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Research Article

Chemopreventive Effects of Naringin against Valproic Acid-Induced Reproductive Toxicity in Male Wistar Rats

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Abstract
Valproic acid (VA) is an anticonvulsant widely used for the treatment of seizures and other neurological disorders but limited by reproductive toxicity. Naringin (NGN), a flavanone glycoside commonly found in fruits and vegetables has been shown to modulate and interact with signaling pathways and molecules resulting in its pharmacological activities, however, reports on its chemopreventive effects against VA-induced reproductive toxicity in male Wistar rats is limited. This study investigated the modulatory potential of NGN against reproductive toxicity induced by VA in male Wistar rats. Animals were grouped into six (n= 6) treatments to receive oral administration of; normal saline (1 mL/kg), VA (500 mg/kg), NGN (10 mg/kg), VA (500 mg/kg) + (Sildenafil (SDF), 100 mg/kg), VA (500 mg/kg) + NGN (10 mg/kg), and VA (500 mg/kg) + NGN (20 mg/kg) respectively for 28 days. Reproductive hormones, semen parameters, biochemical indices, testis and epididymis histology were assessed. Also, immunohistochemical evaluation of inducible nitric oxide (iNOS) levels in the testis was investigated. VA significantly decreased testis weight (32.9%), serum testosterone (80.2%), follicle stimulating hormone (47.7%), sperm count (75.5%) and motility (80.2%) and elevated cyclooxygenase-2 and malondialdehyde levels in the testes when compared with the control. Histology showed vascular congestion. Immunohistochemical evaluation of iNOS showed positive reaction in the testes of VA treated rats. However, NGN administrations reversed VA induced toxicity in the treated rats. Antioxidant levels increased in the testes and the epididymis of NGN treated rats. Further, NGN prevented structural abnormalities in the VA treated rats. Overall, these results provide evidence of a chemopreventive potential of NGN that may serve an adjuvant purpose for clinical uses.

Key Words: Valproic Acid; Sexual Dysfunction; Reproductive Toxicity; Naringin; Antioxidant System; Phytomedicine

*INTRODUCTION

Epilepsy is a serious neurological disease characterized by the transient occurrence of abnormal, excessive and/or synchronous neuronal activity in the brain, associated with various neurobiological, cognitive and psychological signs and/ or symptoms (Lezaic *et al*., 2019; Lipska *et al*., 2021). It is well known that epilepsy itself affects the secretion of pituitary hormones (Herzog *et al*., 2003). Thus, reproductive endocrine dysfunction is not unusually common among people with epilepsy (Herzog, 2008; Taubøll *et al*., 2015). Valproic acid (VA) is one of the drugs of choice in primary generalized tonic-clonic seizures, absence seizures, and myoclonic seizures (Tomson *et al*., 2016; Romoli *et al*., 2019; Lipska *et al*., 2021). Mechanistically, VA acts by potentiating the inhibitory activity of GABAergic action through different systems (Romoli *et al*., 2019). However, in rodents and humans, particularly in male, evidence abounds for its reduced potency and sperm abnormalities (Verrotti *et al*., 2016; Ocek *et al*., 2018; Ogunjimi *et al*., 2018). Although, there are reports of the dose-dependent deleterious effects of chronic VA treatments on testicular morphology in rats (Ourique *et al*., 2016; Verrotti *et al*., 2016), the mechanism of VA-induced reproductive toxicity continue to generate scientific debates in recent times. A major suggestion is that excessive production of reactive oxygen species (ROS) and their reactive metabolites can result in oxidative stress and subsequent cell damage (Tung and Winn, 2011; Ocek *et al*., 2018). In respect, the vicinity of VA-induced damage showed increase in ROS production and apoptosis via altered sexual function and

