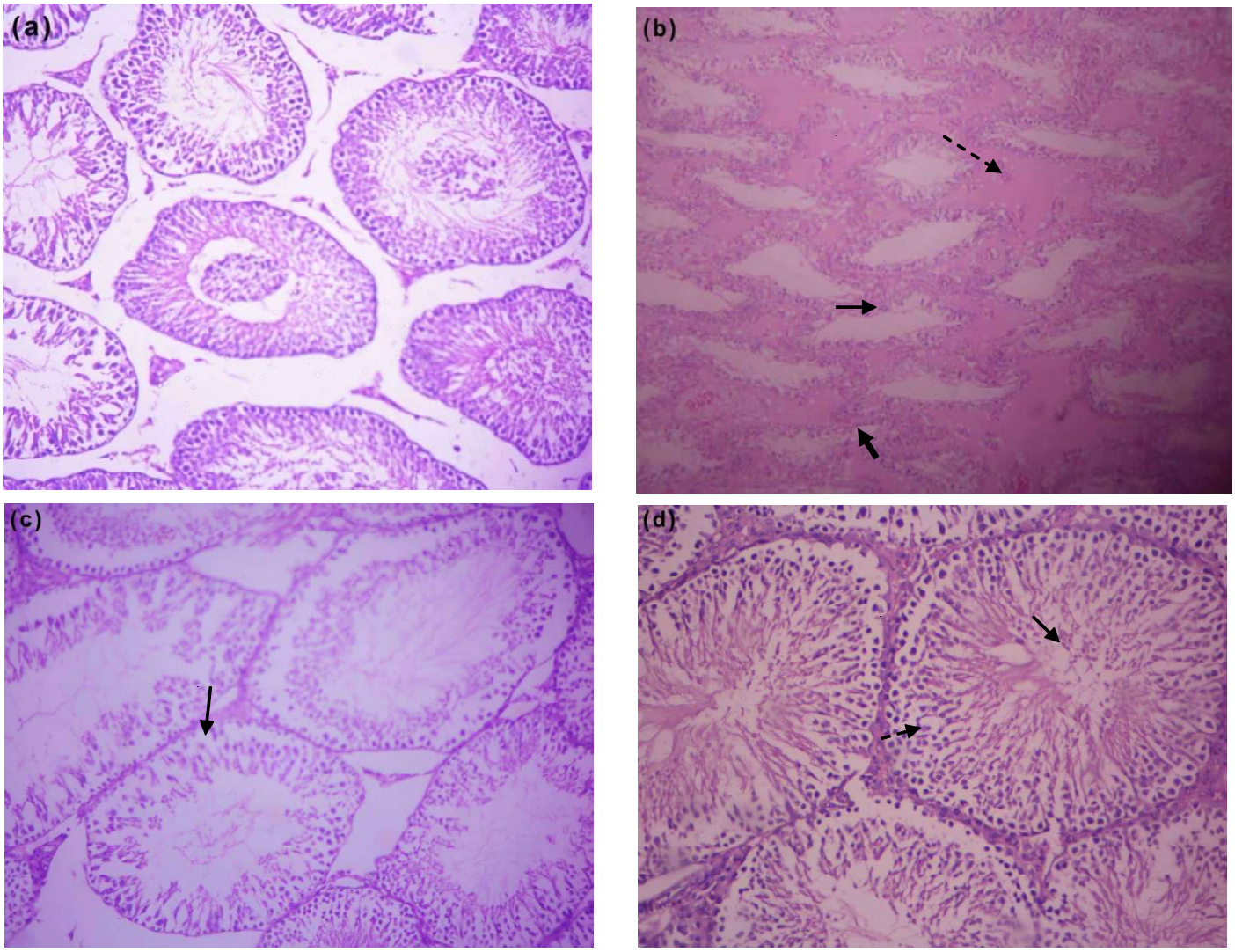

Fig. 2: Histopathological slides of the testes in (a) control groups of rat showing normal seminiferous tubules (b) sodium valproate (400 mg/kg) treated rats showing necrosis(thick arrow), degeneration in germinal cells (thin arrow), and edema (broken arrow) (c) sodium valproate and ellagic acid (25 mg/kg) treated groups showing partial restoration of germinal cells (arrow) with the reduction in necrosis, edema and congestion [d] sodium valproate and ellagic acid (50 mg/kg) treated group showing spermatogenesis (arrow) with well defined germinal cells (broken arrow) similar to that of normal control group. H&E, 100 X magnifications.

TABLE II: The findings of pathological changes in testicular tissues of different treatment groups.

