Metabolic Brain Disease (2022) 37:2827–2838
https://doi.org/10.1007/s11011-022-01054-w

ORIGINAL ARTICLE

# Ferulic acid inhibits catamenial epilepsy through modulation of female hormones

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Received: 19 July 2021 / Accepted: 18 July 2022 / Published online: 6 August 2022


## Abstract

Approximately 40% of women with epilepsy experience perimenstrual seizure exacerbation, referred to as catamenial epilepsy. These seizures result from cyclic changes in circulating progesterone and estradiol levels and there is no effective treatment for this form of intractable epilepsy. We artificially increased progesterone levels and neurosteroid levels (pseudo-pregnancy) in adult Swiss albino female mice (19–23 g) by injecting them with pregnant mares' serum gonadotropin (5 IU s.c.), followed by human chorionic gonadotropin (5 IU s.c.) after 46 h. After this, ferulic acid (25, 50, 100 mg/kg i.p.) treatment was given for 10 days. During treatment, progesterone, estradiol, and corticosterone levels were estimated in blood on days 1, 5, and 10. Neurosteroid withdrawal was induced by finasteride (50 mg/kg, i.p.) on treatment day 9. Twenty-four hours after finasteride administration (day 10 of treatment), seizure susceptibility was evaluated with the subconvulsant pentylenetetrazol (PTZ) dose (40 mg/kg i.p.). Four to six hours after PTZ, animals were assessed for depression like phenotypes using tail-suspension test (TST). Four to six hours following TST, animals were euthanized, and discrete brain parts (cortex and hippocampus) were separated for estimation of norepinephrine, serotonin, and dopamine as well as glutamic acid decarboxylase (GAD) enzyme activity. PMSG and HCG treatment elevated progesterone and estradiol levels, assessed on days 1, 5, and 10 causing a state of pseudo-pregnancy. Treatment with finasteride increased seizure susceptibility and depression-like characteristics possibly due to decreased progesterone and elevated estrogen levels coupled with decreased monoamine and elevated corticosterone levels. Ferulic acid treatment, on the other hand, significantly decreased seizure susceptibility and depression like behavior, possibly because of increased progesterone, restored estradiol, corticosterone, monoamines, and GAD enzyme activity. We concluded anticonvulsant effect of ferulic acid in a mouse model of catamenial epilepsy, evidenced by favourable seizure attenuation and curative effect on the circulating progesterone, estradiol, and corticosterone levels along with restorative effect on GAD enzyme activity and monoamine levels.

**Keywords** Catamenial epilepsy · Progesterone · Estrogen · Corticosterone · Monoamines · Pentylenetetrazole

## Introduction

Catamenial epilepsy, affecting around 40% of women with epilepsy, describes a condition in which seizures worsen during the menstrual cycle (Maguire and Nevitt 2019). Seizures mostly occur during follicular phase (type I seizures), perimenstrual phase (type II seizures), and luteal phase (type III seizures). Type III seizures in the luteal phase are typically observed in non-ovulatory menstrual cycles (Joshi and Kapur 2019). The current clinical practices involve use of non-hormonal (e.g. clobazam or acetazolamide) and hormonal (progesterone) treatment in women with normal menses. However, in women with irregular menses, the treatment involves synthetic hormones such as medroxyprogesterone, triptorelin and goserelin which ceases the menstruation and defends against catamenial seizures (Maguire and Nevitt 2019). However, a double bind, multicenter, phase III trial showed limited efficacy for suppressing these seizures (Herzog et al. 2012). In line with this, Nestrone, a

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progesterone receptor agonist, also reported to increase the seizure frequency in epileptic animals (Shiono et al. 2019). Thus, it would be inappropriate to focus therapies solely on the development of progesterone receptor modulators, since development and progression of catamenial seizures involve the complex interplay of multiple hormones, including progesterone and estrogen (Reddy 2004, 2013).

In addition to that, the antiepileptic drugs (AEDs) (e.g., carbamazepine, phenytoin, topiramate, phenobarbital) have also been reported to stimulate the enzyme induction via CYP3A4 which leads to the enhanced metabolism of endogenous steroidal hormones. This may account for the pharmacoresistance towards AEDs associated with catamenial epilepsy (Isojarvi et al. 2005; Brodie et al. 2013). The affected females additionally tend to have epilepsy-associated depression due to the interaction between female hormones and their central nervous system (CNS). Approximately 1–7% of women with epilepsy suffer from mood disorders related to their menstrual cycle (Guille et al. 2008) and currently, there is no specific drug therapy available to treat catamenial epilepsy as well as comorbid depression, thus worsening the disease prognosis (Reddy 2004; Pahwa et al., 2022; Williamson et al. 2019; Singh et al. 2020, 2022a, b, c; Bandopadhyay et al. 2021).

Ferulic acid, one of the major bioactive phytoconstituent of *Ferula Asafoetida*, is considered useful in the treatment of several fertility related complications concerning women such as sterility, unwanted abortion, pre-mature labor, painful, difficult, and excessive menstruation, and leucorrhoea (Mahendra and Bisht 2012). It has also been reported to increase the secretion of progesterone hormone (Keshr et al. 1999; Mahendra and Bisht 2012; Zia-Ul-Haq et al. 2012). Recently, Thapliyal and colleagues reviewed anticonvulsant effects of ferulic acid in various animal models of epilepsy, but no study was reported/mentioned in view of catamenial epilepsy (Thapliyal et al. 2021). Thus, based on the available data, ferulic acid appears to be extremely valuable in treating catamenial epilepsy through its ability to normalize female reproductive hormone levels (Keshr et al. 1999; Mahendra and Bisht 2012; Zia-Ul-Haq et al. 2012; Singh and Goel, 2015) as well as restoring altered monoamine levels (Thapliyal et al. 2021; Singh et al. 2017c). Furthermore, considering the safety of naturally derived phytoconstituents (Singh et al. 2022a, b, c), the present study was envisaged to elucidate the anticonvulsant effect of ferulic acid in an animal model of catamenial epilepsy (Thapliyal et al. 2021; Singh et al. 2017c).