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reproductive parameters which have been updated in literature (Fu *et al*., 2010; Heidari *et al*., 2018; Koroglu *et al*., 2021). Studies have reported VA-mediated down-regulation of the conversion of the arachidonic acid pathway in both animals and human models (Bosetti *et al*., 2003; Rawat *et al*., 2020).
Flavonoids are polyphenolic compounds commonly present in vegetables and fruits (Solnier and Fladerer, 2021). The flavanone glycoside, naringin (NGN), (a flavanone-7-O-glycoside or 4′, 5, 7-Trihydroxyfl avanone-7-rhamnoglucoside) occurs naturally in citrus fruits (family, Rutacae), being abundant in grapefruit (citrus paradisi) (Sharma *et al*., 2019). Also, NGN is responsible for the bitter taste in grapefruit juice (Sharma *et al*., 2019). The bioactive compounds from the grapefruit juice particularly the flavonoids such as anthocyanins, malvidin, naringin, naringenin, kaempferol, quercetin, etc. have attracted several scientific attention due to their abilities to scavenge free radical molecules (Dhalaria *et al*., 2020). NGN, the glycoside of naringenin, is the most abundant flavonoid present in grapefruit juice (Memariani *et al*., 2021). NGN has been shown to be a very potent antioxidant that stimulates increase the antioxidant system both in vitro and in vivo (Dhalaria *et al*., 2020; Memariani *et al*., 2021). Additionally, NGN possesses anti-inflammatory, antimicrobial, anti-apoptotic, anti-ulcer, anti-osteoporotic, anti-hepatoxic and anti-carcinogenic (Bharti *et al*., 2014; Chen, 2016; Koroglu *et al*., 2021). Naringin and its aglycol form, naringenin, have been reported to play a very important role in preventing reproductive toxicity by attenuating oxidative stress in the testes (Roy *et al*., 2013; Gil *et al*., 2016; Adana *et al*., 2018). Reports on free radical scavenging actions of flavonoids have been updated in literature (Ranawat & Bakshi, 2017; Solnier, & Fladerer, 2021). Such ideal molecules might help to mop the high risk of reproductive toxicity that is associated by VA. There is paucity of information on whether NGN could protect against oxidative damage due to VA-induced reproductive toxicity. This may increase our understanding of the potential interaction between NGN and VA and thus offers positive intervention in patients with epilepsy. The designing of appropriate intervention and or dose translation can be optimized. Therefore, the effect of NGN on valproic acid reproductive toxicity was investigated in Male Wistar Rats.

MATERIALS AND METHODS

Chemicals: Naringin was purchased from Sigma-Aldrich Chemical Co. (St, Louis, Missouri, USA). Sildenafil (SDF) (Vivera®) was purchased from Shreechem Pharmaceuticals Pvt-Ltd. R-914/915, TTC industrial area, Navi Mumbai 400701, India. Rat follicle stimulating hormone (FSH) (Cat. No.: Rshakrfs-010R) and luteinizing hormone (LH) ELISA (Rshakrlh010SR) kits were purchased from (Biovendor, Shibayagi Co., Ltd. (Japan). RAT testosterone (RTC001R) ELISA kit was obtained from Biovendor, Laboratorni, medicinaa.s Karasek (Czech Republic). Sodium hydroxide was obtained from MERCK (Germany). Cyclooxygenase (COX, ovine) assay kit was a product of Cayman Chemical Company (Ann Arbor, Michigan, USA). All other chemicals were of analytical grades.

Animals: Adult male Wistar rats (180 ± 25 g) were obtained from Babcock University animal facility and housed in a unisexual group of metallic cages maintained under standard laboratory conditions of ambient environmental temperature and a light-dark cycle of 12 hours. Animals were allowed free access to tap water and rodent chow (Ladoke Akintola Grower Mash, Osun, Nigeria). They were acclimatized to the experimental conditions for a period of two weeks prior to the commencement of experiments. The study was approved by the Babcock University Health Research Ethics Committee (BUHREC G/130). This experiment followed the procedures as documented by Kilkenny *et al*. (2011). The Guide for the Care and Use of Laboratory Animals published by the U. S. National Institutes of Health (NIH Publication No. 85-23, revised 1996)" for studies involving experimental animals was also followed.

Experimental design: Animals (rats, n = 36) were randomly divided into 6 experimental groups, composed of 6 animals each, and received the following treatments: normal saline (1 mL/kg/day, control), VA (500 mg/kg/day) (Nishimura *et al*., 2000), NGN (10 mg/kg/day), VA + (SDF, 100 mg/kg/day), VA + NGN (10 mg/kg/day), and VA + NGN (20 mg/kg/day). VA was prepared in normal saline (0.9% NaCl solution) and administrated orally at a dose of 500 mg/kg. The dose of 500 mg/kg of VA followed the methods according to Nishimura *et al*. (2000). All treatments including VA, SDF, and NGN were freshly prepared and the experiment lasted four weeks.

Semen Analysis: The left caudal epididymis following a cervical dislocation and the testis were separated and their contents transferred to a petri dish containing normal saline. Longitudinal incisions were made with a fine sharp scalpel blade (1 mm incision) to release the spermatozoa into 1mL of normal saline at 37°C (Raji *et al*., 2005). The sperm motility and counts were evaluated by placing the mixture on a slide and then examining under the microscope attached to a Celestron® Digital Microscope Imager (Torrance, CA 90503) and viewed under X40 objective according to the methods described by Kale and Awodele (2016). The percentages of total and progressive sperm motility were estimated from three different fields in each sample and the mean was used as the final value of motility. Additionally, the sperm count was determined using Neubauer's haemocytometer. Sperm count was expressed as the number of sperm million/mL. The slides were examined under the microscope under oil immersion with X 100 objectives.

