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ORIGINAL ARTICLE

Buckwheat tartary regulates the Gsk-3β/β-catenin pathway to prevent neurobehavioral impairments in a rat model of surgical menopause

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Abstract

Menopause is a natural aging process characterized by decreased levels of sex hormones in females. Deprivation of estrogen following menopause results in alterations of dendritic arborization of the neuron that leads to neurobehavioral complications. Hormone replacement therapy is in practice to manage postmenopausal conditions but is associated with a lot of adverse effects. In the present study, the efficacy of buckwheat tartary (*Fagopyrum tataricum*) whole seed extract was investigated against the neurobehavioral complication in middle-aged ovariectomized rats, which mimic the clinical postmenopausal condition. Hydroalcoholic extraction (80% ethanol) was done, and quantification of major marker compounds in the extract was performed using HPLC. Oral treatment of the extract following the critical window period rescued the reconsolidation process of spatial and recognition memory, as well as depression-like behavior. Gene expression analysis disclosed elevated oxidative stress and neuroinflammation that largely disturb the integrity of the blood-brain barrier in ovariectomized rats. *Gfap* and *Pparγ* expression also showed reactive astrogliosis in the rats subjected to ovariectomy. The extract treatment reverted the elevated oxidative stress, neuroinflammation and expression of the studied genes. Furthermore, protein expression analysis revealed that Gsk-3β was activated differentially in the brain, as suggested by β-catenin protein expression, which was normalized following the treatment with extract and rescued the altered neurobehavioral process. The results of the current study concluded that *Fagopyrum tataricum* seed extract is better option to overcome the neurobehavioral complications associated with the menopause.

Keywords Glycogen synthase kinase 3 · Depression · β-catenin · Spatial memory · Reactive astrogliosis

Introduction

Climacteric is a natural aging course of the ovaries that is experienced by every woman in their late 40s. It declines the level of sex hormones especially estrogen, resulting in the disruption of various physiological functions and neuroendocrine events (Rana et al. 2020). Sexual hormone deprivation following menopause disrupts the neuronal circuits, which is characterized by a reduction in the pyramidal neurons, dendritic arborization, and spine density, resulting in neurobehavioral complications (Gava et al. 2019; Rana et al. 2022a).

Oxidative stress and neuroinflammatory events are the major pathophysiological factors engaged in the development of various psychiatric disorders (Barron et al. 2017). Estrogen possesses both antioxidant and anti-inflammatory characteristics. Preclinical studies also showed altered expression of antioxidant and anti-inflammatory genes in the brain following ovariectomy (Rana et al. 2020, 2022a), shedding light on the disturbance in brain homeostasis. Rather than neuronal, the brain also consists of a non-neuronal cell population such as astrocytes, which are known to participate in neurovascular coupling, synaptogenesis, and waste clearance (Jiwaji et al. 2022). However, in neurodegenerative conditions, astrocytes become reactive and damage the neuronal circuits, either by elevating oxidative stress (Chen et al. 2020) or triggering neuroinflammatory events (Jiwaji et al. 2022). Likewise, sexual hormone deprivation

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following menopause also triggers a reactive astrogliosis process in the discrete regions of the brain, which ultimately leads to neurobehavioral impairments (Rana et al. 2022a). Furthermore, there are shreds of evidence that the blood-brain barrier (BBB) function is also compromised following menopause, which results in inappropriate passage of systemically circulating molecules and cells towards the brain parenchyma (Maggioli et al. 2016; Rana et al. 2022a), thus initiation of neurodegenerative cascades.

Glycogen synthase kinase-3 beta (Gsk-3β) is a serine/threonine kinase that regulates various cellular and neurophysiological processes for a steady flow of neuronal transmission (Rana and Singh 2018). The available experimental evidence revealed that impaired Gsk-3β is deleterious to the cell as it interferes with the cellular defensive mechanisms (Rana et al. 2022b). Estrogen deprivation following menopause activates the Gsk-3β, resulting in the degradation of β-catenin protein, which is majorly engaged in cell protection and cell adhesion mechanisms (Pinto-Almazan et al. 2012; Rana et al. 2022a). Activated Gsk-3β following menopause also decreases the synthesis of brain-derived neurotrophic factors by obstructing the cAMP-response element binding protein signalling pathway (Konishi et al. 2020; Rana et al. 2022a), which results in neurobehavioral impairment.

Hormonal replacement therapy (HRT) is in clinical practice to overcome postmenopausal complications, but its efficacy is restricted only up to a critical window period (Daniel and Bohacek 2010). Chronic treatment of estrogen in middle-aged ovariectomized (*ovct*) rats after the therapeutic period has been shown to enhance BBB permeability (Bake and Sohrabji 2004), thus suggesting an increased risk of cerebrovascular disorders. Moreover, HRT was also reported to be linked with various side effects like breast cancer, cerebral stroke, endometrium cancer, and neurobehavior impairment (Bjarnason 2005; Mehta et al. 2021). Because of the numerous adverse effects linked with the therapy, researchers are increasingly focusing on the plant-based alternatives.

Fagopyrum tataricum (L.) Gaertn (*F. tataricum*), commonly known as buckwheat tartary is a traditionally edible medicinal plant belonging to the Polygonaceae family (Zou et al. 2021). Phytochemical analysis revealed that *F. tataricum* is a good source of proteins, vitamins, dietary fibers, and polyphenols (Zhu 2016; Zou et al. 2021). As a consequence of these bioactive metabolites, *F. tataricum* showed various pharmacological activities such as antibacterial, antihypertensive, anti-inflammatory, anticancer, and antidiabetic (Zhu 2016; Zou et al. 2021). *F. tataricum* extract also improved the amyloid-β-induced memory impairment in mice model of Alzheimer's disease *via* suppressing oxidative stress (Choi et al. 2013). It was also observed that the incorporation of *F. tataricum* in the daily diet reduces the risk of chronic diseases such as diabetes, hypertension, and neurodegeneration (Zhang et al. 2012). However, the potential of *F. tataricum* extract for the improvement of neurobehavioral complications associated with menopause is not known. Hence, in the current study, we hypothesized that *F. tataricum* extract might overcome the neurobehavioral alteration by suppressing oxidative stress and neuroinflammatory events. The middle-aged *ovct* rat model, which resembles the postmenopausal condition was employed.

Materials and methods

Experimental animals and care

The present study was conducted on 10-months old female Sprague Dawley rats. The rats were kept in the animal house of the CSIR-IHBT in standard polycarbonate cages (3 rats/cage) at a temperature of 25 ± 2 °C, 12 h day/night cycle, and 50–60% of relative humidity. The *ad libitum* food and water was provided to animals throughout the experiment. The experimental protocol was duly permitted by the Institutional Animal Ethics Committee. All the trials were conducted in the day cycle.

Extraction and chemical quantification of major flavonoids

The seeds of *F. tataricum* were procured from the Lahaul and Spiti region of Himachal Pradesh, India. The plant material was authenticated and deposited in the herbarium at the CSIR-IHBT, Palampur, Himachal Pradesh, India with specimen voucher number PLP 22,007. The seeds were ground and subjected to extraction by percolation using 80% ethanol at room temperature (RT). The collected extract was filtered and concentrated under reduced pressure using a rotary evaporator (Rotavapor R-300 BUCHI, Switzerland), and finally dried by lyophilization (Labconco FreeZone 6Plus, USA). The dried extract (BXT) was weighed and stored at 4 °C for further use.

Furthermore, to quantify the hydroethanolic extract, rutin and quercetin were used as marker compounds. A standard stock solution of both flavonoids was prepared in 50:50 concentrations of methanol and water (1 mg/mL). The stock was diluted to the desired concentrations, filtered sample through a 0.45 µm membrane, and injected directly into the Shimadzu High-performance liquid chromatography system (HPLC; Tokyo, Japan) equipped with a pump (Model LC-20AT), degasser (Model DGU-20A5), photodiode Array detector (SPDM20A) and an autosampler (SIL-20AC).

The chromatographic separation of compounds was done using an RP-18 Merck column (250 mm x 4.6 mm; 5 µm). The extract solution was prepared in a similar manner. The mobile phase was composed of acetonitrile: water (40:60) with 0.1% of orthophosphoric acid (v/v), which was used with a flow rate of 0.6ml min^{-1} and a run time of 30 min. The standard curve of the compounds was used to quantify the major metabolites of BXT.

Animal surgery and experimental protocol

Surgical removal of the ovaries in experimental animals is a well-accepted model for the study of post menopause-associated complications. Briefly, a cocktail of ketamine (50 mg/kg; Psychotropics India Ltd.) and xylazine (20 mg/kg; Sigma Aldrich, USA) was injected intraperitoneally (*i.p.*) into female rats for induction of anaesthesia. Animals were placed in the supine position after confirmation of anaesthesia. The surgical site was prepared and disinfected with povidone-iodine. A minor cut was made on just the right side of the 5th nipple on the abdomen to isolate the uterine tubes and ovaries. A knot was made between the ovaries and distal part of the uterine tubes through an absorbable suture (SOLUS-910®, 3 – 0, LNW 2437 – 910, Lotus, India). The uterine tubes were returned back to the peritoneal cavity after resection from the ovaries. The peritoneal and muscle layer was stitched with absorbable sutures, while the skin was stitched with non-absorbable sutures (NYLUS®, 3 – 0, LNW 3328, Lotus, India). Further, to minimize the post-surgical discomfort and avoid infection diclofenac sodium (10 mg/kg, *i.p.*) and streptomycin (20 mg/kg, *i.p.*) respectively, were injected into each animal immediately after the surgery and continued for further 3 days (once in 24 h). The animals were housed individually in a postoperative room for 1 week to recover from the surgery, thereafter regrouped in their home cage. After surgery, animals were kept for 12 weeks to develop neurobehavioral impairments (Rana et al. 2020). Successfully recovered animals were divided into 4 groups ($n = 5$/group) as, vehicle control (*ovx*), *bxt100* (administered BXT 100 mg/kg; *p.o.*), *bxt200* (administered BXT 200 mg/kg; *p.o.*) and *bxt400* (administered BXT 400 mg/kg; *p.o.*) and were given respective treatment for subsequent 10 weeks. A group ($n = 5$) served as normal control (*naive*), running parallel to the rest of the experimental groups throughout the experiment. Thereafter, the neurobehavioral function was assessed by the blind observer, in a sound-attenuated room as depicted in the schematic presentation (Fig. 1A). Animals were decapitated after 24 h of the last behavior task. The cortex and the hippocampal regions of the brain were isolated and stored at -80 ºC. The adjusted uterus weight was also calculated from the isolated uterine horns for confirmation of the success of the *ovx* procedure as described by Rana et al. (2020).