## Materials and methods

### Animals

All the studies were performed according to protocols approved by the Institutional Animal Ethical Committee (Approval no. 107/99/CPCSEA –2016-08), Punjabi University, India. Adult female Swiss albino mice (19-23 g; 10–12 weeks old) were used for these studies; four to five mice were kept in a cage, mice had ad libitium access to food and water, and they were maintained on a 12 h light/dark cycle.

### Drugs and chemicals

Pregnant mare's serum gonadotropin (PMSG), human chorionic gonadotropin (HCG), finasteride, β-Cyclodextrin, estradiol, progesterone, pentylenetetrazole, ferulic acid, dopamine, serotonin, and methanol (HPLC grade) were obtained from Sigma Aldrich, St. Louis MO, USA; Heptane sulfonic acid (Merck, India); perchloric acid and tartaric acid (Spectrochem, Mumbai, India); norepinephrine (Troikaa Pharmaceuticals, Ahmedabad, India). Sodium-L-glutamate (S D Fine-Chem limited, Mumbai, India), trichloroacetic acid and pyridoxal-5-phosphate (Sigma Chemical Co., St. Louis MO, USA), ninhydrin and gamma-aminobutyric acid (GABA) (Sigma Chemical Co., St. Louis MO, USA), triton X-100 (scintillation grade) (Loba Chemie, Mumbai, India) were also used in the study. All other chemical reagents were of analytical grade.

### Neurosteroid withdrawal model of catamenial epilepsy and seizure susceptibility

A state of prolonged high serum progesterone level (pseudopregnancy) was induced in mice by sequential injection of pregnant mares' serum gonadotropin PMSG (5 IU s.c.) followed 46 h later by human chorionic gonadotropin HCG (5 IU s.c.) (Fig. 1; Table 1) (Reddy et al. 2001; Pahwa et al., 2021). The day of the second gonadotropin injection was considered day 0. Neurosteroid withdrawal was induced by treatment with finasteride (50 mg/kg, i.p.), a 5α-reductase inhibitor on day 9 that blocks the conversion of progesterone to allopregnanolone, decreased neurosteroid levels, mimicking perimenstrual changes in women.

Animals were randomly divided into six cohorts (n = 6 each). Cohort I was naïve group (saline treatment only) and cohort II was administered PMSG followed by HCG (pseudo pregnant control). We have used the female mice in diestrous phase because basal progesterone level is high in this phase of estrous cycle. Cohort III were administered PMSG followed by HCG 46 h after, followed by finasteride

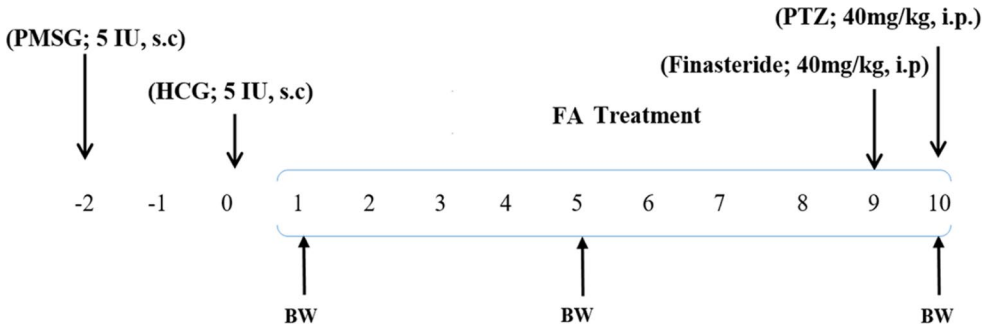


**Fig. 1** Schematic presentation of experimental protocol. Abbreviations: PMSG: Pregnant mare's serum gonadotropin; HCG: Human chorionic gonadotropin; PTZ: Pentylenetetrazole; BW: Blood Withdrawal

(50 mg/kg, i.p.) on day 9. Cohort II and III were labelled as pseudo pregnant (positive control) and finasteride control (negative control) groups, respectively. Following PMSG and HCG, cohort IV to VI also received ferulic acid (25, 50 and 100 mg/kg i.p.) for 10 days. The cohort III to VI received finasteride (40 mg/kg, i.p.) on day 9 of treatment and to assess seizure severity subconvulsant PTZ dose (40 mg/kg, i.p.) was given on day 10 (24 h after finasteride injection) (Fig. 1; Table 1). Latency to forelimb clonic seizures, latency to generalized tonic-clonic seizures (GTCS) as well as seizure severity was observed according to modified Racine's scale; Stage 0: no response; Stage 1: hyperactivity, restlessness and vibrissae twitching; Stage 2: head nodding, head clonus and myoclonic jerks; Stage 3: unilateral or bilateral forelimb clonic seizures; Stage 4: forelimb bilateral clonic seizures without involuntary jumping; Stage 5: GTCS with/out involuntary jumping; Stage 6: hind limb extensor; and Stage 7: death. Seizure scoring was performed by one of the authors (TS) in a blinded manner. The mice are considered fully protected when, there is no sign of any seizure or myoclonic jerks (stage 1 or stage 2). Four to six hours after PTZ injection (animals restore normal behavior two hours following PTZ injection), animals were evaluated for depression-like phenotypes using a tail suspension test (only once on treatment day 10). Four hours following TST, animals were anesthetized, chest cavity was cut opened to collect blood directly from heart and brain sub regions (cortex and hippocampus) were harvested for estimation of monoamines. However, for estimation of hormones on treatment days 1 and 5, blood was withdrawn from retro-orbital sinus. Our pilot studies have shown that 4 h after tail suspension test, circulating hormones and cerebral monoamine levels return to baseline, which explained animal euthanasia on these time intervals Singh and Goel 2016a, b; Kaur et al. 2017; Singh et al., 2017 a; b; c; Sharma et al. 2017; Singh and Goel 2021; Singh et al. 2021; Pahwa et al., 2021).