Sample Collection and Processing: The animals were sacrificed by cervical dislocation 24 hours after the last treatment and whole blood obtained. Blood samples were collected by cardiac puncture into plain bottles and centrifuged at 3500 rpm at room temperature for 7 minutes to separate the serum. The testis and epididymis were carefully excised, cleared of adhering tissues and weighed. The weight was recorded in grams per kilogram body weight (g/kg). A small portion of the right testis and epididymis were carefully excised, fixed in 10% formaldehyde, dehydrated in graded alcohol, and embedded in paraffin. Fine sections were obtained, mounted on glass slides, and counterstained with hematoxylin and eosin (H&E) for histopathologic examination. The remaining right portion of the testis and epididymis were weighed and homogenized in four volumes

of phosphate buffer (0.1 M, pH 7.4). Both serum and the organs homogenates were used for biochemical analysis.

Oxidative stress and Antioxidant parameters: The methods of Beutler (1963) and Varshney and Kale (1990) were followed in estimating the levels of reduced glutathione (GSH) and lipid peroxidation (MDA) in the testes and epididymis respectively. Similarly, the activities of superoxide dismutase (SOD) and catalase (CAT) were determined by the method of McCord and Fridovich (1969) and Aebi (1984) respectively.

Reproductive hormones and COX-2 assays: Serum concentrations of male reproductive hormones and cyclooxygenase-2 (COX-2) were measured using commercially available enzyme-linked immunosorbent assay kits (ELISA, Cayman) for rat and expressed as ng/mL. Briefly, triplicate samples were tested twice per plate (Intra-Assay: CV<8% and inter-assay: CV< 10-12%). The optical density of each well was determined according to the manufacturer's instructions. Thus, testosterone and COX-2 ELISA assays followed the methods of Joshi *et al*. (1979) and Scoditti *et al*. (2014) respectively. Also, rat serum follicle-stimulating hormone (FSH) and luteinizing hormone (LH) were assessed by biotin-conjugated anti-FSH/anti-LH against standard according to Odell *et al*. (1968).

Immunohistochemical Staining: The protein was purchased from Gentaur, genprice Inc., logistics, San Jose, CA 95123, USA. Immunostaining was performed using Animal Research Kit (DAKO ARK™), Peroxidase (DAKO, Carpinteria, CA, USA) to investigate the immunohistochemical localization of inducible nitric oxide synthase (iNOS) in testes following the manufacturer's instruction.

Statistics: Results were expressed as the mean ± standard error of the mean (SEM). Differences between groups were determined by one-way analysis of variance (ANOVA) using Statistical Package for Social Sciences (SPSS, 20.0) software for windows. Post hoc testing was performed for intergroup comparisons using the least significant difference (LSD) (Levine, 2013), followed by Tukey's test for multiple comparism. P value < 0.05 was considered significant. Figures were obtained using GraphPad Prism 6.

RESULTS

Organ-Body weight Ratio: Rats that received only VA (500) demonstrates significantly decreased testis weight by 32.9% while administrations of VA + NGN (10), VA + NGN (20) and VA + SDF + NGN (10) improved testis weight by 53.2%, 51.1% and 40.4% respectively compare with the VA group (Figure 1).

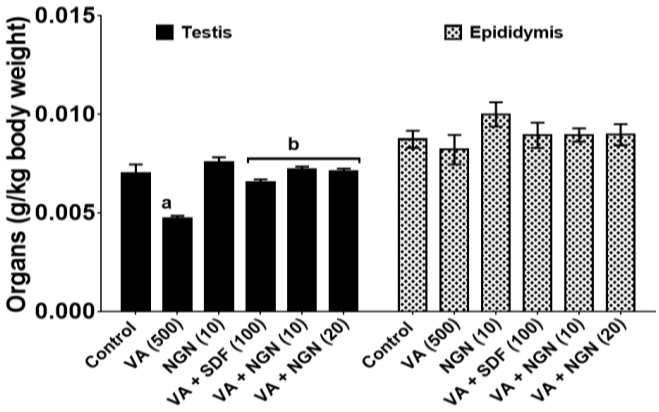

Figure 1:
Effect of NGN on testis and epididymis weights in normal and VA-treated rats. N= 6, Data are expressed as mean ± SEM. NGN: naringin, VA: Vaproic acid, SDF: Sildenafil, NGN: Naringin, VA: Vaproic acid, [a] p< 0.05 vs Control (Saline, 10 mL/kg) group, [b] p< 0.05 vs control VA (500 mg/kg) group.

