Extract of Yokukansan improves anxiety-like behavior and increases serum brain-derived neurotrophic factor in rats with cerebral ischemia combined with amyloid-β42 peptide

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Abstract

OBJECTIVE: To examine the effects of Yokukansan (YKS) extract on two endogenous modulators of anxiety, brain-derived neurotrophic factor (BDNF) and serotonin (5-HT) receptors pharmacologically, in the ischemic rat model of dementia.

METHODS: The cerebral ischemia (CI) was induced by bilateral occlusion of the vertebral and common carotid arteries (4-vessel occlusion ischemia). The CI was combined with the amyloid-β42 peptide (Aβ 42) injected intracerebroventricularly, and referred to as CI+Aβ. Anxiety-like behaviors were assessed by elevated plus maze (enclosed arm), light/dark transition test (dark chamber), and open-field test. Wet-dog shakes were induced by the 5-HT, receptor agonist 2, 5-dimethoxy-4-iodoamphetamine (DOI). The concentration of BDNF in serum was determined by enzyme-linked immuno sorbent assay.

RESULTS: CI + Aβ increased anxiety, as demonstrated by the increase of time spent in the enclosed arms and dark chambers, and locomotion in the outer zone of the open field (thigmotaxis). CI + Aβ decreased the serum concentration of BDNF. YKS reduced the anxiety-like behaviors, suppressed the DOI-induced wet-dog shakes and increased serum BDNF concentrations.

CONCLUSION: Our findings suggest that YKS extract improves CI + Aβ-induced anxiety by antagonizing 5-HT, receptors and increasing BDNF.

Keywords: Brain ischemia; Amyloid beta-peptides; Anxiety; brain-derived neurotrophic factor; Receptor, serotonin, 5-HT2A; Yokukansan

INTRODUCTION

The behavioral changes are not only a consequence of dementia but also a predisposing factor. Anxiety is a component of behavioral and psychological symptoms of dementia (BPSD), which is observed in neurodegenerative conditions such as Alzheimer’s disease and cerebral ischemia (CI). Yokukansan (YKS), a traditional
Kampo medicine (Japanese variant of Chinese traditional medicine that involves the use of herbs), is inspired by the original traditional Chinese formulation Yi-Gan-San. YKS is known to improve neuropsychiatric symptoms and activities of daily living scores in patients with dementia. YKS improves involuntary movement disorders in Huntington’s disease and neuroleptic-induced tardive dyskinesia. Moreover, YKS improves sleep disorders, behavioral and psychological symptoms such as delusions, hallucinations, agitation/aggression, depression, anxiety and irritability in dementia, Alzheimer-type and Parkinsonian patients with dementia without worsening their cognitive function, ability to perform activities of daily living. It has been reported that YKS reduces age-related and preoperative anxiety. Moreover, YKS inhibits aggressive behavior induced by the amyloid-β42 peptide (Aβ42), social isolation, serotonergic neurotoxin p-chloroamphetamine, and that observed in amyloid precursor protein transgenic mice. YKS also alleviates stress-related emotional abnormalities. Moreover, YKS has anxiolytic activity in both innate and memory-dependent fear as a result of aversive stress. YKS exerts multiple potential actions on a variety of the neurotransmitter systems via different mechanism such as noncompetitive inhibition and/or downregulation of 5-HT1a receptors through partial agonistic action; 5-HT release in the PFC, which might be associated with the anxiolytic effects of YKS; increases acetylcholine release, increases dopaminergic activity and blocks α2A-adrenoceptors; positively modulates GABA activity and decreased glutamate release with increased uptake. It has also been reported that YKS could exert its anxiolytic effects via activation of benzodiazepine and GABA receptors, normalization of the hypothalamic-pituitary-adrenal axis, inhibition of NMDA receptors, and suppression of glutamate release in the hippocampus. Currently, the available anxiolytic agents do not provide adequate treatment for dementia-related anxiety. This may be explained by different pathological mechanisms compared with the anxiety of other etiologies, or the lack of efficacy of anxiolytics. There are several animal studies reporting that YKS ameliorates the symptoms of anxiety in various models of dementia. We previously reported anxiolytic activity of YKS in CI model induced in the rat by 4-vessel occlusion, 4-VO. Anxiety is one of the components of BPSD. Accordingly, it becomes crucial to determine any anxiolytic property in drugs effective in treatment of dementia. In this study, we aimed to investigate the anxiolytic activity of YKS extract in elevated plus maze, light/dark transition and open-field tests in addition to effects on wet-dog shakes and serum brain-derived neurotrophic factor (BDNF) in a rat model of ischemia induced by combination of 4-VO with Aβ42 (CI + Aβ).