## Tail suspension test

The tail suspension test was used to evaluate depression-like behavior in animals (Steru et al., 1985; Singh et al. 2017a; b; c) on day 10. The method is based on the observations that a mouse suspended by the tail shows alternating periods of mobility and immobility (mobility reflects efforts to correct the posture, and immobility reflects despair). Immobility time was recorded during a 6 min period; however, the duration of immobility was recorded during the final 4 min

**Table 1** Schematic presentation of experimental protocol, Except naïve, all groups were challenged with sub-convulsant PTZ dose (40 mg/kg, i.p.) on treatment day 10. Abbreviations: PMSG: Pregnant mare's serum gonadotropin; HCG: Human chorionic gonadotropin; FA: Ferulic aid; PTZ: Pentylenetetrazole

| Sr. No | Group Name | Treatment |
|---|---|---|
| 1 | Naive | Naïve |
| 2 | Pseudopregnant control (Pseudo) | PMSG + HCG (Positive control); No finasteride was given. |
| 3 | Finasteride control (Fina) | PMSG + HCG followed by saline treatment for 10 days as well as finasteride on treatment day 9 (Negative control); PTZ on day 10 |
| 4 | FA 25 | PMSG + HCG followed by ferulic acid (25 mg/kg) for 10 days with finasteride administration on treatment day 9; PTZ on day 10 |
| 5 | FA 50 | PMSG + HCG followed by ferulic acid (50 mg/kg) for 10 days with finasteride administration on treatment day 9; PTZ on day 10 |
| 6 | FA 100 | PMSG + HCG followed by ferulic acid (100 mg/kg) for 10 days with finasteride administration on treatment day 9; PTZ on day 10 |

interval of the test. An animal was immobile when it did not show any body movements and hung passively.

## Determination of serum neurosteroids levels

Neurosteroids were estimated in serum samples using previously reported HLPC-UV method (Wei et al. 1990; Pahwa et al., 2021). To prepare the sample, serum (50 µl) was digested with ethyl ether (0.5 ml), vortexed for 3 min and then centrifuged at 3500 rpm for 5 min. The organic layer was transferred to small glass ampoules and were evaporated to dryness at 50 °C. Fifty µl mixture of methanol and water (60: 40 v/v) was added into the organic layer, which was vortexed and vibrated ultrasonically for 60 s. After centrifugation for 2 min at 4000 rpm, 20 µl of supernatant was injected into the system.

Waters HPLC system (Milford, USA) consisted of 515 binary pumps (Waters, USA), 2489 ultraviolet detector (Waters, USA) and a rheodyne manual injector (20 µl) was used. The chromatographic separation performed at room temperature was achieved using Zorbex SB-C18, reversed phase column (4.6 mm x 150 mm x 5 µm) (Agilent, USA) at 254 nm. The mobile phase consisted of a mixture of methanol: tetrahydrofuran: water (26:18:56 v/v/v) with a flow rate of 1 ml/min at room temperature, filtered using 0.45 µm membrane (Millipore, USA) and degassed using Transonic T 570/H, Elma, Germany. The data was acquired and processed in Empower Pro® Operating System (Waters®, Milford, USA). A stock solution (1 mg/ml) of corticosterone, estradiol and progesterone were prepared in methanol. Corticosterone; $y = 44261x + 13{,}104$, $R^2 = 0.996$, estradiol; $y = 0.735\,x + 0.905$, $R^2 = 0.987$, and Progesterone; $y = 63171x - 38{,}604$, $R^2 = 0.999$). The data were acquired and processed in Empower Pro Operating System (Waters, Milford, USA).

## Neurochemical estimations

Monoamine (norepinephrine, dopamine, and serotonin) levels in the cortex and hippocampus areas of the mice brain were estimated using HPLC method, previously standardized in the lab Singh and Goel 2016a, b; Kaur et al. 2017; Singh et al., 2017 a; b; c). Isolated brain parts were weighed and subdivided into two equal portions. One portion was homogenized in ice cold 10% w/v (0.1 M) perchloric acid and centrifuged at 14,000 g for 30 min at 4 °C (REMI C-24BL, Cooling Centrifuge, REMI, India) and was used for estimation of monoamines. The Waters HPLC system (Milford, USA) consisted of 515 binary pumps (Waters, USA), 2465 electrochemical detector (Waters, USA) and rheodyne manual injector (20 µl). The mobile phase consisted of 150 mM sodium dihydrogen phosphate, heptane sulfonic acid (2.28 mM), and EDTA (1 mM) and methanol in 90:10 ratio. The mobile phase pH was adjusted to 4.8 with 5 M sodium hydroxide, filtered through a 0.22 mm membrane filter (Millipore, USA) and degassed using bath sonicator (Transonic T570/H, Elma, Germany). The chromatographic separation was achieved on reversed-phase analytical column (250 mm X 4.6 mm i.d., 5 mm; Sunfire, USA). The mobile phase was eluted at flow rate of 1.2 ml/min. Chromatographic analyses were performed at 35 °C. The standard curves were prepared for concentration ranging 10–100 ng/ml for norepinephrine, dopamine, and serotonin (Norepinephrine: $y = 11684x + 29{,}761$; $R2 = 0.995$, Dopamine: $y = 71752x + 20{,}169$; $R2 = 0.997$, and Serotonin: $y = 87070x + 15{,}648$; $R2 = 0.997$) The data were acquired and processed in EmpowerPros® Operating System (Waters®, Milford, USA). A stock solution (1 mg/mL) of norepinephrine, dopamine, and serotonin were prepared in 0.1 M Perchloric acid. However, another half of the mice brain was used for estimation of total nitrite levels using microplate reader, also reported in previous publication (Singh et al. 2014; Singh et al. 2015; Singh and Goel 2017a, b; c). The brain tissue was homogenized in ice cold 10% w/v (0.05 M, pH 7.4) phosphate buffer and centrifuged at 6000 g for 20 min at 4 °C and clear supernatant was utilized for estimation of total nitrite levels. The method involved the use of the griess diazotization reaction to spectrophotometrically detect total nitrite levels. Fifty µl of brain homogenate (filtered) and the standard (NaNO2/NaNO3) was mixed with 50 µl of the griess reagent in triplicate using a 96-well plate. Cu/Cd alloy was added in the supernatant to convert nitrates to nitrites. The well plate was shaken at 150 rpm for 60 s to ensure proper mixing of the samples/standards followed by room temperature of incubation of plate for 30 min. The absorbance of nitrite-containing samples was measured at 540 nm using a microplate reader (APR-4 Microplate Reader, Logotech, ISE Group, Germany) against a photometric reference (blank: 50 µl HPLC grade water and 50 µl griess reagent). The total nitrite levels were estimated using the straight-line equation for nitrite ($y = 0.0008x + 0.0046$, $R2 = 0.9954$). The results were expressed as ng/g of wet tissue.