Sperm Characteristics: Rats that received VA show reduced sperm count and motility by 75.5% and 80.2% compared with the control saline group (Figure 2). But, the administrations of VA + NGN (10), and VA + NGN (20) however produce improved (p< 0.01) sperm count (88.9% and 112.5%) and motility (120.6% and 152.8%) when compared with control VA group (Figure 2A and 2B).

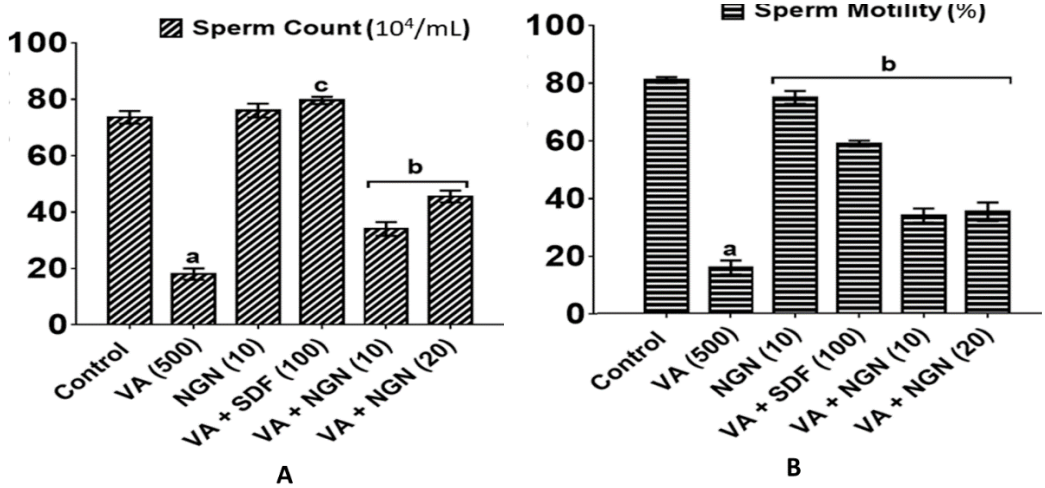

Figure 2:
Effect of naringin (NGN) on (a) sperm count and (b) sperm motility in normal and VA-treated rats. N = 6, Data are expressed as mean ± SEM. NGN: naringin.VA: Vaproic acid, SDF: Sildenafil, NGN: Naringin.VA: Vaproic acid. [a] p< 0.05 vs Control (Saline, 10 mL/kg) group. [b] p< 0.05 or [c] p< 0.01 vs control VA (500 mg/kg) group.

Reproductive hormones: The administrations of VA decreased testosterone, FSH and LH levels by 80.2% ($p< 0.05$), 47.7% ($p< 0.05$) and 19.3% ($p> 0.05$) when compared with the control saline group (Table 1). VA + NGN (10) and VA + NGN (20) however produce increased ($p< 0.05$) testosterone (75.2%, 77%), FSH (32.2%, 54.7%) and LH (20.4%, 26.1%) levels when compared with control VA group.

Table 1:
Effect of Naringin on testosterone, follicle stimulating hormone and luteinizing hormone levels in normal and VA-treated rats.

Treatments	Testosterone (ng/mL)	FSH (mIU/mL)	LH (mIU/mL)
Control	2.74 ± 0.08	0.51 ± 0.01	0.53 ± 0.02
VA (500 mg/kg)	0.54 ± 0.10*	0.27 ± 0.01*	0.43 ± 0.04
NGN (10 mg/kg)	2.05 ± 0.11	0.41 ± 0.01	0.57 ± 0.01
VA + SDF (100 mg/kg)	1.13 ± 0.15†	0.40 ± 0.01†	0.46 ± 0.03
VA + NGN (10 mg/kg)	0.95 ± 0.15†	0.35 ± 0.01	0.51 ± 0.03
VA + NGN (20 mg/kg)	0.96 ± 0.15†	0.41 ± 0.01†	0.54 ± 0.02

Data are expressed as mean ± SEM. N = 6. VA: Vaproic acid, SDF: Sildenafil, NGN: Naringin. FSH: Follicle stimulating hormone, LH: Luteinizing hormone*$p< 0.05$ compared with control (normal saline group), †$p< 0.05$ or ††$p< 0.01$ compared with control VA (500 mg/kg) group.