METHODS

Animals
Male Wistar rats (300-350 g, age > 3 months; Kyudo Co., Saga, Japan) were housed in standard conditions [temperature, (23 ± 2) °C; relative humidity, 60% ± 5%; 12 h light/dark cycle, lights on at 07:00 A.M.]. Food and water were provided ad libitum. All animal care, housing (4-5 rats per group) and experimental procedures (generally, 8-10 rats were included in each group) were conducted in accordance with the guidelines of the Animal Care and Use Committee of Fukuo University (permit numbers 1205559/1405744). All required efforts were taken to ameliorate animals suffering by proper handling, anesthesia, analgesia and postoperative care.

Induction of CI by 4-VO method
The rats were anesthetized with sodium pentobarbital (50 mg/kg, i.p.; Tokyo Kasei, Tokyo, Japan), and immobilized in stereotaxic apparatus (Narishige Scientific Instruments, Tokyo, Japan). In the CI rats, the bilateral vertebral arteries were electrocauterized with a bipolar coagulator (MICRO-3D, Mizuho Industrial Co., Tokyo, Japan), one day later both common carotid arteries were occluded for 10 min using aneurysm clips (single ischemia). Sham rats underwent cauterization of the vertebral arteries, and the common carotid arteries were fitted with oculuders, but not occluded. The rats that did not demonstrate loss of righting reflex during arterial occlusion were excluded from the study. No apparent changes other than loss of righting were observed, except decreased (~25%) body weight. No mortality due to the CI was reported in this study.

Stereotaxic lateral ventricular cannulation for intracerebroventricular (i.c.v.) injection
The rats were anesthetized with sodium pentobarbital and placed in the stereotaxic frame. Guide cannulas [0.71 ± 0.02 mm outer diameter, (0.4 ± 0.02 mm inner diameter, 13 mm length)] were bilaterally implanted at the following coordinates (mm): AP = −0.8 from the bregma, L = ± 1.3 from the mid-sagittal line, and H = 3.3 from the skull surface. The implanted cannula was anchored by two screws driven into the skull, fixed by dental acrylic cement, and then secured with a dummy cannula kept in place by a cap. After surgery, each rat was injected with penicillin in the hindquarter muscle (100 000 units) and individually housed after the operation. The rats that only underwent cauterization of the vertebral arteries and then were fitted with oculuders on the common carotid arteries without occlusion were used as sham-operated controls.

Elevated plus maze test
The elevated plus maze apparatus consisted of a central hub (10 cm × 10 cm × 40 cm) with 5-mm thick polyvinylchloride arms; two open (55 cm × 10 cm × 40 cm)
and two enclosed (50 cm × 10 cm × 40 cm). The arms were arranged such that arms of the same type were opposite to each other. The maze was elevated 50 cm above floor level. The rats were placed in the central hub facing one of the enclosed arms and were allowed to enter the arms freely for 10 min. The time spent in the enclosed arms and the frequency of entering the enclosed arms were recorded by a researcher who was blind to the drug treatment. Entry into an arm was considered when all four paws were placed on the arm floor.

**Light-dark transition test**

The light/dark box consisted of a white and a dark chamber (30 cm × 30 cm × 30 cm) connected by a gate (8 cm × 8 cm) in a middle of a partition separating the two chambers. The box was placed in a dark room, and illumination was provided exclusively by a 40-W white incandescent bulb lamp that was set aside of the apparatus. The rats were placed in the middle of the gate, facing the dark chamber and then released. The number of full-body transitions between the two compartments and the time spent in the dark box was recorded for 10 min.

**Open-field test**

The locomotor activity and anxiety-like behaviors were observed for 10 min in an open-field apparatus composed of a circular floor (60 cm diameter divided by colored lines into 19 equal blocks) enclosed by a parapet (50 cm height) with an upper opening (90 cm diameter), and illuminated by a 100-W bulb placed 80 cm above the center of the apparatus floor. The blocks were divided into two zones: inner (7 blocks) and outer (12 blocks). Rodents usually tend to spend a greater amount of time exploring the periphery of the arena, in contact with the walls (thigmotaxis) rather than the unprotected center area. The rats that are more anxious in the open field spend less time in the inner area. Accordingly, the rats that spend more time exploring the unprotected inner area demonstrate anxiety-like behavior. The locomotion was estimated by counting the number of times of full-body occupancy of each block (all four paws crossed into the block). Anxiety-like behavior was estimated by calculating the ratio of locomotion in the outer zone to that recorded in the total blocks.

