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Proconvulsant effects of estriol-a female ovarian hormone on kianic acid induced seizures in mice

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ABSTRACT

Background: The estriol was evaluated for its effect on the kianic acid kindling model of epileptogenesis in mice followed by evaluation of kindling-induced changes in cognitive and motor functions.
Material and methods: Kindling was induced on every alternate day for 45 days and treatment with kainic acid was given at doses ranging from 15 to 30 mg/kg body weight intraperitoneal (i.p) and estriol was also administered at dosages of 0.005 and 0.1 mg/kg through i.p. After induction of kindling the seizure severity was recorded and a further percentage incidence of animals kindled at the end of 45 days was also recorded. Spatial learning and cognitive alterations were assessed by the Morris water test (MWT) and spontaneous alternation behavior (SAB) while motor function was assessed by grip strength meter.
Results: Estriol increased the rate of kindling in both sexes of mice at a great scale. The percentage incidence of seizures was also intensified. A noticeable decline in the grip strength, spontaneous alteration behavior, and Morris water was observed following KA-kindling in pre-treated estriol groups of mice in both sexes.
Conclusion: Control animals developed a seizure score of 4 after the end of 5 weeks, mice treated with estriol exhibited kindling in the first two weeks only Clomiphene at a dose of 0.9 mg/kg i.p. exhibited anticonvulsant effects. The study displayed that estriol has a powerful anticonvulsant effect.
Keywords: Estriol, kainic acid, seizures, clomiphene, spontaneous alteration behavior, grip strength meter.

INTRODUCTION

Given the heterogeneity and complexity of seizures, its association with estrogens has been difficult to define. Existence of contrasting data on the association between estrogen and epileptic seizures has made it a much more interesting subject. Although it is generally believed that estrogen increase neuronal excitability and mediates proconvulsant effects (Veliskiova, 2006; Edwards et al., 1999; Nicoletti et al., 1985). There are clinical and animal data to show that estrogen also have anticonvulsant effects (Veliskiova, 2007; Reibel et al., 2000; Kalkbrenner and Standley, 2003; Tominaga et al., 2001). Estrone (E1), estradiol (E2) and estriol (E3) are the three biologically active estrogen present. Estradiol is the major estrogen in premenopausal women and is the most investigated among all estrogen in various epilepsy models.

Pretreatment with beta-estradiol in female rats showed neuroprotective effects on status epilepticus induced neuronal damage (Veliskiova, 2000). Estrogen was reported to attenuate clonic seizures induce by kainic acid (Budziszewska et al., 2001).

Earliar studies of estradiol administration in rodents have revealed proconvulsant effects (Nicoletti et al., 1985). Estrogen applied to cortex could increase seizures (Marcus et al., 1966). Estradiol was shown to facilitate kindling (Reddy, 2009). While most of the studies show proconvulsant effects of estradiol, there are also studies which report anticonvulsant and neuroprotective effects.

There are very few studies evaluating the role of estrone and estriol on seizures. Barha and co-workers (2010) recently reported a reduced percentage of animals with hippocampal neuronal loss following estrone (E1)

(Budziszewska, 2001). It was also shown to significantly decrease the percentage of animals with clonic seizures and their mortality in kainate-induced seizures (Ahmad and Vohora, 2014). Thus, both anticonvulsant and neuroprotective effects have been reported with estrone.

So far, estriol (E3), the third estrogen has not been investigated for its effects on seizures. There is insufficient information on how it can affect seizures or neuronal excitability. The present study, thus, evaluated the effects of this hormone on kianic-acid kindling model of epileptogenesis in mice followed by evaluation on kindling induced changes in motor and cognitive functions. Further, since sex can influence the effect of estrogen on seizures, the study would involve both male and female mice.

MATERIAL AND METHODS

Animals

Swiss Albino mice weighing between 25-35g were used. They were housed in polypropylene cages and maintained at 25-30 °C and 50% relative humidity in a natural light/dark cycle. Animals were given food and water ad libitum. The mice were kept separate from the opposite sex all the time. Institutional Animal Ethics Committee (IAEC) of Pinnacle Biomedical Research Institute (PBRI), Bhopal (Reg. No. 1824/PO/ERe/S/15/ CPCSEA) had approved the study and study approval reference number is PBRI/IAEC/PN-16026.