Lipid Peroxidation (MDA) Assay: Malondialdehyde (MDA) levels in the VA-treated rats were significantly elevated in the testes (152.9%, $p< 0.01$) but remained unchanged in the epididymis when compared with control saline group (Table 2). In the treated rats that received VA + NGN (10) and VA + NGN (20), lowered ($p< 0.01$) MDA levels in the testes was observed when compared with the control VA group.

Antioxidant Parameters: The level of GSH decreased ($p< 0.05$) in the testes and epididymis of VA-induced rats compared with the control saline group (Table 2). Administration of VA + NGN (10), and VA + NGN (20) to rats improved GSH levels in the testes (60.3%, and 87.3%) and in the epididymis (35.1% and 66.5%) compared with the control VA group.

Administration of VA did not significantly alter SOD and CAT activities in the testes and epididymis in all rats (Table 2). However, the CAT activities in the testes of VA + NGN (10), and VA + NGN (20) treated groups were elevated ($p< 0.05$) by 120.5%, and 68.1% in the treated rats compared with control VA group. Also, CAT activities in the epididymis increased ($p< 0.05$) when compared with the control VA group.

Table 2:
Effect of Naringin on lipid peroxidation and antioxidant enzymes in normal and VA-treated rats.

TREATMENTS (mg/kg)	**MDA Testis** (nmol/mg protein)	**MDA Epididymis** (nmol/mg protein)	**GSH Testis** (µmol/mg protein)	**GSH Epididymis** (µmol/mg protein)	**SOD Testis** (µmol/min/ mg protein)	**SOD Epididymis** (µmol/min/ mg protein)	**CAT Testis** (µmol/min/ mg protein)	**CAT Epididymis** (µmol/min/ mg protein)
Control	0.57±0.06	0.72±0.01	0.31±0.01	0.57±0.01	0.16±0.01	0.16±0.01	0.14±0.01	0.48±0.01
VA (500)	1.45±0.04**	0.87±0.01	0.13±0.01*	0.25±0.01*	0.17±0.01	0.15±0.01	0.17±0.01	0.31±0.01
NGN (10)	0.62±0.03	0.70±0.01	0.22±0.01	0.35±0.01	0.15±0.01	0.14±0.01	0.38±0.01*	0.62±0.01
VA + SDF (100)	0.94±0.08	0.62±0.01	0.21±0.01†	0.25±0.01	0.13±0.01	0.15±0.01	0.38±0.01††	0.37±0.01
VA + NGN (10)	0.71±0.03†	0.80±0.01	0.22±0.01†	0.33±0.01	0.15±0.01	0.13±0.01	0.37±0.01†	0.46±0.01†
VA + NGN (20)	0.70±0.08†	0.81±0.01	0.25±0.01††	0.41±0.01†	0.15±0.01	0.14±0.01	0.28±0.01†	0.51±0.01†

Data are expressed as mean ± SEM. N = 6. VA: Vaproic acid, SDF: Sildenafil, NGN: Naringin. MDA: Malondialdehyde, GSH: Reduced Glutathione, SOD: Superoxide Dismutase, CAT: Catalase.*$p< 0.05$ compared with control (normal saline group), †$p< 0.05$ or ††$p< 0.01$ compared with control VA (500 mg/kg) group.

COX-2 Assay:
Administration of VA alone elevated ($p< 0.05$) COX-2 level by 93.1% compared with the control saline group (Figure 3). Although neither NGN (10) nor NGN (20) was able to lower the increased COX-2 level in the treated animals, although, SDF reduced ($p< 0.05$, 30.2%) VA-induced COX-2 level in the treated animals.

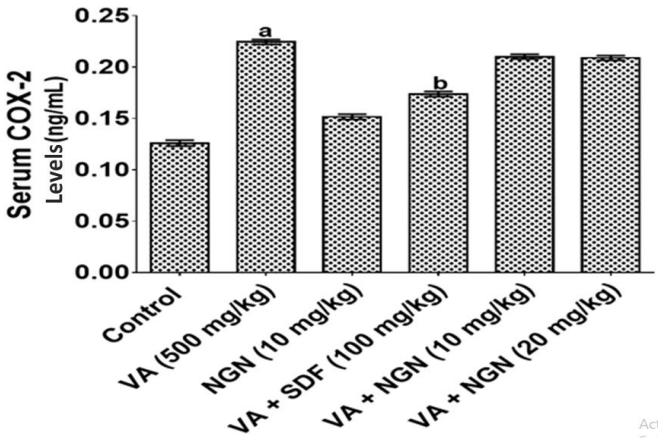

Figure 3:
Effect of NGN on Serum cyclooxygenase-2 (COX-2) level in normal and VA-treated rats.Data are expressed as mean ± SEM. NGN: Naringin, VA: Vaproic acid, SDF: Sildenafil. N = 6. VA: Saline Vaproic acid. [a] $p < 0.05$ vs Control (Saline, 10 mL/kg) group. [b] $p < 0.05$ vs control VA (500 mg/kg) control group.