Morris water maze test

The Morris water maze (MWM) test was done to assess spatial memory impairment. The test was conducted in a large black circular tank, filled with clean water (25 ± 2 ºC) up to 30 cm in depth. A camera (Sony color and Lente, CAMCOLORPAL) connected with video tracking software (SMART V3.0., Panlab, Barcelona) was fitted at the top centre of the tank. The extra-maze visual clues remained fixed throughout the experiment in the room. The total duration of the test was 5 days. The first 4 days were the acquisition/learning phase in which each rat received 4 alternative trials with 5 min inter-interval daily. On day 5th probe trial was performed as described earlier (Rana et al. 2020). There was no alternation in the consolidation process of memory observed in our previous studies (Rana et al. 2020, 2022a), hence only the time spent on day 5th was recorded in the present work.

Recognition memory analysis

A novel object recognition test (NORT) was performed to analyze the recognition memory. The test was conducted next day after the completion of MWM. The test apparatus consists of a black wood square box (65 L x 65 B x 45 H cm), opened top with a camera fitted (HD Logitech C525) in the centre of the box. This test was performed in three phases i.e., habituation, familiarization, and recognition phase. Each rat was kept in the centre of a maze devoid of objects during the habituation phase and allowed to explore it for 5 min at 2-time points with a 4 h inter-interval time each day until habituation. Following the 24 h of the habituation, familiarization and recognition phases was carried out as described by Rana et al. (2022a). The object exploration time was recorded to find out the object discrimination ratio as mentioned by Rana et al. (2022a).

Behavioral analysis

The immobility time was calculated to find out the depression-like behavior in rats using the forced swimming test (FST) (Mazumder et al. 2019). The test was carried out the day after the NORT in a 50 cm height Plexiglas cylinder, filled up to 30 cm with clean water (25º C ± 2). A camera (HD Logitech C525) was placed in front of the cylinder, which connected with video tracking software. The event count mode of the software was used to track the amount of time each rat spent immobile after being placed in the centre of a cylinder and given 6 min to swim.

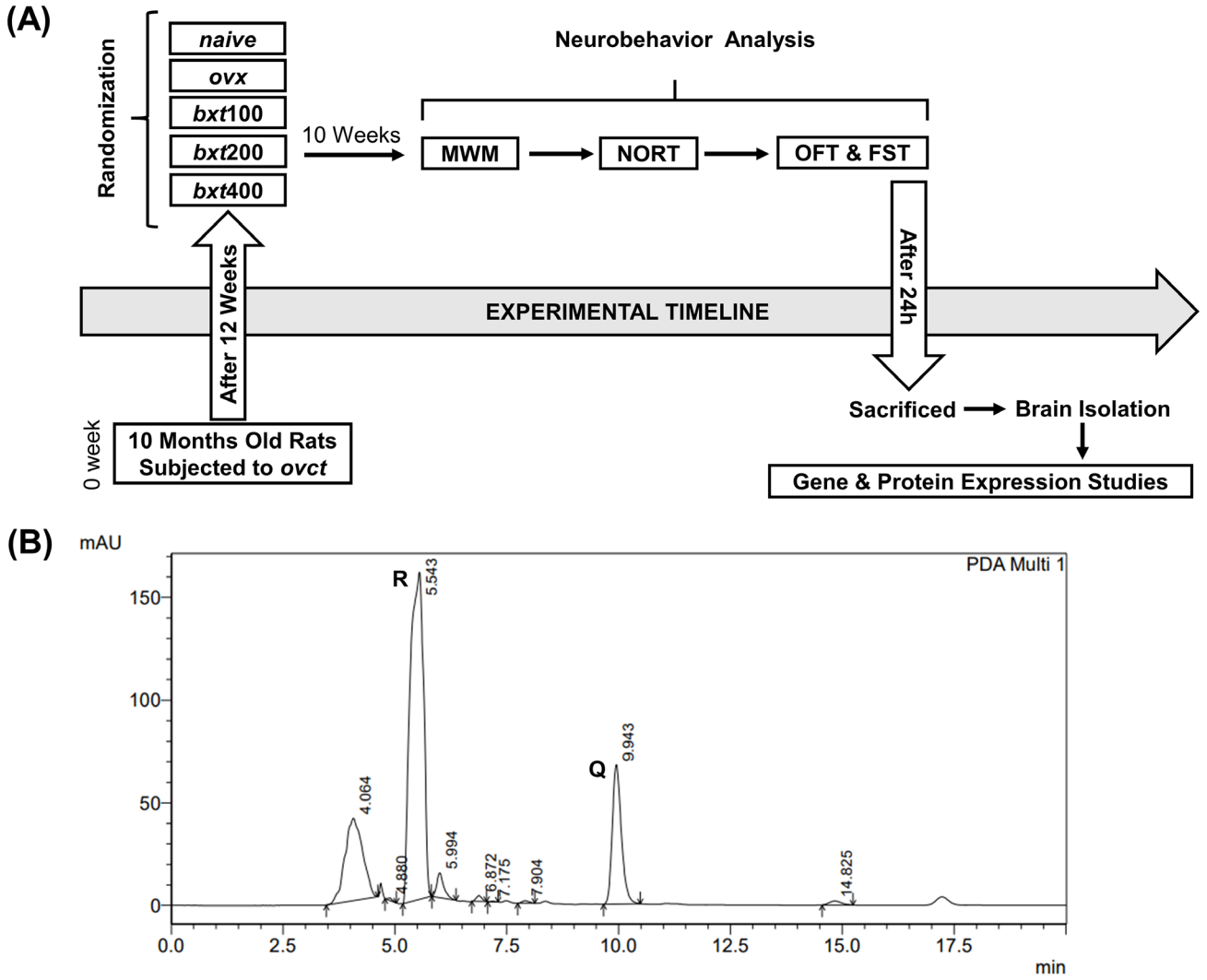

Fig. 1 **(A)** Schematic representation of the experimental timeline. Ovariectomization was performed on the 10-month-old SD female rats and kept further for 12 weeks for the development of the neurobehavioral complications. After 12 weeks, animals were randomly divided into different groups and treated with BXT/vehicle for 10 weeks. Thereafter, cognitive and behavioral analysis were performed, and animals were sacrificed after 24 h for gene and protein expression studies. (**B**) HPLC-PDA chromatograph of BXT showing peaks of rutin (**R**) and quercetin (**Q**) at 5.543 and 9.943 min respectively. ***ovct***: ovariectomy; **MWM**: Morris water maze; **NORT**: Novel object recognition test; **OFT**: Open field test; **FST**: Forced swim test; ***naïve***: Normal control; ***ovx***: Group subjected to ovariectomy and treated with vehicle; ***bxt100***: Group subject to ovariectomy and treated orally with 100 mg/kg dose; ***bxt200***: Group subject to ovariectomy and treated orally with 200 mg/kg dose and; ***bxt400***: Group subject to ovariectomy and treated orally with 400 mg/kg dose

Locomotor activity was assessed 1 h before the FST through an open field test (OFT) to avoid erroneous results. A camera was installed at the top centre of a Plexiglas box (100 L x 100 B x 35 H cm) that had transparent walls with an open top and a grey bottom for the test.

The box was virtually divided into 25 squares (5 × 5) of equal size by using software and allowed the individual rat to explore the box for 5 min. Mean total square entries were calculated for total locomotion.

Protein expression

Total protein from both the isolated brain regions was extracted by radioimmunoprecipitation assay buffer (1:10 w/v), supplemented with protease inhibitor. Total protein concentration was determined by the Bradford assay, and 40 µg protein was further used for expression study. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis was performed to separate the total protein after denaturation in 1X Laemmli buffer and transferred onto 0.45 µm poly (vinylidene fluoride) membrane (Amersham™ Hybond™, GE Healthcare, Germany). Further, to avoid the non-specific

binding of antibodies, the membrane was incubated in 5% non-fat dried milk (Himedia, India) at RT for 1 h. After blocking, the membrane was washed with PBST thrice and probed with primary antibodies [Gsk-3β (1:1500,# MA5-15109, Invitrogen, USA), p-Gsk-3β (Tyr^{216}) (1:1000,# orb5359, Biorbyt, UK) and β-catenin (1:4000,# PA5-19469, Invitrogen, USA) overnight at 4 °C. Following incubation, the membrane was washed with PBST and incubated with a secondary antibody which was conjugated with horseradish peroxidase (1: 10,000) for 3 h at RT. After clearing the membrane with PBST, it was exposed to Clarity ECL-HRP substrate (Bio-Rad Laboratories, Hercules, USA) to visualize bands under Azure cSeries (Azure Biosystem California, USA). The intensity of the bands was analyzed by ImageJ software. β-tubulin (1:2000, # PA5-16863, Thermo Fisher Scientific, USA) was used as a housekeeping protein for normalization as defined earlier (Sharma et al. 2021).