## Glutamic acid decarboxylase (GAD) activity

GAD activity was assayed according to the method reported (Wolf and Klemisch 1991). Enzyme activity was expressed as micro-gram GABA/ mg protein of wet tissue.

## Statistical analysis

The statistical analysis was performed using GraphPad prism® version 8 (Graph-Pad Software Inc., San Diego, CA,

USA). Data obtained was analyzed by one-way analysis of variance (ANOVA) followed by Student-Newman-Keul's test to determinate statistically significant differences among all the groups. Each value was expressed as mean ± SEM, and statistical significance was considered at $p < 0.05$.

## Results

### Ferulic acid decreased seizure susceptibility following PTZ injection

The mean seizure severity score was significantly ($P < 0.05$) increased in finasteride control animals in comparison to pseudopregnant control animals. However, ferulic acid (50 and 100 mg/kg) treatment significantly ($P < 0.05$) reduced mean seizure severity score as compared to finasteride control group (Fig. 2 A).

### Ferulic acid increased mean latency to forelimb clonic seizures (stage 3) and GTCSs (stage 5) following PTZ injection

The mean latency time to the onset of clonic convulsions (seizure stage 3) was significantly ($P < 0.05$) decreased in finasteride control group as compared to pseudopregnant control group. The mean latency to onset of clonic convulsions (seizure stage 3) remains unaffected in ferulic acid (25, and 50 mg/kg) treated animals as compared to finasteride control group. However, ferulic acid (100 mg/kg) significantly ($P < 0.05$) increased mean latency time to onset of forelimb clonic seizures (seizure stage 3) as compared to finasteride control group (Fig. 2B).

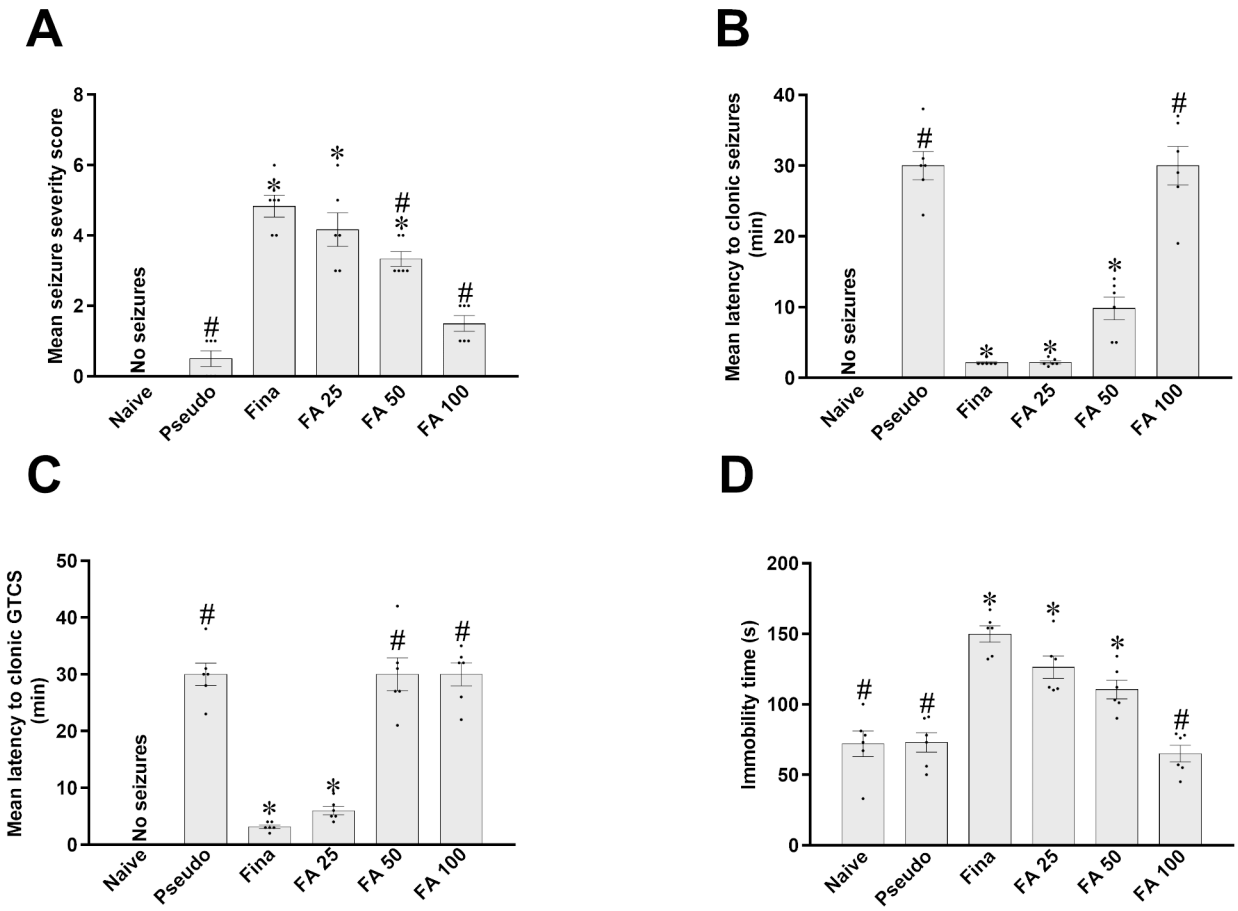


**Fig. 2** Effect of different treatments on (a) seizure severity score, (b) mean latency to develop forelimb clonic seizures (stage 3 seizures), (c) mean latency to develop generalized tonic-clonic seizures (GTCS), and (d) immobility time. Each value is expressed as mean ± standard error mean (n = 6). The significance level was considered at $P < 0.05$ (Student Newman Keuls Post hoc test). *: significant as compared to pseudo pregnant control; # significant as compared to finasteride control. Abbreviations: Naïve: saline treatment only; Pseudo: Pseudopregnant control; Fina: Finasteride control; FA 25, 50 and 100: Ferulic acid 25, 50 and 100 mg/kg; i.p

The mean latency time to the onset of GTCSs was also significantly ($P < 0.05$) decreased in finasteride control group as compared to pseudopregnant control group. The onset of GTCSs remain unaffected in ferulic acid (25 mg/kg) as compared to finasteride control group. However, ferulic acid (50 and 100 mg/kg) significant ($P < 0.05$) increased mean latency time to the onset of GTCSs as compared to finasteride control group (Fig. 2 C).