HISTOLOGY: Animals treated with VA showed testicular atrophy with aggregates of inflammatory red cells in the vessels. While NGN (10 mg/kg) did not ameliorate this defect, NGN (20 mg/kg) or SDF (100 mg/kg) attenuated atrophic tissues and vascular congestions caused by VA intoxication (Figure 4). Also, in the epididymis, VA produces stereociliated epithelium and congestions in the blood vessels of rats, whereas, animals' epithelia were normalized following NGN pretreatments (Figure 5).

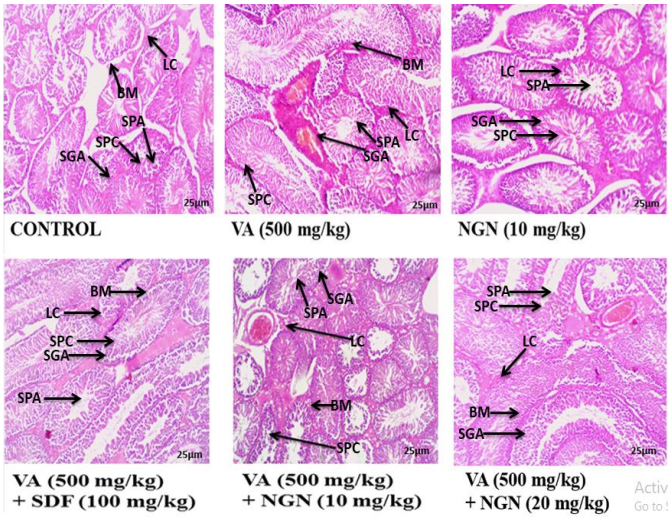

Figure 4:
Histology section of testis in normal and VA-treated rats. Control (normal saline 10 mL/Kg), NGN (10 mg/kg), VA + SDF (100 mg/kg), and VA + NGN (20 mg/kg) show sections of testicular tubules lined by spermatogenic series cells and containing numerous luminal spermatozoa with no abnormality seen. VA (500 mg/kg) and VA + NGN (10 mg/kg) show tubules lined by spermatogenic series cells and containing numerous luminal spermatozoa with aggregates of inflammatory red cells seen in the vessels (vascular congestion). VA: Vaproic acid, SDF: Sildenafil, NGN: Naringin. SGA: Spermatogonia; SPA: Spermatozoa; SPC: Spermatocytes: BM: Basement Membrane; LC: Leydig Cells (H & E stain, X400).

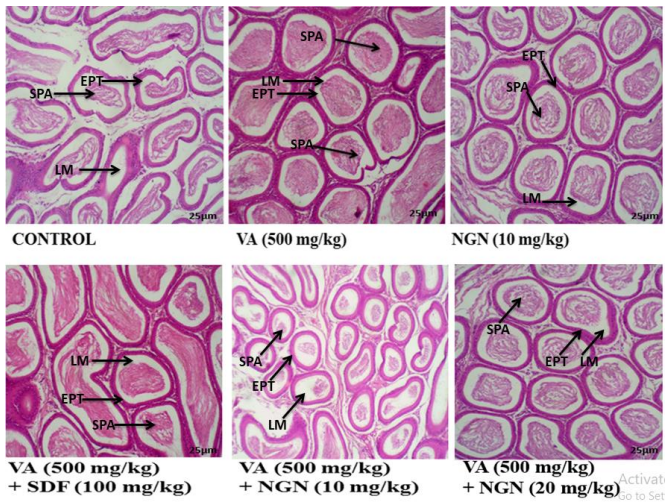

Figure 5:
Histology section of epididymis in normal and VA-treated rats. Control (normal saline 10 mL/Kg), NGN (10 mg/kg), VA + SDF (100 mg/kg), VA + NGN (10 mg/kg), and VA + NGN (20 mg/kg) show presence of numerous spermatozoa with no abnormality (normal epididymis). VA (500 mg/kg) showed sterociliated epithelium, spermatozoa in lumen, basal cells, loose connective tissue and congested blood vessel seen. VA: Vaproic acid, SDF: Sildenafil, NGN: Naringin. SPA: Spermatozoa; EPT: Epithelium of the Epididymis; LM: Lumen. (H & E stain, X400).