Quantitative real-time PCR analysis (qRT-PCR)

Total RNA was isolated from both the regions of the brain as described by Sharma et al. (2021).

Briefly, the tissue after being homogenized in Trizol reagent (Sigma Aldrich, USA), mixed well with chloroform (0.2mL/mL of Trizol) and then centrifuged at 12,000 g in a pre-cooled centrifuge (Sigma 3–18 K, USA) for 15 min to separate the aqueous layer. Total RNA was precipitated out by mixing the chilled isopropanol (0.5mL/mL of Trizol) with an aqueous layer and centrifuged for 10 min at 12,000 g. The pellet following centrifugation was washed with 75% ethanol (1mL/mL of Trizol), resuspended in nuclease-free water, and finally kept at -20 °C. Nano-Drop™ (Thermo Fisher Scientific, USA) was used to analyze the purity and quantity of the total RNA. The mRNA was reverse transcribed into cDNA by Verso cDNA Synthesis Kit (Thermo Scientific) as per the manufacturer's protocol. The Primer 3.0 software was used for designing the targeted gene primers (Table 1). *Gapdh* was used as a housekeeping control in qRT-PCR analysis. Further, each sample was technically repeated thrice, to minimize any sampling error and the average value was considered in the final interpretation. The relative fold change of each gene in *ovx*, *bxt100*, *bxt200*, and *bxt400*, over the *naive* group, was calculated through 2^ΔΔCT method.

Statistical analysis

The data were represented as a mean ± standard error. The distribution of data was analyzed by the Shapiro-Wilk test. One-way analysis of variance (ANOVA) with Tukey's *post hoc* test was applied for normally distributed data, while the Kruskal-Wallis test along with Mann-Whitney U *post hoc* was performed for parameters with skewed data. Further, Cohen's d was applied to compute the effective size for pairwise comparison, while Eta square (η2) was employed for one-way ANOVA. The effective size for Mann Whitney U test was computed by $r = Z/\sqrt{N}$ (r = correlation coefficient, Z = standardized value for U value, and N = number of samples), while $E_R^2 = \frac{H}{(n2-1)/(n+1)}$, applied for Kruskal-Wallis test. The statistical significance of the data was considered at $P < 0.05$.

Results

Extraction and quantification

The hydroalcoholic extraction of *F. tataricum* seed powder yielded 8.5% w/w of dried extract. HPLC analysis of the extract showed rutin and quercetin to be the major metabolites present in the extract. The calibration curves of rutin ($y = 13641x + 110{,}171$; $R^2 = 0.9675$) and quercetin ($y = 36790x - 146{,}054$; $R^2 = 0.9852$) were made using reference standards. The quantity of rutin and quercetin was found to be 2.0 ± 0.01 and 0.25 ± 0.0007% w/w of the seed powder, respectively (Fig. 1B).

Effect of BXT on the uterine weight

There was significant reduction in the uterus weight/body weight detected in the *ovx* group ($F_{(4,20)} = 15.239$, $P < 0.001$, $\eta 2 = 0.753$), in relation to the *naive* group ($P < 0.001$, $d = 2.553$). However, no statistical change in uterine weight in *bxt100* ($P = 1$, $d = 0.516$), *bxt200* ($P = 0.999$, $d = 0.481$)

Table 1 Primer sequence of the genes used for qRT-PCR

Genes	Forward primer (5' → 3')	Reverse primer (5' → 3')
Tjp1	AAAAGGACGTTTATCGCCGCATT	TCCCCTCAGAGACCCACACCAG
Ocln	TTGCTTCATCGCTTCCTTG	TCCATCTTTCTTCGGGTTTT
Pparg	CATGACCAGGGAGTTCCTCAA	AGCAAACTCAAACTTAGGCTCCAT
Gfap	GAAATGGAGCTGAAAGTGTC	AAGGTTAGCAGAGGTGACAA
Il2	CAGTGACGCTTGTCCTCCTT	AGCACCTGTAAGTCCAGCAA
Il1b	CACCTTCTTTTCCTTCATCTTTG	GTCGTTGCTTGTCTCTCCTTGTA
Nfe2l2	GGAAACCTTACTCTCCCAGT	AGCCGTACAAAACAAACACT
Nqo1	TCCATCCTGGTTTCAATTAC	TTGTATTCATTTCCCAGGAC
Gapdh	GGTGGACCTCATGGCCTACA	CAGCAACTGAGGGCCTCTCT

and *bxt400* (P = 1, d = 0.080) groups was observed when compared to the *ovx* group. The *naïve* group showed the significant increase in the same as compared to the *bxt100* (P < 0.001, d = 2.629), *bxt200* (P < 0.001, d = 2.399) and *bxt400* (P < 0.001, d = 2.569) groups.

Effect of BXT on cognitive functions in *ovct* rats

In MWM, during the probe trial, *ovx* group ($\chi^2_{(4)} = 12.097$, P = 0.007, E^2_R = 0.587) showed the significant alternation in the memory, as indicated by the less time spent in the targeted zone than the *naive* group (P = 0.009, r = 0.825). While, *F. tataricum* seed extract improved altered spatial memory at all the doses i.e., *bxt100* (P = 0.047, r = 0.627), *bxt200* (P = 0.028, r = 0.693) and *bxt400* (P = 0.047, r = 0.627) indicated by more time spent in the targeted zone compared to the *naive* group. Chronic treatment of *F. tataricum* seed extract at all the groups *i.e., bxt100* (P = 0.032, r = 0.693), *bxt200* (P = 0.032, r = 0.693) and *bxt400* (P = 0.032, r = 0.693) helps to overcome from the altered spatial memory, but still significant difference was observed for the same compared to *naive*$^-$ group. However, an insignificant difference in the spatial memory index was observed when a comparison was done between all the treatment groups (Fig. 2A).

Similar to the spatial memory, the *ovx* group also showed significant alteration ($F_{(4,20)} = 9.747$, P < 0.001, η2 = 0.660) in the episodic memory as indicated by a decrease in discrimination ratio in relation to the *naive* group (P = 0.001, d = 4.057). However, chronic treatment of *F. tataricum* seed extract at all doses i.e., *bxt100* (P = 0.003, d = 2.755), *bxt200* (P < 0.001, d = 3.055) and *bxt400* (P < 0.001, d = 3.053) helps to overcome the altered episodic memory suggested by an increased discrimination ratio compared to *ovx* group. However, no change in the discrimination ratio was observed among different *F. tataricum* seeds extract treated groups (Fig. 2B).

Effect of BXT on depression-like behavior in *ovct* rats

Initially, locomotion was studied in all the rats to avoid the false results of depression-like behavior by calculating the total mean square entries in the OFT (Fig. 2C). There was no significant alteration in the total mean square entries observed in any of the experimental group ($F_{(4,20)} = 1.213$,

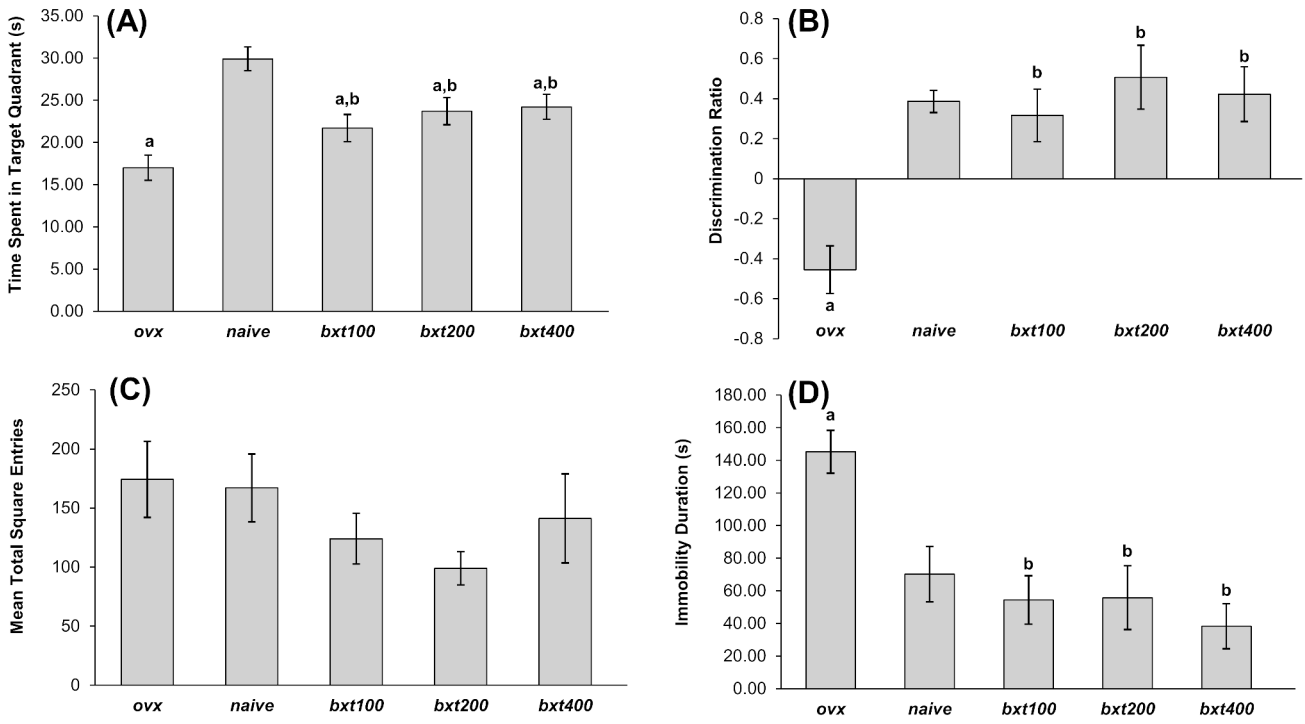

Fig. 2 Effect of BXT on the neurobehavior alternation in *ovct* rats. (**A**) Reconsolidation memory analysis in the MWM by calculating the time spent in a targeted zone on day 5th; (**B**) Recognition memory analysis by evaluating the discrimination ratio in NORT; (**C**) Total locomotion analysis by OFT and; (**D**) Depression-like behavior analysis by calculating the immobility duration in the FST. [a]P < 0.05 as compared to *naïve*, [b]P < 0.05 as compared to *ovx*. ***naïve***: Normal control; ***ovx***: Group subjected to ovariectomy and treated with vehicle; ***bxt100***: Group subject to ovariectomy and treated orally with 100 mg/kg dose; ***bxt200***: Group subject to ovariectomy and treated orally with 200 mg/kg dose; ***bxt400***: Group subject to ovariectomy and treated orally with 400 mg/kg dose; **MWM**: Morris water maze; **NORT**: Novel object recognition test; **OFT**: Open field test; **FST**: Forced swim test; and **s**: Seconds