### Ferulic acid treatment decreased immobility duration

The duration of immobility was significantly ($P < 0.05$) increased in finasteride control group as compared to pseudopregnant control groups. However, ferulic acid (100 mg/kg) treatment significantly ($P < 0.05$) reduced immobility time as compared to finasteride control group (Fig. 2D).

### Ferulic acid treatment restored corticosterone, progesterone, and estradiol levels

The corticosterone levels were significantly different ($P < 0.001$) between different treatment cohorts. A significantly ($P < 0.05$) enhanced levels of corticosterone was observed in finasteride control group as compared to pseudopregnant control group and naïve group on days 1, 5 and 10. However, the corticosterone levels observed in the ferulic acid (25, 50, and100 mg/kg) and was significantly ($P < 0.05$) reduced as compared to finasteride control group on days 5 and 10 (Fig. 3 A; Supplementary Fig. 1).

A significant ($P < 0.05$) increase in the progesterone level was observed in the pseudopregnant and finasteride control groups as compared to naïve animals on days 1, 5 and 10. However, ferulic acid (25, 50 and 100 mg/kg) treatment significant ($P < 0.05$) enhanced progesterone level as compared to finasteride control group on day 10 (Fig. 3B; Supplementary Fig. 1).

A significant ($P < 0.05$) increase in the estradiol level was observed in the pseudopregnant and finasteride control animals as compared to naïve animals on days 1, 5 and 10. However, ferulic acid (100 mg/kg) significantly ($P < 0.05$) reduced estradiol levels as compared to finasteride control group on day 10 (Fig. 3 C; Supplementary Fig. 1).

### Ferulic acid treatment increased GAD enzyme activity

The GAD enzyme activity was significantly ($P < 0.05$) was significantly increased in pseudopregnant as compared to finasteride control group. The ferulic acid treatment (25, 50 and 100 mg/kg) significantly ($P < 0.05$) enhanced the GAD enzyme activity as compared to finasteride control group (Fig. 3D).

### Ferulic acid treatment restored norepinephrine levels

The cortical ($P < 0.0001$) and hippocampal ($P < 0.0001$) norepinephrine levels were significantly different in various treatment cohorts. A significantly ($P < 0.05$) reduced cortical and hippocampal norepinephrine levels were observed in finasteride control group as compared with pseudopregnant control group and naïve group. However, ferulic acid treatment (25, 50, and 100 mg/kg) significantly ($P < 0.05$) restored cortical and hippocampal norepinephrine levels in comparison to finasteride control group (Fig. 4 A, 4B).

### Ferulic acid treatment restored dopamine levels

The dopamine levels were significantly ($P < 0.0005$) different between various treatment cohorts. A significantly ($P < 0.05$) reduced cortical and hippocampal dopamine levels were observed in finasteride control group as compared with pseudopregnant and naïve control groups. However, the hippocampal dopamine levels were significantly ($P < 0.05$) enhanced in ferulic acid (25, 50, and 100 mg/kg) treated animals as compared to finasteride control group (Fig. 4 C, 4D).

### Ferulic acid treatment restored serotonin levels

The cortical ($P < 0.0001$) and hippocampal ($P < 0.0001$) serotonin levels were significantly different between different treatment cohorts. A significantly ($P < 0.05$) reduced cortical and hippocampal serotonin levels were observed in finasteride control group as compared with pseudopregnant control group. However, the cortical and hippocampal serotonin levels were significantly ($P < 0.05$) restored in ferulic acid (25, 50, and 100 mg/kg) treated animals as compared to finasteride control group (Fig. 4 C, 4D).

## Discussion

In summary, we revealed the anticonvulsant effect of ferulic acid in a mouse model of catamenial epilepsy, evidenced by favourable seizure attenuation and curative effect on the circulating progesterone, estradiol, and corticosterone levels along with restorative effect on GAD enzyme activity and monoamine levels. The study outcome and justification of the methodologies used are discussed herein.

Neurosteroid withdrawal, a well-accepted model of catamenial epilepsy, was used for this study. In consistent