TESTES iNOS IMMUNOHISTOCHEMISTRY:
VA-treated rats show positive reactions at all levels of the germinal cells. The primary spermatogonia in NGN (10 or 20 mg/kg) and SDF (100 mg/kg) however were negative to iNOS immunostaining (Figure 6).

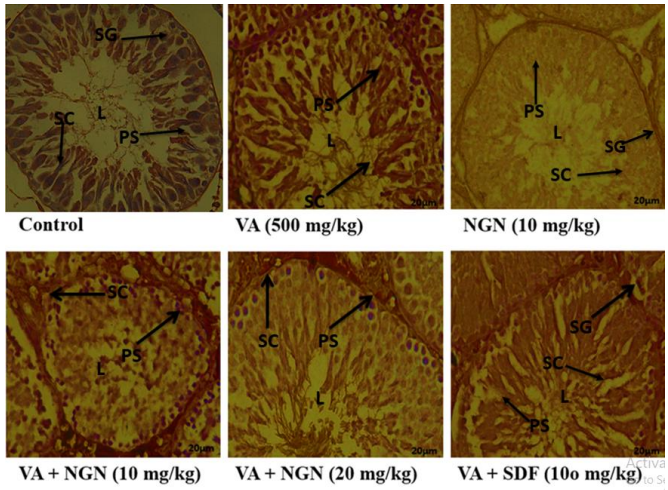

Figure 6:
Photomicrograph of the testes showing inducible nitric oxide synthase (iNOS) immunohistochemical staining following the administration of naringin (NGN) in normal and vaproic acid (VA) treated rats. Control (normal saline, 10 mL/Kg) group shows negative reactivity to iNOS staining. VA (500 mg/kg) shows positive reaction at all levels of the germinal cells. NGN (10 mg/kg) shows negative reactivity in the entire germ layer and the interstitial cells in group compared with the other groups. The primary spermatogonia in VA + NGN (10 mg/kg), VA + NGN (20 mg/kg) and VA + SDF (100 mg/kg) show negative reactions when compared with others and the cells of interstitium. VA: Vaproic acid, SDF: Sildenafil, NGN: Naringin. SG: Spermatogonium. SC: Sertoli cell. LD: Leidig Cell (X400).