**Wet-dog shakes test**

Wet-dog shakes were induced by the 5-HT1, receptors agonist 2,5-dimethoxy-4-i-odoamphetamine (DOI; 5 mg/kg i.p.). The number of characteristic, paroxysmal, intermittent but rhythmic, ‘wet dog-like’ shakes of the head, neck, and trunk were counted for a 10-min period after DOI administration.

**Measurement of serum BDNF by enzyme-linked immuno sorbent assay (ELISA)**

The level of serum BDNF was determined using the E-Max ImmunoAssay system (Promega, Madison, WI) according to the manufacturer’s protocol. Briefly, standard 96-well flat-bottom Corning ELISA plates were incubated with carbonate coating buffer containing monoclonal anti-BDNF overnight at 4 °C. The next day, the plates were blocked with Block & Sample 1X Buffer 1 for 60 min at room temperature to block the nonspecific binding. Serial dilutions of known amounts of BDNF ranging from (0–500 pg/mL) were performed in duplicate for the standard curve for each set of rat serum. For both the standards and the samples, 100 μL was added to each well in duplicate and incubated for 120 min at room temperature. The wells were then incubated with anti-human BDNF polyclonal antibody (120 min at room temperature). Then, the wells were incubated with anti-IgY conjugated to HRP for 60 min BDNF at room temperature. A TMB One solution was used to develop color in the wells for 10 min at room temperature. The reaction was stopped with 1 N HCl to the wells. The absorbance was read at A450 within 30 min in an automatic multi-well spectrophotometer (Tecan, Männedorf, Switzerland). Serum protein was measured using Pierce™ BCA Protein Assay Kit (Thermo Fisher Scientific, Waltham, MA, USA). The amount of BDNF was divided by total serum protein for normalization and expressed as pg/mg protein.

**Drugs and treatment schedule**

The dried extract powder of YKS was supplied by Tsumura & Co., Japan (TJ-54, voucher No. 29101690). YKS contains the following seven herbs: Attractylodis lancea- De Candolle, rhizome (Asteraceae), Sojutsu, (S. jujuba; Fabaceae), Toki ((Polyhoreum), Bukuryo (4.0 g); Cnidium officinale-Makino, rhizome (Umbelliferae), Senkyu (5.0 g); Uncaria rhynchophylla-Miquel, thorn (Rubiacaeae), Chotoke (3.0 g); Angelica acutiloba-Kitagaw, root (Apiaceae), Toki (3.0 g); Bupleurum falcatum Linné, root (Umbelliferae; bupleurum root), Saiko (2.0 g), and Glycyrrhiza uralensis-Fisher, root (Fabaceae), Kanzo (1.5 g). The identification and authentication of each plant material had been conducted according to the Japanese Pharmacopoeia and the company’s standard. The extracts were manufactured according to GMP, subjected to factory release test. The quality control is based on Good Manufacturing Practice defined by the Ministry of Health, Labour and Welfare of Japan. Samples of the extracts are retained in Tsumura & Co. The following active components had been identified in YKS by three-dimensional HPLC: ferulic acid; liquiritin apio side; liquiritin; 4E, 6E, 12E-tetradecatrien-8, 10-diyne-1, 3, 14-triol; formononetin-7-O-glucoside; liquiritigenin; glycyrrhizin; geissoschizine methyl ether; hirsute; xanthotoxin; hirsute; saikosaponin b2; saikosaponin b1; 12-isovaleryl-2E, 8E, 10-Etriene-4,6-diyyne-1, 14-diol; 14-isovaleryl-2E, 8E, 10-Etriene-4,6-diyyne-1,
YKS extract was dissolved in distilled water, administered p.o. after the last Aβ42 injection (commenced seven days after 4-VO), and continued for seven days. The behavioral tests were conducted 60 min after the last YKS dose. The effect of three different doses (100, 300 and 1000 mg/kg, p.o.) were tested in the elevated plus maze and light-dark transition tests. In the light of the results of these tests and the data available in the literature, the dose of 1000 mg/kg was examined in the other experiments.

The 5-HT1 receptors antagonist Ketanserin, and agonist DOI (Sigma-Aldrich, St. Louis, MO, USA) were dissolved in distilled water, rendered isotonic and administered (5 mg/kg i.p.) 30 min before the tests. In the interaction experiments, DOI was administered 30 and 60 min after ketanserin/vehicle and YKS respectively.