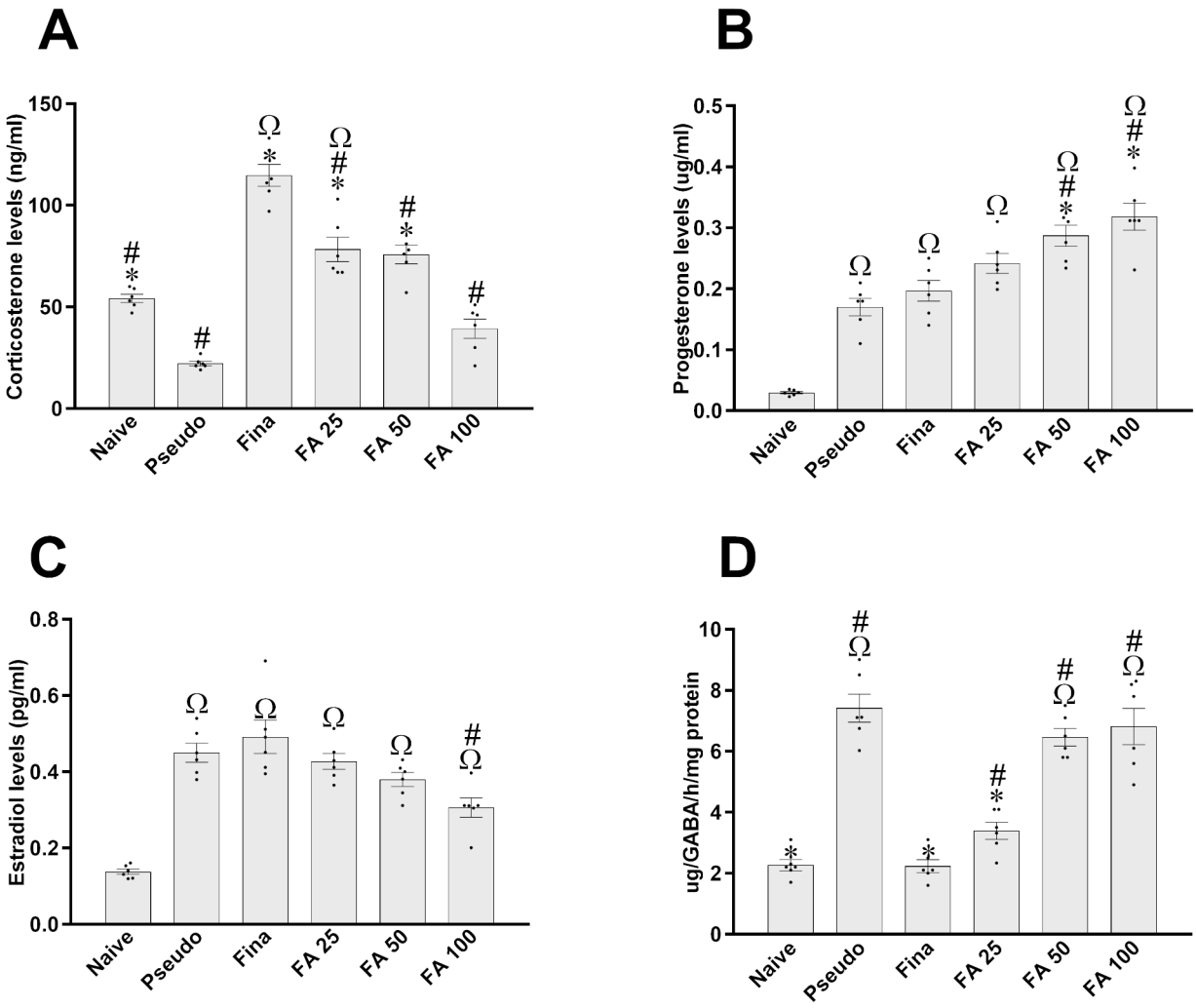


**Fig. 3** Effect of different treatments on corticosterone, progesterone, estradiol, and GAD enzyme activity on day 10. Each value is expressed as mean ± standard error mean (n = 6). The significance level was considered at $P < 0.05$ (Student Newman Keuls Post hoc test). *: significant as compared to pseudo pregnant control; # significant as compared to finasteride control; Ω: significant as compared to naïve. Abbreviations: Naïve: saline treatment only; Pseudo: Pseudopregnant control; Fina: Finasteride control; FA 25, 50 and 100: Ferulic acid 25, 50 and 100 mg/kg; i.p

with the previous studies, gonadotropin regimen significantly increased progesterone levels (pseudo-pregnancy) as observed on the different treatment days (Reddy et al. 2001; Reddy 2009; Pahwa et al., 2021). To simulate neurosteroid withdrawal, animals were additionally injected with finasteride, on the treatment day 9, like what happens during the menstruation. A neurosteroid withdrawal was associated with the decreased seizure thresholds and an increase in the seizure severity scores following PTZ injection, also reported previously (Reddy and Rogawski 2001; Reddy et al. 2001). Progesterone withdrawal might have decreased its major metabolite, allopregnanolone, which exerts its anticonvulsant effects through the modulation of the γ -aminobutyric acid type-A (GABA-A) receptor. Despite being ineffective clinically, animal studies have shown that the biotransformation of progesterone to its neurosteroid derivative allopregnanolone produces increased GABA-A receptor mediated inhibition in the brain and prevents epilepsy (Joshi and Kapur 2019). The results agreed with an earlier study in which finasteride failed to protect against convulsions after a subconvulsant PTZ challenge in the control animals (Reddy and Rogawski 2001; Reddy et al. 2001; Pahwa et al., 2021). However, ferulic acid treatment increased the seizure latency in comparison to the finasteride control animals. Pseudo pregnant positive control animals also showed resistance to the subconvulsant PTZ challenge as compared to finasteride control animals, possibly owing to the elevated progesterone levels. The observed anticonvulsant