P = 0.337, η2 = 0.195). A remarkable increase in the immobility time was detected in the *ovx* group ($F_{(4,20)} = 7.067$, P = 0.001, η2 = 0.585) in comparison to the *naive* group (P = 0.023, d = 2.214), thus suggested depression-like behavior. However, treatment with *F. tataricum* seed extract at all the tested doses in *bxt100* (P = 0.005, d = 2.909), *bxt200* (P = 0.006, d = 2.401) and *bxt400* (P = 0.001, d = 3.555) groups helped to overcome from the depression-like behavior compared with the *ovx* group. There was no statistical difference in immobility time observed when a comparison done between the treatment groups (Fig. 2D).

Effect of BXT on Gsk-3β activity in the discrete brain regions of *ovct* rats

There was no change in the Gsk-3β expression ($\chi^2_{(4)} = 0.923$, P = 0.921, $E^2_R = 0.038$), observed in the cortex region of the brain in any of the experimental groups (Fig. 3A&B). However, there was a marked upregulation in the expression of p-Gsk-3β (Tyr^{216}) in the cortex of *ovx* group ($F_{(4,20)} = 22.313$, P < 0.001, η2 = 0.816) as compared to the *naive* group (P < 0.001, d = 4.165). *F. tataricum* seed extract treatment at all the doses in *bxt100* (P < 0.001, d = 4.008), *bxt200* (P < 0.001, d = 3.899) and *bxt400* (P < 0.001, d = 3.918) groups showed a remarked downregulation of p-Gsk-3β (Tyr^{216}) in the cortical region compared to the *ovx* group.

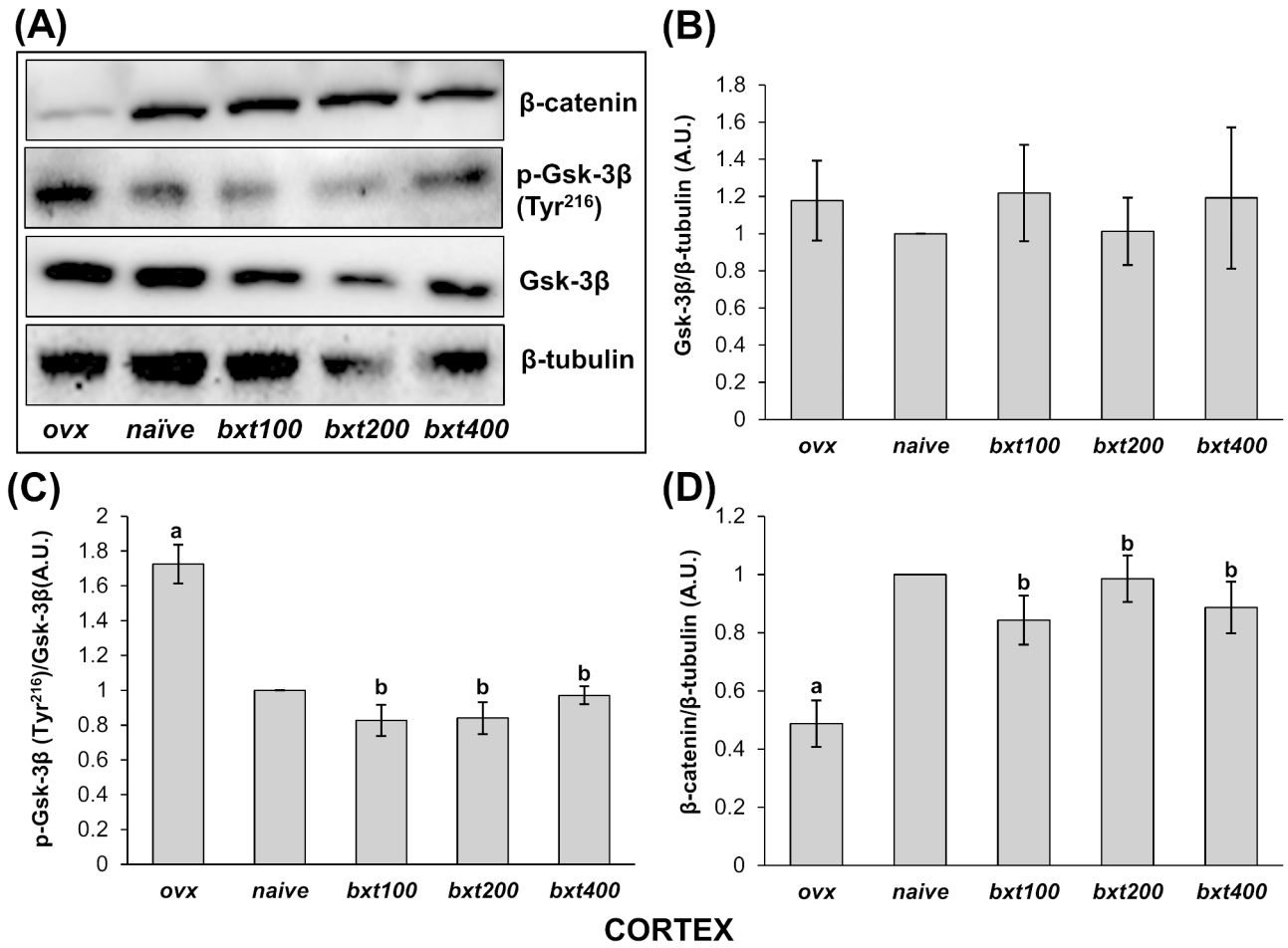

Fig. 3 Effect of BXT on the protein expression of cortex region in *ovct* rats. (**A**) Representative western blotting image of Gsk-3β, p-Gsk-3β (Tyr^{216}), β-catenin, and β-tubulin; (**B & D**) Graphic representation of relative fold change of Gsk-3β and β-catenin in the *ovx*, *bxt100, bxt200*, and *bxt 400* in relation to naïve control. The Gsk-3β and β-catenin were normalized with β-tubulin; (**C**) Graphic representation of relative fold change of p-Gsk-3β (Tyr^{216}) in the *ovx*, *bxt100, bxt200*, and *bxt 400* in relation to naïve control. The p-Gsk-3β (Tyr^{216}) was normalized with Gsk-3β. [a]P < 0.05 as compared to *naïve*, [b]P < 0.05 as compared to *ovx*. ***naïve***: Normal control; ***ovx***: Group subjected to ovariectomy and treated with vehicle; ***bxt100***: Group subject to ovariectomy and treated orally with 100 mg/kg dose; ***bxt200***: Group subject to ovariectomy and treated orally with 200 mg/kg dose; ***bxt400***: Group subject to ovariectomy and treated orally with 400 mg/kg dose; **β-catenin**: Beta-catenin; **Gsk-3β**: Glycogen synthase kinase 3 beta; **p-Gsk-3β (Tyr^{216})**: Phosphorylated glycogen synthase kinase at tyrosine 216 amino acid; **β-tubulin**: Beta tubulin and; **A.U.**: Arbitrary unit

There was no change in the level of p-Gsk-3β (Tyr^{216}) in all the treatment groups (Fig. 3A&C).

Similar to that of the cortical region, there was no change in the expression of Gsk-3β in the hippocampus (Fig. 4A&B) among all the experimental groups ($\chi^2_{(4)} = 5.306$, P = 0.257, $E^2_R = 0.221$). The *ovx* group ($F_{(4,20)} = 15.494$, P < 0.001, η2 = 0.755) showed the significant upregulation in the expression of p-Gsk-3β (Tyr^{216}) in the hippocampus region as compared to the *naive* group (P < 0.001, d = 3.446). However, treatment with *F. tataricum* seed extract at all the doses in *bxt100* (P < 0.001, d = 4.406), *bxt200* (P < 0.001, d = 4.333) and *bxt400* (P < 0.001, d = 2.166) groups significantly decreased the expression of p-Gsk-3β (Tyr^{216}) compared to *ovx* group. There was no change in the expression of p-Gsk-3β (Tyr^{216}) between the treatment groups (Fig. 4A&C).