DISCUSSION

Evidence abounds that VA, a very popular antiepileptic drug, causes serious reproductive endocrine dysfunction (Verrotti *et al*., 2016; Ogunjimi *et al*., 2018; Lezaic *et al*., 2019; Lipska *et al*., 2021). Although, the report of dissociative sexual function is also found with other anti-epileptic drugs but more with VA (Lundberg *et al*., 2001; Herzog, 2008). It has been found that about 60% of men with epilepsy experienced reproductive problems (Penovich, 2000). In this study, we assessed the possible chemopreventive benefits of NGN against reproductive toxicity induced by VA in male rats. In an experimental model, the NGN was found to be relatively safe after a chronic administration (Li *et al*., 2014). Treatment with VA is widely used for both children and adult epilepsy (Romoli *et al*., 2019). Studies have demonstrated that VA reproductive toxicity is independent of seizure in various experimental rodent models (Sukhorum and Iamsaard, 2017; Heidari *et al*., 2018). VA has been found to cause testis, epididymis, and prostate atrophies while altering spermatogenesis in a dose-dependent manner (Nishimura *et al*., 2000; Ourique *et al*., 2016; Adana *et al*., 2018). Reports have shown that VA altered sexual function in animals given a dose of 500 mg/kg/day (Nishimura *et al*., 2000). Reports from animal studies have indicated VA treatments could have a direct influence on both fertility and sperm morphological characteristics (Fu *et al*., 2010; Heidari *et al*., 2018). Semen quality and fertility in VA-treated rodents were found to reduce after chronic administrations in low doses (Nishimura *et al*., 2000; Ourique *et al*., 2016). Some studies have also found reduced sperm quality in men who had taken valproate (Jamalan *et al*., 2016; Ocek *et al*., 2018). Our results were further consistent with those of other studies that showed weight reduction in testis and/ or epididymis of animals following VA administrations (Nishimura *et al*., 2000; Sukhorum and Iamsaard, 2017). Also, sperm count and motility in the rats that received VA intoxication was reduced. NGN at the doses used demonstrated effectiveness by increasing sperm count and motility in the treated animals. Neuroendocrine disruptions leading to altered reproductive hormones have been reported in male and female patients with epilepsy (Herzog, 2008; Ogunjimi *et al*., 2018). Thus, VA toxicity is implicated in both androgen biosynthesis and hypogonadal hypophyseal axis during epilepsy (Lundberg *et al*., 2001; Taubøll *et al*., 2015; Tomson *et al*., 2016). One clinical manifestation with such hormonal changes is usually a severe defect in sperm production secondary to germ cells tumour (Taubøll *et al*., 2015; Romoli *et al*., 2019). Testosterone and FSH or LH levels are used to assess the testicular functions as part of reproductive function. From our results, VA administrations reduced testosterone, FSH, and LH levels in normal rats. NGN (10 and 20 mg/kg) co-administrations together with VA increased significantly testosterone and FSH, but not LH levels in the treated animals. Abnormal increase or decrease in serum FSH concentrations has been linked to a variety of widespread toxicity of reproductive origin (Adana *et al*., 2018). The decreased levels of testosterone observed in our study following VA intoxication might be related directly, in part, to a decreased in the FSH levels. Also, it might be because of a direct inhibitory action of VA on sperm cells production (Fu *et al*., 2010). The increased reproductive hormone levels observed in the NGN treated rats however demonstrate its tendency to centrally or peripherally ameliorate the effect of VA in vivo. This also explains the possibility of involvement of the hypophyseal axis in a centrally mediated feedback effect which would subsequently lead to a significant rise in serum testosterone levels (Hegazy *et al*., 2016). Antioxidant effects of NGN or Naringenin in oxidative stress in glioma cell lines have been reported (Chen *et al*., 2016; Hegazy *et al*., 2016). Previous reports show that VA exerts its toxic effect mostly on the testes (Nishimura *et al*., 2000; Sukhorum and Iamsaard, 2017). Mechanistically, VA has such ability to increase the MDA levels in serum and tissue while it incapacitates the activity of antioxidants in the endocrine system (Tung *et al*., 2011; Verrotti *et al*., 2016). Both NGN and naringenin have been reported to offer protective effects and thus preserve the reproductive system including the testis, epididymis, prostate as well as reproductive hormones against drug and chemical insults both in vitro and in vivo (Roy *et al*., 2013; Mostafa *et al*., 2016; Sahin *et al*., 2017; Adana *et al*., 2018). In this study, VA intoxication resulted in elevated MDA level, depletion of GSH, SOD and CAT in the experimental animals. However, NGN pretreatment attenuates elevated MDA levels in the testes. Administrations of NGN prior to VA increase GSH levels in the testes and in the epididymis of the treated groups. The elevations of SOD and CAT activities in the rat testes of NGN treated animals were observed. Moreover, CAT activity in the epididymis was elevated as well. VA adverse effects may cause arachidonic acid turnover that could later reduce concentrations of bioactive products in the rat testes (Bosetti *et al*., 2003). Recently, it was suggested that altered prostaglandin production levels in epilepsy may occur before and following antiepileptic drug indication (Rawat *et al*., 2020). Interestingly, NGN has been shown to decrease inflammatory biomarkers including TNF-α released from LPS-stimulated macrophages and improves survival in injured mice (Gil *et al*., 2016). In this study, VA-induced toxicity elevated COX-2 levels in rats, but unfortunately, NGN (10 or 20 mg/kg) did not nullify this increase in the treated animals. This is further supported by altered iNOS immunohistochemistry by VA administration. Although, NGN at the doses used in this study moderately affect iNOS expression, future research to quantify iNOS expression is essential. This altered pattern of iNOS staining might result from changes in activity of Leydig cells (Komarova *et al*., 2005). Studies to show germ cell tumours have been confirmed by immunohistochemistry. Our results are in support of molecular evidence that showed testicular damage in the VA-mediated tumour protein expressions (Komarova *et al*., 2005; Tung *et al*., 2011). These findings correlated also with the histologic changes that accompanied valproate intoxication represented in this study (Figure 4). Thus, the changes in testosterone and FSH levels and morphologic changes in the testes and epididymis attest to both peripherally and centrally mediated adverse effects of VA in rats.

CONCLUSION

Overall, the results of this study provide evidence for chemopreventive benefits of naringin against valproic acid toxicity through reproductive function, and antioxidant defense system. Also, this potential places naringin as a promising candidate that may serve an adjuvant purpose with valproic acid and/or antiepileptic drugs.

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