Experimental design

Induction of kindling

Kainic acid (KA), from Sigma-Aldrich, dissolve in 0.9% saline at 5 mg/ mL to keep the injection volume below 0.5 mL, and was prepared fresh on the day of each experiment. Mice were given intraperitoneal (i.p.) injections of KA, at doses ranging from 15 to 30 mg / kg body weight. Behavioral seizure activity was monitored for 2 h and classified according to a modified Racine scale (Racine, 1972): stage 0, normal behavior; stage 1, immobility; stage 2, repetitive movements, myoclonic twitch, or head bobbing; stage 3, bilateral forelimb clonus and rearing; stage 4, continuous rearing and falling; and stage 5, generalized tonic–clonic seizure.

Grip strength test

The neuromuscular function was determined with the aid of a grip strength meter. The mouse was allowed to hold the grip with it forepaws. The mouse was then pulled back horizontally until it released its grip. The grip strength reading was read directly read from the digital meter (Ali et,al., 2004).

Spontaneous alternation behavior (SAB)

SAB in a plus maze was assessed using the method of (Vohora et al., 2005). After being placed in the central platform, mice were allowed to traverse the maze freely for 6 minutes. The number and sequence of entries was recorded; an alternation is defined as entry into four different arms on an overlapping quintuple set. Five consecutive arm choices within the total set of arm choices constitute a quintuple set.

Spatial memory: Morris water maze test

Spatial learning and cognitive alterations were assessed using the water maze test described by Morris in 1984 and further tested by Charles V and Michael T (2006). It consists of a circular plastic pool of 73 cm in diameter and filled with water, kept at 27 ±2°C, at a depth of 56 cm. The pool was divided conceptually into four quadrants and a platform (6 × 6 cm) was placed 1 cm below the water surface in the center of one of the four quadrants. A mouse was released into the water at one of four randomly selected positions near and facing the wall. Mice were trained with 4 trials per day for 5 days (at 1 min intervals). In probe trials, mice were allowed to swim for 360 s. The pool remained in the same position inside of the room, due to the fact that an animal's ability to locate the platform depends on the use of visible keys available around the pool. A record should be made of latency, defined as the time elapsed from the moment of release to the moment it climbed on the platform. It was considered that an animal has found the platform when it stays on it for 5 s.

Statistical analysis

The data obtained were analyzed by analysis of variance (ANOVA) followed by Dunnett's multiple comparison test. $P<0.05$ was considered to be significant.

RESULTS

The consequence of estriol and clomiphene in the kainic acid model of mice on the incidence of kindling. Kindling was induced chemically in both sexes by using repetitive treatment of KA at a sub-convulsant dose (25 mg/kg i.p) every alternate day (Fig 1 Effect of estriol and clomiphene on the incidence of animals kindled following repeated treatments).

A group of animals of both sexes who were given clomiphene and diazepam followed by KA illustrated a very significant reduction in the percentage of incidence (Fig 1). They illustrated a similar performance in the severity of the seizure scale too (Fig 2a, b).

When a group of animals of both sexes was pre-treated with estriol at dosages of (0.005 and 0.01 mg/kg i.p.), it

was observed that it reduces the kindling induction time from 5 weeks to 3-2 weeks for male and female mice respectively. Furthermore, it also elevates the percentage incidence of seizures (Fig. 1), on the other hand, Clomiphene (0.9 mg/kg i.p) exhibited a significant (P<0.01) reduction of percentage incidence of KA-induced kindling and postpones the development of kindling (Fig. 1, 2).

Reciprocated dosage with KA at a subconvulsive dose (25 mg/kg i.p.) every alternate day, induced chemical kindling in both the sexes (Figure1). We observed that animals treated with Estriol (0.005 and 0.01 mg/kg i.p.) showed a significant (P<0.01) increase in the incidence of KA-induced rapid kindling.