Effect of BXT on β-catenin expression in the discrete brain regions of *ovct* rats

β-catenin is the downstream target of Gsk-3β, thus any change in the action of Gsk-3β is intended to hamper their downstream signalling protein. There was a notable down-regulation in the expression of β-catenin observed in the cortex region of the brain in the *ovx* group ($F_{(4,20)} = 7.853$, P < 0.001, η2 = 0.610) compared to the *naive* (P < 0.001, d = 4.075). Treatment with the *F. tataricum* seed extract at 100 mg/kg (P = 0.022, d = 1.944), 200 mg/kg (P = 0.001,

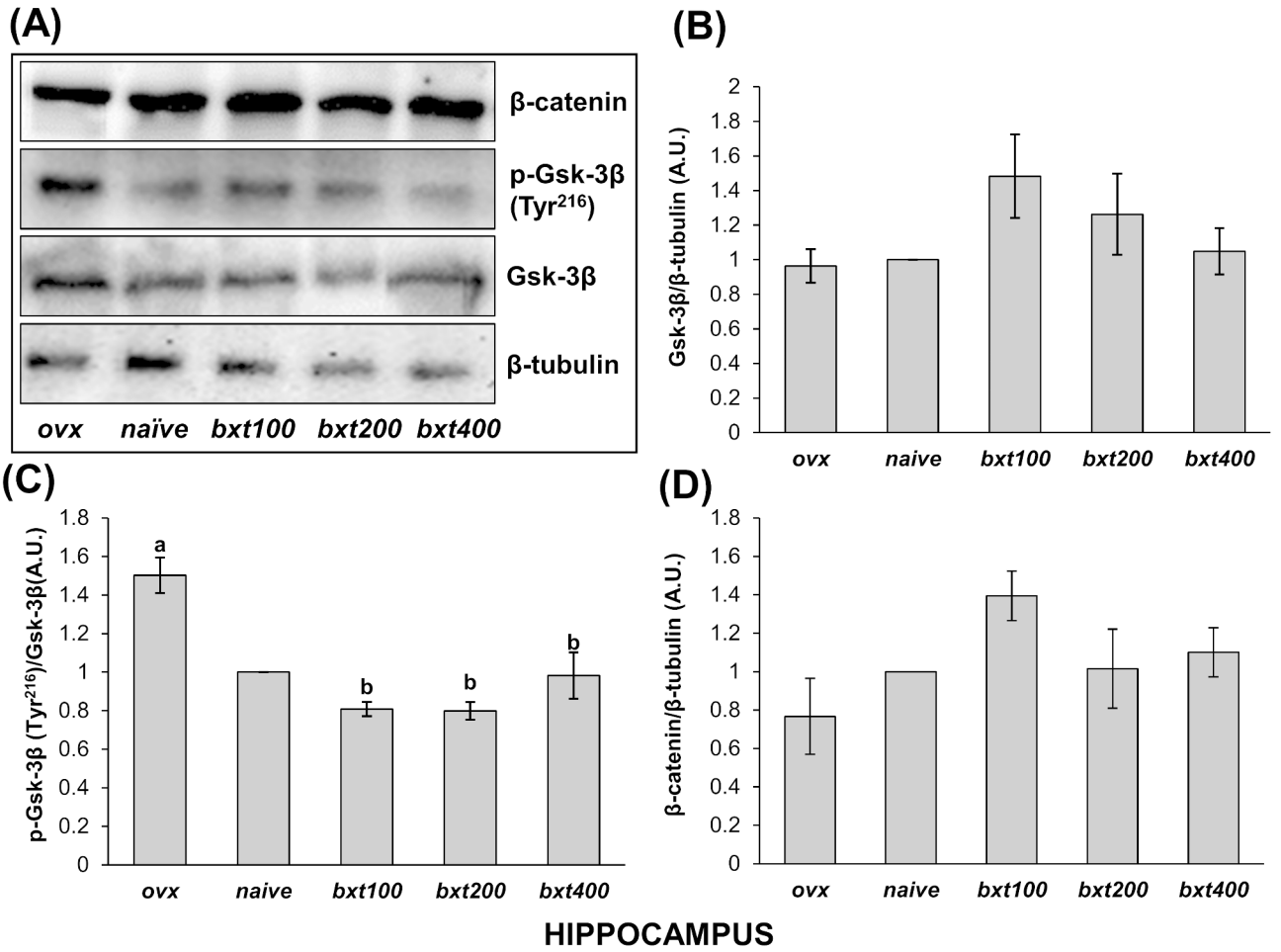

Fig. 4 Effect of BXT on the protein expression of hippocampus region in *ovct* rats (**A**) Representative western blotting image of Gsk-3β, p-Gsk-3β (Tyr^{216}), β-catenin, and β-tubulin (**B & D**) Graphic representation of relative fold change of Gsk-3β and β-catenin in the *ovx*, *bxt100, bxt200*, and *bxt 400* in relation to naïve control. The Gsk-3β and β-catenin were normalized with β-tubulin; (**C**) Graphic representation of relative fold change of p-Gsk-3β (Tyr^{216}) in the *ovx*, *bxt100, bxt200*, and *bxt 400* in relation to naïve control. The p-Gsk-3β (Tyr^{216}) was normalized with Gsk-3β. [a]P < 0.05 as compared to *naïve*, [b]P < 0.05 as compared to *ovx*. ***naïve***: Normal control; ***ovx***: Group subjected to ovariectomy and treated with vehicle; ***bxt100***: Group subject to ovariectomy and treated orally with 100 mg/kg dose; ***bxt200***: Group subject to ovariectomy and treated orally with 200 mg/kg dose; ***bxt400***: Group subject to ovariectomy and treated orally with 400 mg/kg dose; **β-catenin**: Beta-catenin; **Gsk-3β**: Glycogen synthase kinase 3 beta; **p-Gsk-3β (Tyr^{216})**: Phosphorylated glycogen synthase kinase at tyrosine 216 amino acid; **β-tubulin**: Beta tubulin and; **A.U.**: Arbitrary unit

d = 2.797) and 400 mg/kg (P = 0.009, d = 2.119) significantly upregulated the expression compared to the *ovx* group, thus suggested an inactivation of Gsk-3β. Further analysis revealed an insignificant change in the expression of β-catenin among all the extract-treated groups (Fig. 3A&D). Interestingly, insignificant change ($\chi^2_{(4)} = 6.192$, P = 0.185, E^2_R = 0.258) in the expression of β-catenin was detected in the hippocampus among all the experimental groups (Fig. 4A&D).

Effect of BXT on the expression of oxidative stress-related genes

Nfe2l2 acts as a major transcription factor for the gene which possesses the anti-oxidant response elements in the promoter region, hence its expression was studied. A significant downregulation in the expression of *Nfe2l2* was noticed in the cortex region of *ovx* group ($\chi^2_{(4)} = 13.001$, P = 0.011, E^2_R = 0.541) compared to the *naive* (P = 0.005, r = 0.880). Treatment of the extract in *bxt100* (P = 0.009, r = 0.825), *bxt200* (P = 0.016, r = 0.759) and *bxt400* (P = 0.009, r = 0.825) showed a marked upregulation of the *Nfe2l2* expression compared to the *ovx* group. Treatment helps to maintain the normal transcription level of the *Nfe2l2*, indicated by insignificant change among the extract treated groups and the *naïve* (Fig. 5).

The hippocampus region also showed a considerable downregulation of the *Nfe2l2* expression in the *ovx* group ($\chi^2_{(4)} = 17.125$, P = 0.002, E^2_R = 0.713) compared to *naive* group (P = 0.005, r = 0.880). However, a notable upregulation in *Nfe2l2* expression was observed following treatment of *F. tataricum* seed extract in *bxt100* (P = 0.009, r = 0.825), *bxt200* (P = 0.016, r = 0.825) and *bxt400* (P = 0.009, r = 0.825) groups in relation to the *ovx* group. However, there was no alternation in the hippocampal expression of *Nfe2l2* detected when a comparison was done between the extract-treated groups (Fig. 6).

Further the expression of anti-oxidant response element containing gene *i.e., Nqo1* was also studied. There was a marked downregulation in *Nqo1* expression in the cortical region of *ovx* group ($\chi^2_{(4)} = 21.559$, P < 0.0001, E^2_R = 0.898) observed when compared with the *naive* group (P = 0.005, r = 0.880). However, treatment of *F. tataricum* seed extract helped to overwhelmed the oxidative stress *via* upregulating the expression of *Nqo1* in *bxt200* (P = 0.009, r = 0.825) and *bxt400* (P = 0.009, r = 0.825) groups compared to *ovx* in the cortex. However, no alternation in the cortical *Nqo1* expression was detected in *bxt100* (P = 0.076, r = 0.561) group compared to the *ovx* group. The cortical expression of *Nqo1* was also significantly upregulated in *bxt200* (P = 0.005, r = 0.880) and *bxt400* (P = 0.005, r = 0.880), while it downregulated in the *bxt100* (P = 0.005, r = 0.880) group as compared to *naive* group. A significant upregulation in the