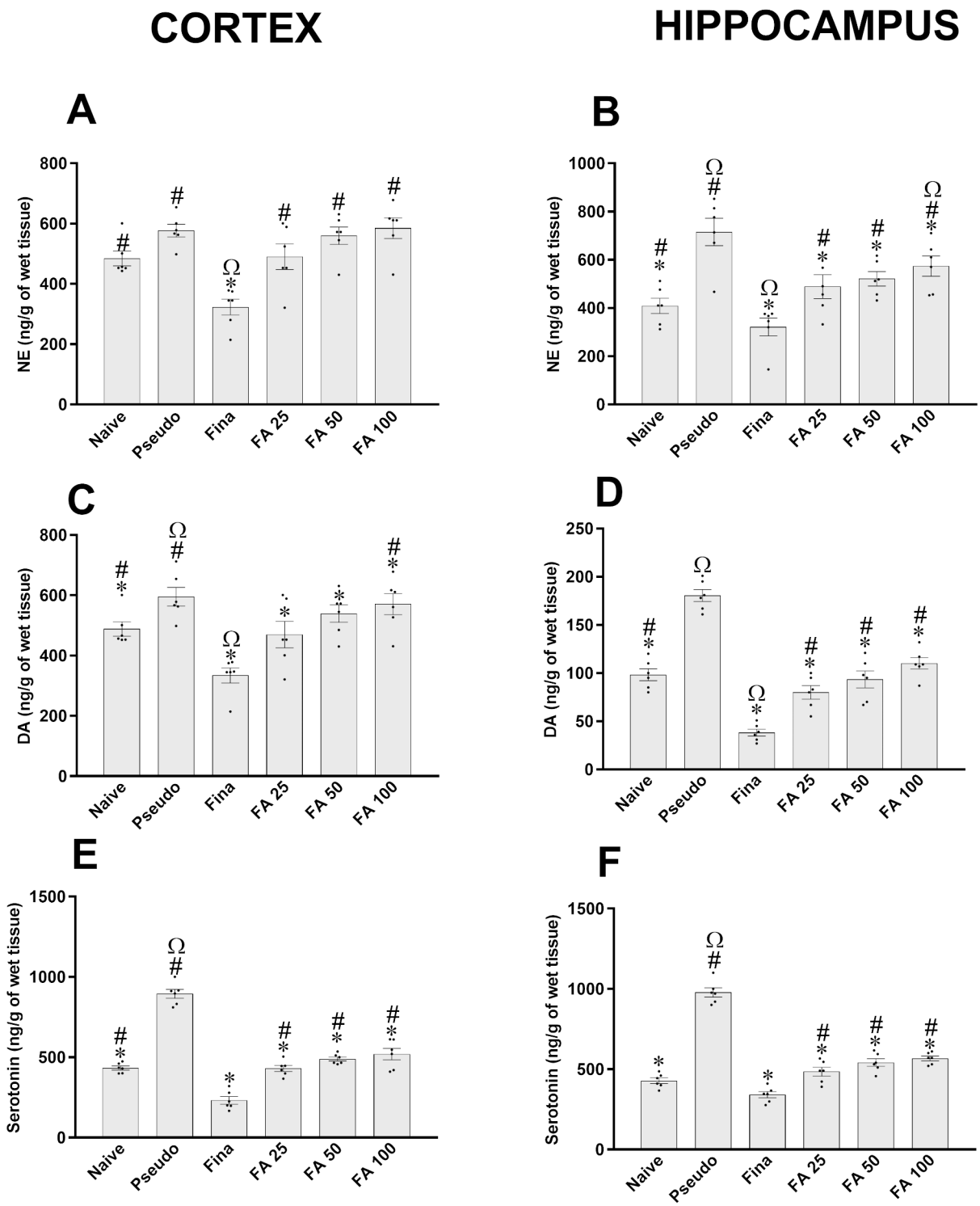


**Fig. 4** Effect of different treatments on cortical and hippocampal monoamine (norepinephrine, dopamine, serotonin (5-HT)) levels in mice brain. Each value is expressed as mean ± standard error mean (n = 6). The significance level was considered at $P < 0.05$ (Student Newman Keuls Post hoc test). *: significant as compared to pseudo pregnant control; # significant as compared to finasteride control; Ω: significant as compared with naïve. Abbreviations: Naïve: saline treatment only; Pseudo: Pseudopregnant control; Fina: Finasteride control; FA 25, 50 and 100: Ferulic acid 25, 50 and 100 mg/kg; i.p

effect of ferulic acid might be discussed in reference to the observed favourable hormonal changes as follows.

Progesterone plays a crucial role in the progression of catamenial epilepsy and has an anti-convulsant properties (Joshi and Kapur 2019). It is possible that the medicinal plants/constituents, like ferulic acid, have fertility enhancing effects due to their progesterone elevating/slowing the reduction of progesterone levels (Salih and Jaafar 2013; Keshr et al. 1999; Mahendra and Bisht 2012; Zia-Ul-Haq et al. 2012). This was also confirmed by the slowed reduction of progesterone levels in ferulic acid treated animals as compared to the finasteride-treated animals. Beside progesterone, estrogen also plays a promising role in the development of catamenial seizures. It generally has proconvulsant and epileptogenic properties in the rodents and humans (Logothetis et al. 1959). Among all the three biologically active estrogens (i.e., estrone (E1), estradiol (E2) and estriol (E3), estradiol plays a major role in the development and progression of catamenial epilepsy (Velíšková 2006, 2007). Therefore, the alteration in serum estradiol levels were monitored on different days during treatment. Our results showed the decreased estradiol levels in ferulic acid treated animals as compared to finasteride control animals. Recent study has also reported the ferulic acid regulate the progesterone and estrogen levels (Li and Shi 2021). Hence, the anticonvulsant effects of the ferulic acid on PTZ provoked seizures was further explained by the effect of progesterone and estradiol levels along with the previously reported antiepileptic mechanisms (Singh et al. 2017; Thapliyal et al. 2021; Pahwa et al., 2022)..

Studies in both the experimental and clinical settings have shown that corticosterone, in addition to the two major neurosteroids, can affect seizure activity. There are multiple evidences that the corticosterone triggers epileptogenesis in animals and is known for its proconvulsant effects (Kling et al. 1993; Roberts and Keith 1994; Karst et al. 1999; Hopper et al. 2018; Basu et al. 2021). There was a significant decrease in the corticosterone levels in ferulic acid treated animals, possibly due to the high levels of progesterone since it decreases the serum corticosterone levels (Baykara et al., 2013; Hooper et al., 2018; Basu et al. 2021). Thus, considering the aforementioned findings, it can be stated that decreasing corticosterone levels in ferulic acid treated animals may help treating catamenial epilepsy.

GAD is a rate limiting enzyme responsible for the synthesis of the major inhibitory neurotransmitter GABA, and its down regulation is associated with increased seizure related phenotypes (Lloyd et al. 1986; Patel et al. 2021). There are reports that estrogen and progesterone modulate the GAD enzyme activity (Wallis and Luttge 1980). Increased estrogen reduces the GAD activity whereas increased progesterone elevates GAD enzyme activity. Therefore, we also assessed GAD enzyme activity in the brain (cortex and hippocampus collectively). The present study showed elevated GAD enzyme activity in pseudopregnant animals as compared to naïve and finasteride control animals. Ferulic acid treatment increased the GAD enzyme activity, and this increase could be due to the increased progesterone or decrease estradiol levels, hence justifying our findings.