Estriol at both doses has produced a maximum rate of incidence of kindling before the completion of the duration of treatment in both the treatment groups that is in male and female. Whereas treatment with Clomiphene (0.9 mg/kg i.p.) showed a significant (P<0.01) reduction in the incidence of KA-induced kindling. While the combination of Clomiphene with the lowest dose of Estriol (0.005 mg/kg i.p.) has also significantly (P< 0.01) increased the incidence of KA-induced kindling irrespective of the sexes. Further, treatment with diazepam (3 mg/kg i.p.) exhibited a significant (P<0.01) reduction in the incidence of KA-induced kindling in both sexes (Figure 5.1). Besides we detected in Figures 2a and 2b while observing the Effect of Estriol and Clomiphene on seizure severity in 35 days during the development of KA-induced kindling in mice, that administration of sub-convulsive dose (25 mg/kg IP.) of KA on alternate day induced seizure severity of 4.0 in male while 4.3 in female mice on 35th day (Figure 2a; Figure 2b). There was a significant (P<0.01) increase in mean seizure score as compared to the control group (Group 1) in both sexes but more in females (Figure 2a; Figure 2b). Animals treated with Estriol (0.005 mg/kg i.p.) showed a seizure severity of 4 in males and 4.2 in female mice on the 14th day but a higher dose (0.01 mg/kg i.p.) showed a seizure severity of 4 in males while 5 in female on 7th day i.e. it reduces the time of induction of kindling. There was a significant (P<0.01) increase in mean seizure score (4 on day 14th; 4 on the 7th day) in males at lower and higher doses respectively. While in the case of females, there was also a significant (P<0.01) increase in mean seizure score (4.2 on day 14th; 5 on the 7th day) in females at lower and higher doses respectively. The addition of Clomiphene (0.9 mg/kg i.p.) to estriol (0.005 mg/kg i.p.) has shown seizure severity of 3.8 and 4.2 on day 21st in males and females respectively. There was a significant (P<0.01) increase in mean seizure score in males and females respectively. Clomiphene at a dose (0.9 mg/kg i.p.) showed seizure severity of 1.4 in males and 1.6 in females on the 35th day while there were significant (P<0.01) changes in mean seizure score in both sexes as compared to group 2. Diazepam at a dose of (3 mg/kg i.p.) showed a seizure severity of 0.91 in both the sexes on 35th day.

The consequence of estriol and clomiphene on grip strength (GS), spontaneous alteration behavior, and Morris water maze following repeated treatment with a sub-convulsant dose of KA for 5 weeks in mice. A noticeable decline in the grip strength, spontaneous alteration behavior, and Morris water was observed following KA-kindling in pre-treated estriol groups of mice in both sexes(p<0.01, Fig. 3,4,5). Clomiphene and diazepam were found to be unsuccessful in reversing the estriol effects in KA-kindled mice for grip strength (GS), spontaneous alteration behavior, and Morris water maze tests.