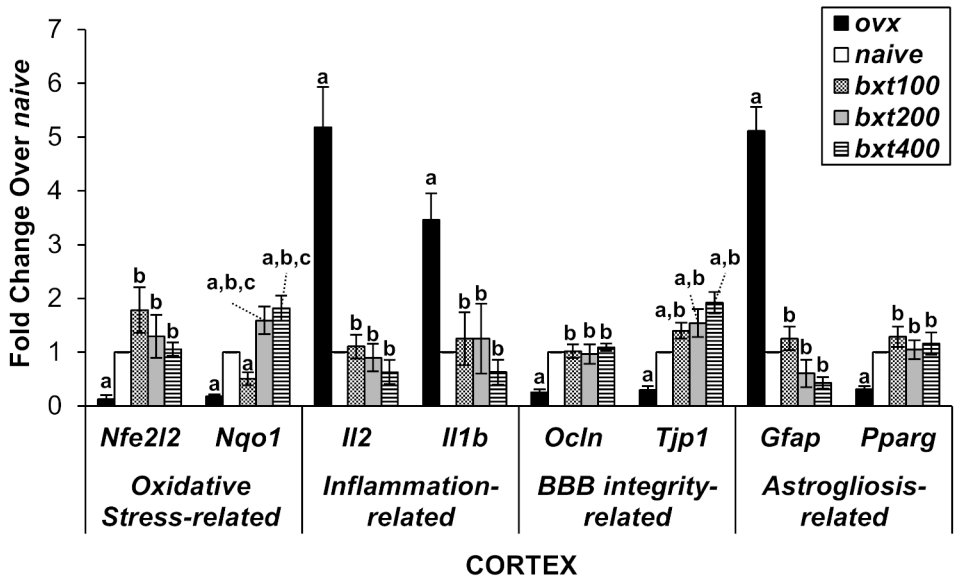

Fig. 5 Effect of BXT on the mRNA expression of oxidative stress-related (*Nfe2l2* & *Nqo1*), Inflammation-related (*Il2* & *Il1b*), BBB integrity-related (*Ocln* & *Tjp1*) and Astrogliosis-related (*Gfap* & *Pparg*) genes in the cortex region of *ovct* rats. [a]P < 0.05 as compared to *naïve*, [b]P < 0.05 as compared to *ovx*, [c]P < 0.05 as compared to *bxt100*. ***naïve***: Normal control; ***ovx***: Group subjected to ovariectomy and treated with vehicle; ***bxt100***: Group subject to ovariectomy and treated orally with 100 mg/kg dose; ***bxt200***: Group subject to ovariectomy and treated orally with 200 mg/kg dose; ***bxt400***: Group subject to ovariectomy and treated orally with 400 mg/kg dose; ***Nfe2l2***: Nuclear factor erythroid 2-related factor 2; ***Nqo1***: NAD(P)H Quinone Dehydrogenase 1; ***Il2***: Interleukin 2; ***Il1b***: Interleukin one beta; ***Ocln***: Occludin; ***Tjp1***: Tight junction protein 1; ***Gfap***: Glial fibrillary acidic protein and; ***Pparg***: Peroxisome proliferator-activated receptor gamma

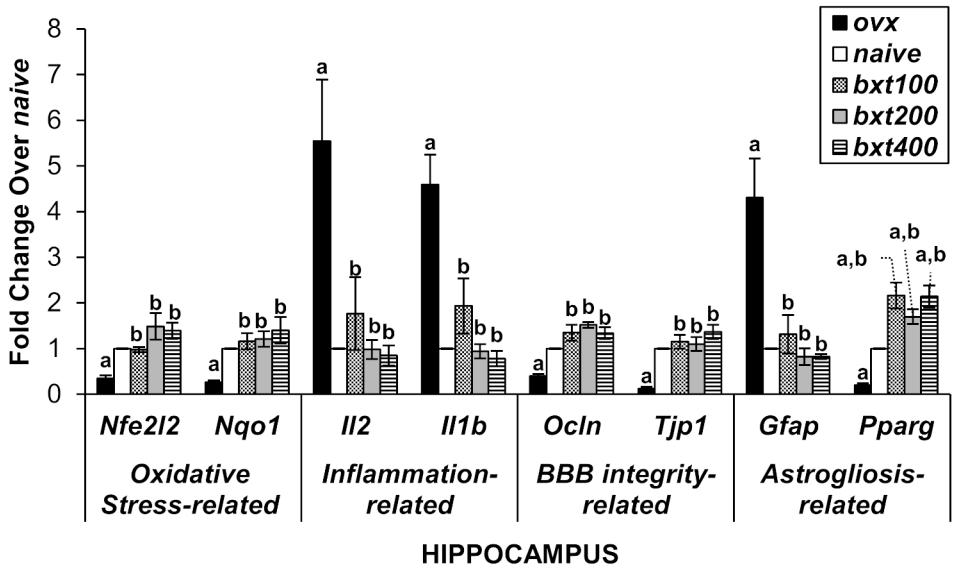

Fig. 6 Effect of BXT on the mRNA expression of oxidative stress-related (*Nfe2l2* & *Nqo1*), Inflammation-related (*Il2* & *Il1b*), BBB integrity-related (*Ocln* & *Tjp1*), and Astrogliosis-related (*Gfap* & *Pparg*) genes in the hippocampus region of *ovct* rats. [a]P < 0.05 as compared to *naïve*, [b]P < 0.05 as compared to *ovx*. ***naïve***: Normal control; ***ovx***: Group subjected to ovariectomy and treated with vehicle; ***bxt100***: Group subject to ovariectomy and treated orally with 100 mg/kg dose; ***bxt200***: Group subject to ovariectomy and treated orally with 200 mg/kg dose; ***bxt400***: Group subject to ovariectomy and treated orally with 400 mg/kg dose; ***Nfe2l2***: Nuclear factor erythroid 2-related factor 2; ***Nqo1***: NAD(P)H quinone dehydrogenase 1; ***Il2***: Interleukin 2; ***Il1b***: Interleukin one beta; ***Ocln***: Occludin; ***Tjp1***: Tight junction protein 1; ***Gfap***: Glial fibrillary acidic protein and; ***Pparg***: Peroxisome proliferator-activated receptor gamma

cortical *Nqo1* expression was detected in the *bxt200* (P = 0.009, r = 0.825), *bxt400* (P = 0.009, r = 0.825) group when compared with the *bxt100* group. There was no statistical change observed in the expression of *Nqo1* in the cortex when a comparison was done between *bxt200* and *bxt400* groups (Fig. 5).

Similarly, to the cortex, the hippocampus region of *ovx* group ($F_{(4,20)} = 6.653$, P = 0.001, η2 = 0.570) also showed the significant downregulation of *Nqo1* expression as compared to the *naive* group (P = 0.044, d = 11.403). However, groups treated with *F. tataricum* seed extract including, *bxt100* (P = 0.011, d = 3.130), *bxt200* (P = 0.007, d = 3.477) and *bxt400* (P = 0.001, d = 2.452) showed a notable upregulation in the *Nqo1* expression as compared with *ovx* group. However, no change in the expression of *Nqo1* among different treated groups was observed in the hippocampus (Fig. 6).

Effect of BXT on the expression of inflammation-related genes

A remarkable upregulation in *Il2* expression was observed in the cortex region of *ovx* group ($F_{(4,20)} = 25.219$, P < 0.001, η2 = 0.843) when compared with the *naive* group (P = 0.001, d = 3.515). While treatment with the *F. tataricum* seed extract reduced the cortical *Il2* expression in *bxt100* (P < 0.009, d = 3.287), *bxt200* (P < 0.016, d = 3.410) and *bxt400* (P < 0.009, d = 3.668) groups in relation to *ovx* group (Fig. 5). Similarly, the hippocampal region (Fig. 6) also showed a significant upregulation in the expression of *Il2* in the *ovx* group ($F_{(4,20)} = 7.927$, P < 0.001, η2 = 0.613), which was reduced in the extract treated groups i.e., *bxt100* (P = 0.01, d = 1.533), *bxt200* (P = 0.002, d = 2.131) and *bxt400* (P = 0.001, d = 2.189). The *naive* group showed significant downregulation in *Il2* expression (P = 0.002, d = 2.148), as compared to *ovx* group.

Apart from *Il2*, the cortex region also a showed remarkable upregulation in the *Il1b* expression in the *ovx* group ($\chi^2_{(4)} = 10.441$, P = 0.034, $E^2_R = 0.435$) as compared to the *naive* group (P = 0.005, r = 0.880). A notable downregulation in the cortical expression of *Il1b* was detected in *bxt100* (P = 0.016, r = 0.759), *bxt200* (P = 0.047, r = 0.627) and *bxt400* (P = 0.009, r = 0.825) groups compared with the *ovx* group (Fig. 5). The hippocampal region also showed significant downregulation of *Il1b* expression in *F. tataricum* seed extract treated *bxt100* (P = 0.002, d = 1.880), *bxt200* (P < 0.001, d = 3.412) and *bxt400* (P < 0.001, d = 3.556) groups compared to *ovx* group ($F_{(4,20)} = 15.021$, P < 0.001, η2 = 0.750). However, there was a remarkable upregulation in the hippocampal *Il1b* expression detected in the *ovx* group (P < 0.001, d = 3.455) in relation to the *naive* group (Fig. 6).

There was an insignificant alteration in the expression of *Il1b* observed when a comparison was made between the extract-treated groups in both studied brain regions (Fig. 5&6). The results suggested that sexual hormone deprivation after menopause triggers the neuroinflammatory events, which were reverted by the seed extract following the critical window period.

Effect of BXT on the expression of BBB integrity-related genes

An alteration in the integrity of the BBB disturbs its selective permeability in various neurodegenerative conditions. Sexual hormone deprivation following experimental menopause has also been reported to interfere with the transcription process of junction complex-associated genes (Rana et al. 2022a). The cortical region showed a remarkable reduction in the expression of *Ocln* in the *ovx* group ($F_{(4,20)} = 10.647$, $P < 0.001$, $\eta 2 = 0.680$) as compared with the *naive* group ($P < 0.001$, $d = 9.565$). However, treatment with the seed extract significantly upregulated the cortical *Ocln* expression in *bxt100* ($P < 0.001$, $d = 3.516$), *bxt200* ($P < 0.001$, $d = 2.410$) and *bxt400* ($P = 0.001$, $d = 6.205$) groups compared to the *ovx* group (Fig. 5). The hippocampus region also showed a notable upregulation in *Ocln* expression in the extract-treated *bxt100* ($P < 0.001$, $d = 3.354$), *bxt200* ($P < 0.001$, $d = 8.815$) and *bxt400* ($P < 0.001$, $d = 4.262$) groups compared to the *ovx* group ($F_{(4,20)} = 18.573$, $P < 0.001$, $\eta 2 = 0.787$). There was a remarkable ($P = 0.004$, $d = 7.896$) downregulation in the hippocampal *Ocln* expression detected in *ovx* group in relation to the *naive* group (Fig. 6).