Parameters	*Control*	*SV (400 mg/kg)*	*SV+EA (10 mg/kg)*	*SV+EA (25 mg/kg)*	*SV+EA (50 mg/kg)*
Necrosis in germinal cells	–	+ +	+	+	–
Atrophy in seminiferous tubules	–	+	–	–	–
Degeneration in germinal cells	–	+ +	+	–	–
Desquamation in germinal cells	–	+	+	+	+
Vacuolization in sertoli cells	–	+ +	–	–	–
Reduction in germinal cells	–	+ + +	+ +	+	+
Disorganization in germinal cells	–	+	–	–	–
Interstitial edema and capillary congestion	–	+	+	+	–
Multi-nucleated giant cell formation	–	+	–	–	–

(SV, sodium valproate; EA, ellagic acid, – absent; + mild; ++ moderate; +++ severe)

in the Johnsen's score was noted in the groups which received ellagic acid at 10, 25 mg/kg, though the statistical significance was evident only at 50 mg/kg dose, thus showing its protective action.

Discussion

In the present study, we induced reproductive toxicity with sodium valproate and evaluated the possible protective role of ellagic acid in restoring the reproductive functions of male rats. For that, the changes in sperm morphology, sperm count, sperm motility and histopathological status of testis were examined. Ellagic acid at doses of 10, 25 and 50 mg/kg, was found to be effective in preventing the sperm abnormalities induced by sodium valproate in a dose dependent manner. Even though only a partial protection was noted at lower doses, 50 mg/kg dose of ellagic acid produced complete normalization of the studied parameters.

There is an increasing concern about the possible effects of sodium valproate on reproductive endocrine function (3, 11). These include testicular atrophy, reduced spermatogenesis with atrophy of the prostate, epididymis and seminal vesicles (3). In agreement with the above reports, we have demonstrated that, at a dose of 400 mg/kg/day, sodium valproate produced reproductive toxicity and associated histological changes in male rats. Sodium valproate treatment had affected the general health condition of rats that was reflected as a decrease in the final body weight (9). The reduction in testis weight observed may be the reflection of the structural changes such as epithelial sloughing, atrophic changes and reduction in the germ cell numbers due to cytotoxicity (12). Ellagic acid improved the body and testis weights of the animals, especially at 50 mg/kg dose showing its protective effect.

Sperm count is one of the most sensitive tests for spermatogenesis and is highly correlated with fertility (4, 13). We have demonstrated that sodium valproate-induced testicular toxicity has resulted in a significant decrease in the sperm count and sperm motility. The decrease in the sperm count and sperm motility observed in the sodium valproate treated rats were reversed by ellagic acid co-administration (10, 25 and 50 mg/kg), showing its protective effects. The maximum protective effect was seen with ellagic acid at 50 mg/kg dose. Further, there was an increase in the head and tail abnormalities of sperms that were exposed to sodium valproate. Ellagic acid was effective in ameliorating these changes induced by this anticonvulsant drug (14). Soliman et al., (1999) have reported a decrease in plasma levels of testosterone, FSH and LH after exposure to sodium valproate that decreased the germ cell number (15). Even though we have not evaluated the testosterone level, the ellagic acid may be influencing testosterone synthesis. Sodium valproate is known to impair mitochondrial functions that may be one of the reasons for the abnormalities in sperm count, motility and morphology. This may be prevented by ellagic acid treatment (16, 17, 18).

Histopathological sections revealed that sodium valproate treatment caused necrosis, atrophy in seminiferous tubules, multi-nucleated giant cell formation, interstitial edema with congestion, reduction in germinal cell count and impaired spermatogenesis (3, 4). A reduction in Johnsen's testicular score was also observed in histological

structure of sodium valproate treated rats. The degree of damage caused by sodium valproate was partially prevented by ellagic acid at lower doses (10 and 25 mg/kg) with partial restoration of germinal cells. It is in agreement with the report that cisplatin-induced decrease in germinal cell layer thickness and the deteriorated histopathologic findings of testis were partially ameliorated by ellagic acid treatment (8). However, at 50 mg/kg dose of ellagic acid, appearance of well defined germinal cells and restoration of spermatogenesis was evident. The histo-architecture of the testis and Johnsen's scores was restored to near normal.

The mechanism involved in the protective effect of ellagic acid against sodium valproate induced reproductive toxicity is unknown. Sodium valproate can disrupt the cellular mechanisms in different ways that can lead to toxicity. It may induce free radical formation and lipid peroxidation, which are chemical mechanisms capable of disrupting the structure and function of testis (1). The antioxidant and free radical scavenging properties of ellagic acid may play an important role in preventing the toxic effects of drugs (19). Sodium valproate has been shown to have apoptosis-promoting effect on human and rat granulosa cells by increasing caspase-3 activity (3). The compounds that have anti-apoptotic properties like ellagic acid may be beneficial against gonadotoxins. Future studies are needed to explore the exact protective mechanism of ellagic acid.