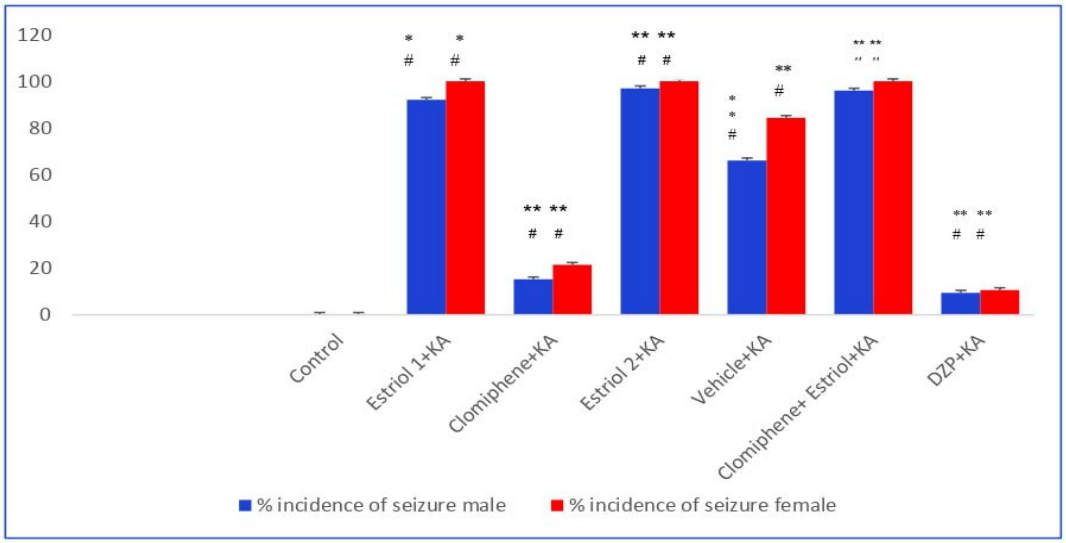
DISCUSSION

It has been commonly accepted that estrogens are excitatory to the central nervous system (CNS) and mediate pro-convulsant effects (Edwards et al., 1999; Nicoletti et al., 1985; Veliskiova, 2006). However, recently it has become clear that they also have anticonvulsant (Veliskiova, 2007; Kalkbrenner and Standley, 2003; Tominaga et al., 2001) and neuroprotective (Brann et al., 2007) effects. These opposite effects of estrogens on seizures depend upon treatment duration, latency before seizure testing, dose, hormonal status and/or seizure type and model used, etc.

In the present study, we endeavored to determine how Estriol (E3), the third estrogen, affects seizure susceptibility in a rodent model of epileptogenesis. We selected estriol due to two reasons: firstly, this is the hormone that has virtually been neglected in epilepsy research even though it is one of the three principal estrogens produced by the body (Reddy, 2009) and secondly, it has been recently considered to be one of the safest hormones in post-menopausal women undergoing hormone replacement therapy (HRT) (Draca, 2006; Takahashi et al., 2000) as well as offered neuroprotection in patients with multiple sclerosis (Zemlyak et al; 2002) (Ahmed and Vohora, 2014).

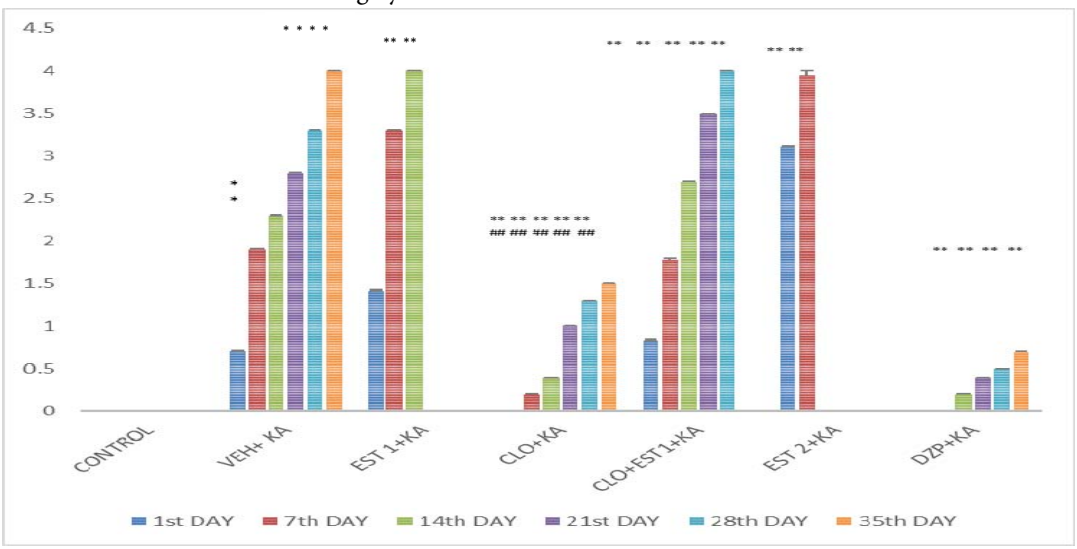
From the result, we concluded that estriol at both doses significantly reduced the time for induction of kindling and when control animals developed a seizure score of 4 after the end of 5 weeks, mice treated with estriol exhibited kindling in the first two weeks only (Fig 1 and 2). The estriol-treated mice also showed a significant increase in the % incidence of animals kindled (Fig 1) and

Figure 1. Drug therapy (Estriol and Clomiphene) their consequences on prevalence regarding kindled mice with reciprocated dose of Kainic acid.