In addition, the ferulic acid treated animals also exhibited reduced immobility as compared to the finasteride-treated controls. The high progesterone levels observed in the ferulic acid -treated animals may have contributed to its antidepressant effects, since progesterone has been reported to have similar antidepressant effects (Andrade et al. 2010; Li et al. 2012, 2013). The ferulic acid treatment has also restored monoamine levels in the cortex and hippocampus as well as decreased circulating corticosterone levels as reported previously (Mishra and Goel 2013; Kaur et al. 2017). The depletion of monoamines (serotonin, norepinephrine, and/or dopamine) in the central nervous system is associated with depression clinically (Delgado 2000). This hypothesis was further underpinned by the mechanism of action of antidepressants; agents that elevate the levels of monoamines in the brain and are effective in alleviating depression (Morilak and Frazer 2004; Bhatia et al., 2022). Similarly decreasing corticosterone levels also explained antidepressant like effects following ferulic acid treatment. The clinical findings suggested that in patients with depression or epilepsy patients associated with depression, elevated corticosterone levels are important biomarker of mood disorders (Ising et al. 2005).

## Conclusions

The present study concluded the antiepileptic effects of ferulic acid in a model of catamenial epilepsy pertaining to its restorative effects on circulating hormones, cerebral monoamine, and GAD enzyme activity levels (Fig. 5). Therefore, herbal formulations containing ferulic acid may be used as monotherapy or adjuvant therapy along with available AEDs for the treatment of epilepsy combined with comorbid depression in the women with epilepsy.

**Authors' contributions** HKD and TS performed experiments. HKD wrote the first draft of manuscript. RKG conceived the idea, edited, and wrote the manuscript.

**Funding** The authors are deeply grateful to the Council of Scientific and Industrial Research (CSIR), Pusa, New Delhi, India for providing financial assistance (Vide F. No. 38 (1339)/12/EMR-II) for the project to Dr. Rajesh Kumar Goel.

**Data Availability** Data will be available on reasonable request.

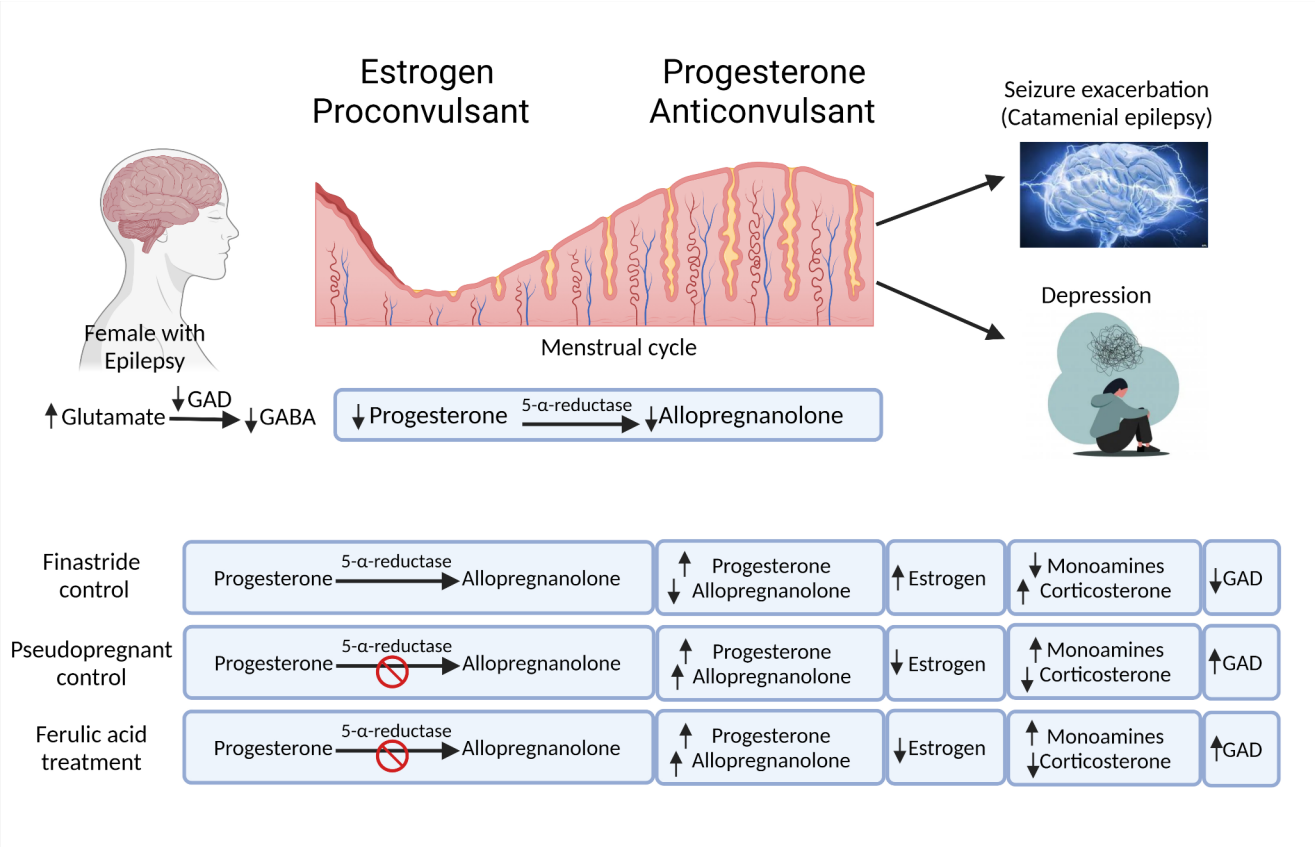


**Fig. 5** Schematic representation of the anticonvulsant mechanism of ferulic acid Abbreviations: GAD: Glutamate decarboxylase; GABA: Gamma aminobutyric acid

**Code Availability** Not applicable.

## Declarations

**Conflicts of interest/Competing interests** None.

**Ethical approval** The animal experiments were obtained the approval from the Animal Ethics Committee of Punjabi University, India.

**Consent for publication** All authors have their consents for publication.

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**Supplementary Information** The online version contains supplementary material available at https://doi.org/10.1007/s11011-022-01054-w