In conclusion, this study suggests that sodium valproate causes testicular toxicity, but the flavonoid compound ellagic acid is protective in terms of sperm count, motility and morphology. The histo-architecture of testis was also restored by ellagic acid confirming its protective effect. This action of ellagic acid may be closely related to its antioxidant and anti-apoptotic property, which needs further research. Therefore, ellagic acid may be useful in epileptic patients on long-term sodium valproate treatment if proved effective in clinical trials.

References

1. Tamber MS, Mountz JM. Advances in the diagnosis and treatment of epilepsy. *Semin Nucl Med* 2012; 42: 371–386.
2. McNamara OJ. Pharmacotherapy of the epilepsies. In: Brunton L.L. (eds.). Goodman & Gilman's The Pharmacological Basis of Therapeutics, 12th Ed. Mc Graw Hill. 2011; 583–607.
3. Cansu A. Antiepileptic drugs and hormones in children. *Epilepsy Res* 2010; 89: 89–95.
4. Bairy L, Paul V, Rao Y. Reproductive toxicity of sodium valproate in male rats. *Indian J Pharmacol* 2010; 42: 90–94.
5. Girish C, Pradhan SC. Drug development for liver diseases: focus on picroliv, ellagic acid and curcumin. *Fundam Clin Pharmacol* 2008; 22: 623–632.
6. Turk G, Sonmez M, Ceribasi AO, Yuce A, Atessahin A. Attenuation of cyclosporine A-induced testicular and spermatozoa damage associated with oxidative stress by ellagic acid. *Int Immunopharmacol* 2010; 10: 177–182.
7. Ceribasi AO, Turk G, Sonmez M, Sakin F, Atessahin A. Toxic effect of cyclophosphamide on sperm morphology, testicular histology and blood oxidant-antioxidant balance, and protective roles of lycopene and ellagic acid. *Basic Clin Pharmacol Toxicol* 2010; 107: 730–736.
8. Turk G, Atessahin A, Sonmez M, Ceribasi AO, Yuce A. Improvement of cisplatin-induced injuries to sperm quality, the oxidant-antioxidant system, and the histologic structure of the rat testis by ellagic acid. *Fertil Steril* 2008; 89: 1474–1481.
9. Narayana K. An aminoglycoside antibiotic gentamycin induces oxidative stress, reduces antioxidant reserve and impairs spermatogenesis in rats. *J Toxicol Sci* 2008; 33: 85–96.
10. Johnsen SG. Testicular biopsy score count a method for registration of spermatogenesis in human normal values and results in 335 hypogonadal males. *Hormones* 1970; 1: 225.
11. Cansu A, Ekinci O, Serdaroglu A et al. Effects of chronic treatment with valproate and oxcarbazepine on testicular development in rats. *Seizure* 2011; 20: 203–207.
12. Narayana K, Prashanthi N, Nayanatara A, Bairy LK, D'Souza UJ. An organophosphate insecticide methyl parathion (o-o-dimethyl o-4-nitrophenylphosphorothioate) induces cytotoxic damage and tubular atrophy in the testis despite elevated testosterone level in the rat. *J Toxicol Sci* 2006; 31: 177–189.
13. Meistrich ML, Finch M, Da Cunha MF, Hacker U, Au WW. Damaging effects of fourteen chemotherapeutic drugs on mouse testis cells. *Cancer Res* 1982; 42: 122–131.
14. Sharma RK, Roberts GT, Johnson FM, Malling HV. Translocation and sperm abnormality assays in mouse spermatogonia treated with procarbazine. *Mutat Res* 1979; 67: 385–388.
15. Soliman GA, Abla Abd, el-Meguid. Effects of antiepileptic drugs carbamazepine and sodium valproate on fertility of male rats. *Dtsch Tierarztl Wochenschr* 1999; 106: 110–113.
16. Toth GP, Stober JA, Zenick H, Read EJ, Christ SA, Smith MK. Correlation of sperm motion parameters with fertility in rats treated subchronically with epichlorohydrin. *J Androl* 1991; 12: 54–61.
17. Ponchaut S, Veitch K. Valproate and mitochondria. *Biochem Pharmacol* 1993; 46: 199–204.
18. Isojarvi JI, Lofgren E, Juntunen KS et al. Effect of epilepsy and antiepileptic drugs on male reproductive health. *Neurology* 2004; 27: 247–253.
19. Girish C, Koner BC, Jayanthi S, Rao KR, Rajesh B, Pradhan SC. Hepatoprotective activity of picroliv, curcumin and ellagic acid compared to silymarin on paracetamol induced liver toxicity in mice. *Fundam Clin Pharmacol* 2009; 23: 735–745.