The group consisted of 24 mice (12 M + 12 F) $$ P <0.01, when compared with normal control (Group I), && P<0.01, when compared with toxic control (Group 2), significant by ANOVA followed by Dunnett's multiple comparison test.

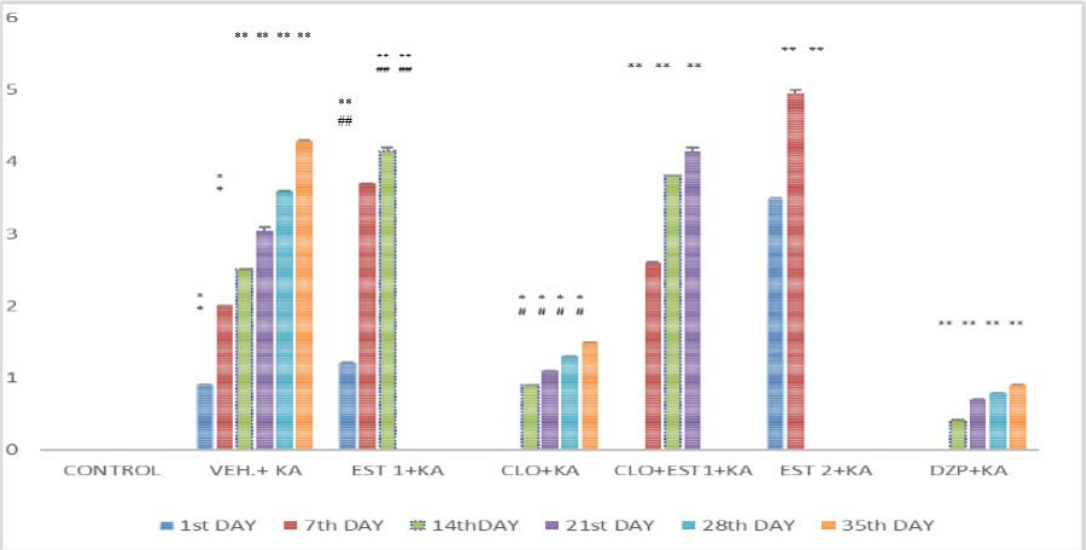
Figure 2a. Drug therapy (Estriol and Clomiphene) their consequences on seizure severity in 35 days (male mice) through the course of induction of kindling by KA.



The group consisted of 12 Male mice. ** P <0.01, when compared with normal control (Group I), ## P<0.01, when compared with toxic control (Group 2), significant by ANOVA followed by Dunnett's multiple comparison test.