Furthermore, the cortical expression of *Tjp1* was also significantly downregulated in *ovx* group ($\chi^2_{(4)} = 19.699$, $P = 0.001$, $E^2_R = 0.820$) when compared with the *naïve* group ($P = 0.005$, $r = 0.880$). *F. tataricum* seed extract-treated *bxt100* ($P = 0.009$, $r = 0.825$), *bxt200* ($P = 0.009$, $r = 0.825$) and *bxt400* ($P = 0.009$, $r = 0.825$) groups, showed remarkable upregulation in the cortical expression of *Tjp1* compared with *ovx* group (Fig. 5). There was notable upregulation in *Tjp1* cortical expression observed when comparison done between *bxt100*, *bxt200* and *bxt400* groups with *naive* group ($P = 0.005$, $r = 0.880$). The hippocampus region (Fig. 6) also showed a significant upregulation of *Tjp1* expression in *bxt100* ($P < 0.001$, $d = 4.120$), *bxt200* ($P < 0.001$, $d = 3.929$) and *bxt400* ($P = 0.001$, $d = 4.857$) groups compared to *ovx* group ($F_{(4,20)} = 16.015$, $P < 0.001$, $\eta 2 = 0.762$). There was a marked downregulating in the hippocampal expression of *Tjp1* in *ovx* group compared to the *naive* group ($P < 0.001$, $d = 12.671$).

Effect of BXT on the expression of reactive astrogliosis-related genes

GFAP is the marker of reactive astrogliosis, which has been reported to be upregulated during different neurodegenerative conditions and promote the linked disease progression. Similarly, in the cortex region, *Gfap* expression was notable upregulated in the *ovx* group ($F_{(4,20)} = 58.47$, $P < 0.001$, $\eta 2 = 0.921$) as compared to the *naive* group ($P < 0.001$, $d = 5.842$). However, treatment with *F. tataricum* seed extract in *bxt100* ($P < 0.001$, $d = 4.915$), *bxt200* ($P < 0.001$, $d = 5.550$) and *bxt400* ($P < 0.001$, $d = 6.460$) groups showed remarkable reduction in the cortical *Gfap* expression as compared to *ovx* group (Fig. 5). Similarly, the hippocampus region of *ovx* group also showed a notable upregulation in the expression of *Gfap* ($F_{(4,20)} = 11.75$, $P < 0.001$, $\eta 2 = 701$) in relation to *naive* group ($P < 0.001$, $d = 2.437$). A remarkable downregulation in the hippocampal expression of *Gfap* observed in *F. tataricum* seed extract treated *bxt100* ($P < 0.001$, $d = 1.973$), *bxt200* ($P < 0.001$, $d = 2.514$) and *bxt400* ($P < 0.001$, $d = 2.561$) groups when compared with the *ovx* group. There was insignificant alternation in *Gfap* expression in the hippocampus among extract-treated groups (Fig. 6).

Further, the expression of *Pparg* in the isolated brain regions was also studied as its downregulation is known to promote the reactive astrogliosis process. A notable downregulation in the expression of *Pparg* was detected in the cortex of *ovx* group ($F_{(4,20)} = 6.546$, $P = 0.002$, $\eta 2 = 0.563$) compared with the *naive* group ($P = 0.029$, $d = 7.795$). However, *F. tataricum* seeds extract treated *bxt100* ($P = 0.002$, $d = 3.146$), *bxt200* ($P = 0.018$, $d = 2.523$) and *bxt400* ($P = 0.006$, $d = 2.518$) groups showed a notable upregulation in the cortical expression of *Pparg* in relation to the *naive* group (Fig. 5). Similarly, the expression of *Pparg* was significantly downregulated in the hippocampus region of the *ovx* group ($F_{(4,20)} = 23.633$, $P < 0.001$, $\eta 2 = 0.825$) as compared to *naïve* group ($P = 0.036$, $d = 11.849$). *Pparg* expression in the hippocampus was significantly upregulated in *bxt100* ($P < 0.001$, $d = 4.350$), *bxt200* ($P < 0.001$, $d = 7.596$) and *bxt400* ($P < 0.001$, $d = 4.984$) groups compared to *ovx* group (Fig. 6). A remarkable upregulation in the expression of *Pparg* was detected in the *bxt100* ($P < 0.001$, $d = 2.610$), *bxt200* ($P < 0.001$, $d = 4.406$) and *bxt400* ($P < 0.001$, $d = 2.98$) groups compared to *naïve* group.

Discussion

Estrogen deprivation following menopause is associated with neurobehavioral impairment. The present study examined the protective effect of an extract from *F. tataricum*

seeds following a critical therapeutic period against the neurobehavioral alteration in a rat model of experimental menopause. *F. tataricum* seed extract suppressed oxidative stress, neuroinflammation and maintained the integrity of the BBB to combat the neurobehavioral alteration.

In middle life, every woman experiences several dramatic alterations in the endocrine system due to menopause, which is characterized by a decrease in the level of sexual hormones (Gava et al. 2019). Rather than restricted to reproductive functions, these hormones play a vital role in several neurological events such as maintenance of BBB integrity, synaptic plasticity, regulation of inflammation (Rana et al. 2022a), and mitochondrial tasks (Aggarwal et al. 2020). The available preclinical and clinical data suggested an elevated risk of neurobehavioral complications in women after menopause (Aggarwal et al. 2020; Rana et al. 2020). Estrogen insufficiency after menopause causes uncertainty in the neurotransmitters system, which exposes women to depressive disorders (Newhouse et al. 2008). At the preclinical level, an FST test was performed to access depression-like behavior. In line with the previous finding (Rana et al. 2020), FST showed a significant increase in the immobility period in the *ovx* group, which suggested altered behavior. Estrogen deficiency has been reported to impact directly the cholinergic system of the basal forebrain, bioenergetic mitochondrial system, and the dopaminergic system which is majorly engaged in the cognitive process (Conde et al. 2021). Estrogen deprivation also altered the structural synaptic plasticity and dendritic spine number of pyramidal neurons in the discrete regions of the brain, resulting in cognitive process alternation (Hara et al. 2015; Rana et al. 2022a). In line with the literature, cognitive batteries such as MWM and NORT in the current study suggested impairment in the cognitive process may be because of altered structural synaptic plasticity.

Sexual hormones are pleiotropic and also possess antioxidant properties (Torrens-Mas et al. 2020), due to the initiation of the transcription of stress response genes to overcome oxidative stress (Borras et al. 2021). These protective mechanisms are lost or reduced after menopause, resulting in an imbalance in redox homeostasis and the initiation of oxidative stress (Rana et al. 2020). Among all systems of the body, the nervous system is more susceptible to elevated stress due to its lipophilic nature, high energy demand, and less antioxidant capacity (Singh et al. 2019). Moreover, a decline in the systemic level of estrogen impairs neuronal glucose metabolism, creates a hypometabolic condition, alters mitochondrial function, and enhances oxidative stress (Ding et al. 2013). Elevated stress triggers neurobehavior alterations in women after menopause (Ding et al. 2013). In the present study, expression of the studied antioxidant genes, *Hmox1* and *Nqo1* was altered in both the regions of *ovct* rats, suggesting the elevation of oxidative stress. Both the antioxidant markers protect the cells from oxidative stress by showing their superoxide scavenging activity (Drolet et al. 2021; Rana et al. 2021), thus any alteration in their regulation enhances the risk of destructive cascades in the cell.

Elevated oxidative stress and deficiency of sexual hormones collectively trigger inflammatory cascades toward the progression of neurodegenerative events. The expression of pro-inflammatory markers such as *Il6*, *Il2*, and *Il1b* was reported to be increased in the cortex and hippocampus regions of the *ovct* rats (Rana et al. 2020, 2022a). The elevated level of stressors in the brain triggers glucocorticoid synthesis by stimulating the hypothalamus-pituitary axis (Kasahara and Inoue 2015), which eventually leads to neurodegenerative outcomes. Similarly, in the current study, upregulation of pro-inflammatory cytokines in both the regions of the *ovct* rat brain may be responsible for the development of neurobehavioral alterations. Due to the critical therapeutic period and/or associated side effects of HRT, its use is always restricted. So, *F. tataricum* seed extract as an alternative therapy was selected over HRT in the current study to investigate the therapeutic potential against neurobehavioral complications after menopause.

All the species of the *Fagopyrum* contain an appreciable content of flavonoids like rutin, quercetin, vitexin, isovitexin, orientin, isoorientin, and many more. In line with the previous studies (Zhu 2016; Zou et al. 2021), our results also showed the presence of rutin and quercetin as major flavonoids. Numerous studies suggested the neuroprotective effect of flavonoids against neurodegenerative conditions *via* suppression of oxidative stress, neuroinflammation, and apoptosis (De Andrade Teles et al. 2018; Maher 2019). *In vitro* and *in vivo* studies also proved the low toxicity effect and cytostatic proprieties of the flavonoids, making them a viable candidate for alleviating the menopause-associated neurobehavioral alteration without posing any additional health risks (Amer et al. 2012). Flavonoids contain phenolic rings in the structure, which prevents the cell from auto-oxidation either by inhibiting reactive oxygen species formation or interrupting the propagation of free radical reactions (Tan et al. 2018). Moreover, flavonoids were also reported to initiate the transcription of oxidative stress response genes *via* activating or maintaining a normal pool of Nrf2 in the cell (Bakoyiannis et al. 2019). A preclinical study in the rat model of Alzheimer's disease showed improvement in cognitive impairment following *F. tataricum* extract *via* suppressing the elevated level of oxidative stress (Choi et al. 2013), suggesting the anti-oxidative nature of the extract. Similarly, normalization of *Hmox1* and *Nqo1* was also observed following the treatment of BXT, which suggested

the maintenance of cellular homeostasis by suppressing oxidative stress.