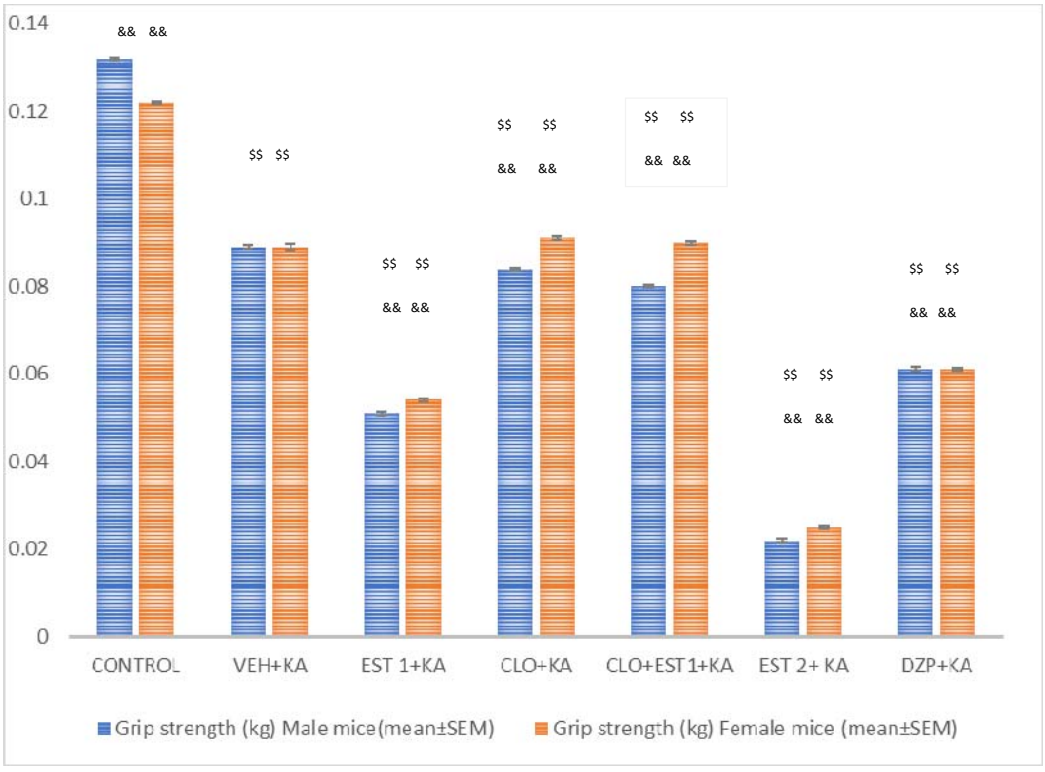
higher seizure severity. A human dose of estriol was used in our study and surprisingly this dose produced such a marked proconvulsant effect, which is ironic and thought for concern regarding the patients who are at risk for seizure disorders. Moving forward the behavior indicative of more kindled females than male mice in the above study, the explanation for this could be related to changes in the estrous cycle and exacerbation of catamenial seizures in females (Scharfman et al., 2003). Their estrous cycle dates were not included in our study. Wahnschaffe and Loscher (1992) reported, however, that natural changes in the sex hormone levels during the estrous cycle don't affect seizure susceptibility in the amygdala-kindling model of epilepsy. Henceforth, as we have used another model, it is indicative that the seizure susceptibility was affected by changes in hormone levels in female mice.

Figure 2b. Effect of Estriol and Clomiphene on seizure severity during induction of kindling by KA in female mice.



The group consisted of 12 Female mice. ** P <0.01, when compared with normal control (Group I), ## P<0.01, when compared with toxic control (Group 2), significant by ANOVA followed by Dunnett's multiple comparison test.

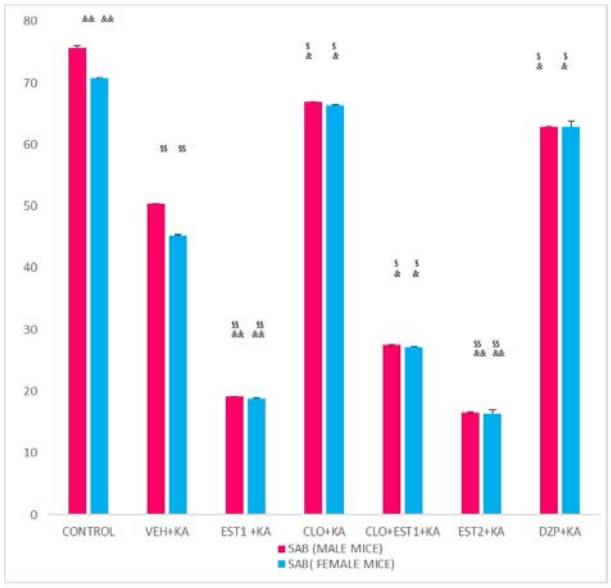
Figure 3: Drug therapy (Estriol and Clomiphene) their consequences on the grip strength of mice with reciprocated dose of Kainic acid.



The group consisted of 24 mice (12 M + 12 F) $^{\$\$}$ P <0.01, when compared with normal control (Group I), $^{\&\&}$ P<0.01, when compared with toxic control (Group 2), significant by ANOVA followed by Dunnett's multiple comparison test.