Rather than an antioxidant, BXT also showed anti-inflammatory activity, suggested by decreased genomic expression of inflammation-related genes. Chronic treatment of flavonoids had earlier been reported to suppress inflammatory events by inhibiting the various pro-inflammatory enzymes such as inducible nitric oxide synthase, cyclooxygenase-2, lipoxygenase, and Nf-κb (Gomes et al. 2008; Pan et al. 2010). Nrf2 not only behaves as a transcription factor of antioxidant response genes but also acts as an inhibitor of pro-inflammatory markers (Rana and Singh 2018). Apart from Nrf2, Nf-κb is another transcription factor known for its role in inflammatory events (Rana et al. 2020). Elevated oxidative stress in the cell disrupts Nf-κb regulatory system, leading to its translocation from the cytosol to the nucleus, which further triggers the synthesis of pro-inflammatory genes (Ahmed et al. 2017). The expression of pro-inflammatory markers was reported to be downregulated following the quercetin treatment *via* altering the transcriptional activity of Nf-κb in patients diagnosed with stable coronary artery disease (Chekalina et al. 2018). Similarly, there may be a chance that treatment of BXT in the current study suppressed the transcriptional activity of Nf-κb and activated the Nrf2 to overcome the observed neurobehavioral impairments.

Along with the neuronal circuit, sexual hormone deprivation also disrupts homeostasis in the glial cells population, which is essential for normal activity of the nervous system (Arevalo et al. 2013; Lu et al. 2020; Rana et al. 2022a). Imbalance in the glucose metabolism after menopause triggers the astrocytes to lose their normal functions, promote oxidative stress and neuroinflammatory events which subsequently damage the neighbouring neuronal cells (Rizor et al. 2019; Rana et al. 2022a). *Gfap* is an intermediated filament of the astrocyte, reported being elevated in patients affected with bipolar disorders, shedding light on reactive astrogliosis (Ferensztajn-Rochowiak et al. 2016). The peroxisome proliferator-activated receptor gamma (*Pparg*), is a transcription factor belonging to the superfamily of nuclear receptors (Iglesias et al. 2017; Rana et al. 2022a). *Pparg* regulates neuroinflammatory events by controlling the expression of genes responsible for astrocyte transformation (Iglesias et al. 2017; Rana et al. 2022a). In line with the literature, the *Gfap* expression was significantly elevated, while *Pparg* was downregulated in the brain of *ovct* rat, shedding light on the reactive astrogliosis in both regions of the brain. However, flavonoids treatment in various neurodegenerative disorders was reported to downregulate the *Gfap* (Xu et al. 2013; Ren et al. 2016) and/or upregulate the *Pparg* (Beekmann et al. 2015; Ayaz et al. 2019) expression to maintain the normal population of the astrocyte, which in turn suppresses oxidative stress, neuroinflammation, and triggers the synthesis of neurotrophic factors. Hence, normalization of *Gfap* and *Pparg* expression following the treatment of BXT may be responsible to overcome ongoing pathophysiological events *via* suppressing astrogliosis.

Sexual hormone deprivation, chronic oxidative stress, and neuroinflammation following menopause also disrupted the selective permeability of the BBB *via* altering the synthesis of genes associated with the junction complex (Rana et al. 2022a). It further enhances the paracellular movement of systemically circulating substances toward the brain parenchyma, which also provokes the disease progression by cooperating with the ongoing pathological events (Selvakumar et al. 2013; Rana et al. 2022a). Flavonoids have been reported to prevent neurobehavioral complications in various neurodegenerative conditions linked with BBB dysfunction by suppressing oxidative stress and neuroinflammation (Kim et al. 2022). A study showed that quercetin treatment maintained the BBB integrity in the brain of female rats exposed to polychlorinated biphenyls *via* upregulating the expression of genes associated with the maintenance of junction complex (Selvakumar et al. 2013), thus suggesting the neuroprotective nature of the flavonoid. Moreover, quercetin treatment also prevented BBB damage following reperfusion therapy in cerebral ischemia by initiating the transcriptional activity of the β-catenin (Jin et al. 2019), which ultimately initiated the transcription of various neuroprotective genes (Rana and Singh 2018). In line with previous literature genomic expression of the *Tjp1* and *Ocln* was significantly attenuated following the treatment with BXT in *ovct* rats.

The transcription regulation is a complex mechanism, which requires the cross-talk between various signalling molecules to maintain homeostasis in the body. Gsk-3β is one of the signalling molecules which participates in the transcription process indirectly by controlling the activity of transcription factors to maintain synaptic plasticity, neurogenesis, circadian rhythm, and neurotransmission (Rana et al. 2020, 2022a). However, alteration in the normal functioning of Gsk-3β has been reported to trigger various psychiatric complications (Jope et al. 2017). Similarly, following experimental menopause, lack of sexual hormone impedes Gsk-3β normal functioning in the brain, resulting in a loss in dendritic arborization and spine density, which disrupts the neural circuit (Rana et al. 2022a). Activated Gsk-3β compromised the cell-protective mechanism either by structural modification or by degrading the downstream proteins such as Nrf2 and β-catenin (Rana et al. 2022a, b). β-catenin is the main constituent of Wnt signalling pathway, which not only engages in cell fate determination, but also suppresses inflammatory events and oxidative stress by interacting with Nf-κb (Ma and Hottiger 2016),

and transcription factors associated with Forkhead box O family (Karimaian et al. 2017). Furthermore, β-catenin also regulates the transcription of junction complex associated genes, suggesting its cell-adhesion properties (Tran et al. 2016). Activated Gsk-3β reduces the cellular pool of β-catenin (Rana et al. 2022b), resulting in the elevation of oxidative stress and disruption in the BBB, which progressively affects neurobehavioral functions (Xiao et al. 2017). Activated Gsk-3β also degrades the Nrf2 protein by cullin1/Rbx1- mediated Nrf2 ubiquitination pathway (Chen et al. 2016), which disrupts cellular homeostasis by elevating oxidative stress. Gsk-3β is also known to provoke neuroinflammatory events *via* initiating the transcriptional activity of Nf-κb. The association between the upregulated expression of Nf-κb and the development of mood disorder following menopause has been well explored (Koshkina et al. 2019) in *ovct* rats. However, treatment of BXT inactivated the Gsk-3β and overcome neurobehavioral complications in *ovct* rats. Rutin, a major compound of BXT has shown an inhibitory effect on Gsk-3β activity and sustained the regular pool of Nrf2 and β-catenin to prevent hemorrhagic stroke in a zebrafish model (Rana et al. 2022b). Similarly, other studies also suggested the inhibitory activity of quercetin on Gsk-3β, resulting in the suppression of oxidative stress and inflammatory events in various diseases (Lee and Yoo 2013; Zhou et al. 2015). Hence, based on the literature and results of the current study it can be correlated that estrogen deprivation following *ovct* elevated oxidative stress and neuroinflammatory events leading to neurobehavioral complications. However, BXT treatment helped to overcome oxidative stress and neuroinflammatory events by suppressing the Gsk-3β activity to prevent neurobehavioral complications in *ovct* rats.

Conclusion

The results concluded that sexual hormones deprivation following *ovct* disrupted the neuronal circuit by elevating the level of oxidative stress and inflammatory events in the discrete brain regions of rats. Further, molecular analysis revealed that *ovct* activated the Gsk-3β, which degraded downstream proteins to initiate neurodegenerative events. However, BXT treatment following the critical window periods suppressed the activity of Gsk-3β, and preserved a normal pool of β-catenin that hampered the chain of ongoing pathophysiological events and rescued the neurobehavioral impairments.

Abbreviations

BBB	Blood-brain barrier
FST	Forced swimming test
GFAP	Glial fibrillary acidic protein
Gsk-3β	Glycogen synthase kinase-3 beta
HRT	Hormonal replacement therapy
Il1b	*Interleukin-1beta*
Il2	*Interleukin-2*
MWM	Morris water maze
Nfe2l2	*Nuclear factor erythroid 2-related factor 2*
Nf-κb	Nuclear factor-κB
NORT	Novel object recognition test
Nqo1	*NAD(P)H dehydrogenase (quinone) 1*
Ocln	Occludin
OFT	Open field test
Pparg	*Peroxisome proliferator-activated receptor gamma*
RT	Room temperature and
Tjp1	*Tight junction protein 1*

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Authors contribution AKR performed the animal surgery, behavior experiments, protein expression, and wrote the manuscript. SS carried out the gene expression studies. RK characterized the *F. tataricum* seed extract. DS conceptualized the idea, analysed the data, wrote, and edited the manuscript.

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Data Availability The data will be made available on a reasonable request to the authors.

Additional declarations

Conflict of interest All the authors declare no conflict of interest.

Ethical clearance The study protocol (IHBPT-06; 05/01/16) was duly approved by the Institutional Animal Ethics Committee of the CSIR-IHBT established by the Committee for the Purpose of Control and Supervision of Experiments on Animals, Ministry of Fisheries, Animal Husbandry and Dairying, Government of India.

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