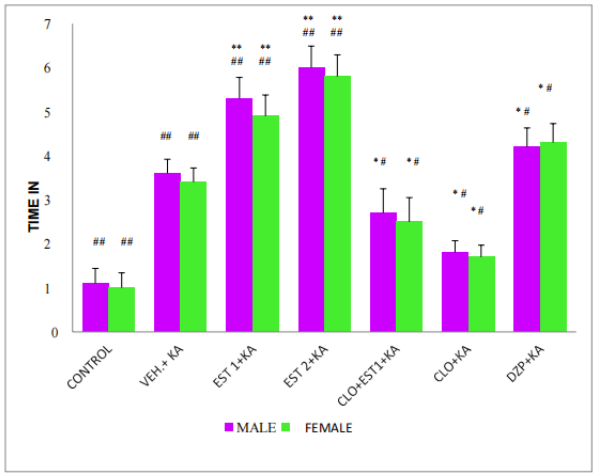
The role of Estradiol, the most extensively investigated estrogen, on seizure activity is thought to be partly mediated through estrogen receptors (ERS) (Levin, 1999; McEwen, 2001). Pretreatment with Clomiphene Citrate, an antagonist of ERS, couldn't reverse the marked proconvulsant effects of Estriol. However, it demonstrated significant anti-epileptogenic effects against the development of PTZ-kindling. The anti-epileptogenic effects of Clomiphene observed in our study were even comparable to diazepam.

Figure 4. Drug therapy (Estriol and Clomiphene) their Consequences on the spontaneous alteration behaviour of mice with reciprocated dose of Kainic acid.



The group consisted of 24 mice (12 M + 12 F) $$ P <0.01, when compared with normal control (Group I), && P<0.01, when compared with toxic control (Group 2), significant by ANOVA followed by Dunnett's multiple comparison test.

Figure 5. Drug therapy (Estriol and Clomiphene) their Consequences on the Morris water test of mice with reciprocated dose of Kainic acid



The group consisted of 24 mice (12 M + 12 F) $$ P <0.01, when compared with normal control (Group I), && P<0.01, when compared with toxic control (Group 2), significant by ANOVA followed by Dunnett's multiple comparison test.

Though not many studies have investigated the effects of Clomiphene on seizures, our findings are in agreement with a case report (Herzog, 1988) where Clomiphene benefited a 36-year-old man from developing seizures. Other clinical evidence also shows the anticonvulsant effects of Clomiphene in both epileptic men and women (Check et al., 1982; Andrew et al., 1999). Nicoletti and co-workers (1985) demonstrated a mild anticonvulsant effect of Clomiphene against kainic acid (KA)-induced seizures in rats and a proconvulsant effect when Clomiphene was used at higher doses. It has been postulated that Clomiphene may improve seizures by either normalizing the serum testosterone levels, by raising the serum levels of other anticonvulsant drugs, or by action at a cerebral level (Herzog, 1988).

AED therapy and epilepsy are associated with motor and cognitive dysfunction, for this reason, we also evaluated the effects on grip strength, spontaneous alteration behavior, rotarod, and Morris water test following KA-induced kindling in mice. A convincing decline in the strength, spontaneous alteration behavior, rotarod, and Morris water test was noted after KA-induced kindling indicating a decline in motor function and spatial memory, clomiphene and diazepam were unsuccessful in reversing their effect. A contrasting effect of estriol on SAB concerning the other two estrogens (estradiol and estrone) was reported as estriol reduced it (Brann et al., 2007, Gibbs and Gabor, 2003). Our study suggests that estriol may have a distinct effect on cognitive functions than the other two estrogens, estrone, and estradiol. Clomiphene has recently been speculated to treat cognitive impairment (Tan et al., 2003).

CONCLUSION

From our study, we concluded that estriol has a contrasting powerful proconvulsant effect from the other two estrogens (estrone, and estradiol). It enhances the development of KA-induced kindling which was not reversed by clomiphene and diazepam. Its administration in patients who has a history or are susceptible to seizures is not advisable.

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CONFLICT OF INTEREST

None declared.

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