Aβ42 (Anaspec, Inc.; San Jose, CA, USA) was dissolved in N-(2-hydroxyethyl) piperazine-N-2-ethanesulfonic acid-buffered solution to a final concentration of 10 μM, followed by incubation at 37 °C for seven days to form aggregates. The aggregated Aβ42 300 (pmol/10 μL) was bilaterally injected into awake rats via an injection cannula [(0.35 ± 0.01) mm outer diameter, (0.17 ± 0.02) mm inner diameter, 14 mm length], protruding 1 mm from the tip of an implanted guide cannula, and connected by PE tubing (1.09 mm outer diameter, 0.38 mm inner diameter; Intramedic, Visalla, CA, USA) to a perfusion pump (CMA/200; CMA Microdialysis AB, Stockholm, Sweden) driven at a rate of one μL/min. The Aβ42 was injected once-daily started 10 min after induction of CI and continued for seven days.

The rats used in this study were adolescent. Considering that during the adolescence, 10.5 rat days equals one human year, the seven-day (subchronic) injection period in rat will correspond to 238 d (i.e. chronic) in human. The rats with CI + Aβ are hereafter referred to as ischemic rats in this study. The study included five main groups: (a) sham, and ischemic rats received vehicle; (b) YKS, 1000-1000 mg/kg p.o.; (c) diazepam, 20 mg/kg p.o.; (d) or ketanserin, 5 mg/kg i.p.; (e) the experimental schedule is depicted in Figure 1.

**Statistical analysis**

The data were checked for homogeneity of variance and evaluated by one-way analysis of variance (ANOVA). Tukey’s post hoc was applied for multiple comparisons whenever ANOVA detected significant differences. The criterion for statistical significance was \( P < 0.05 \). Data are expressed as the mean ± standard error of mean. The analyses were performed using IBM SPSS 21 (IBM Corp., Armonk, NY, USA) for Windows.

**RESULTS**

**Anxiety-like behavior**

Figure 2 shows that CI + Aβ induced an anxiogenic behavior characterized by increased (by 28%, \( P < 0.01 \)) time spent in the enclosed arm of the elevated plus maze. The effects of CI + Aβ are expectedly different from the effect produced by either CI or Aβ on their own. In this study, YKS at 100 and 300 mg/kg did not display any significant effect, whereas at 1000 mg/kg YKS decreased (by 20.2%, \( P < 0.01 \)) the time spent in the enclosed arm, an effect not significantly different from that produced by diazepam (19.8%, \( P < 0.01 \)). Interestingly, the dose 1000 mg/kg also reflects that used clinically. The human equivalent dose (HED) of the dose used in the rat can be calculated according to Sharma and McNeill, 2009:

\[
\text{HED} = \text{animal dose (mg/kg)} \times \left(\frac{\text{animal weight (kg)}}{\text{human weight (kg)}}\right)^{0.7}
\]

\[
= 1000 \text{ mg/kg} \times \left(\frac{0.325/60}{0.178/70}\right)^{0.7} \quad (\text{mean body weight of rats used in this study} = 325 \text{ g})
\]

= 178.70 mg·kg⁻¹·day⁻¹

Assuming the human weight to be 60 kg, the HED is 10.72 g/d. Applying a safety factor of 10, the starting dose in humans is 1.7 g/d (approximately 2 g extract) which is close to the minimum daily starting dose used clinically. Since the water extract yield of YKS is 15.9%, the calculated HED of YKS corresponds to 11 g of the crude YKS mixture.

Figure 3 shows that ketanserin reduced the time spent in the enclosed arm by 33% (\( P < 0.01 \)). The anxiolytic effect of YKS extract on CI + Aβ-induced anxiety was also investigated after activating 5-HT2 receptors by DOI and compared with ketanserin. It can also be seen from Figure 3 that ketanserin significantly (\( P < 0.01 \)) decreased the time spent by the ischemic rats received
The basic measure is the animal's preference for dark, enclosed places over bright and exposed places. Time spent in the light half of the box, and the related exploratory activities, are reliable parameters for assessing the anxiolytic effect. Figure 4 shows that CI + Aβ increased (by 45%, *P < 0.001) the time spent in the dark chamber of the light/dark transition test. On the other hand, YKS at 100 and 300 mg/kg did not display any significant effect, whereas at 1000 mg/kg decreased (by 27%, *P < 0.01) the time spent in the dark chamber, an effect not significantly different from that of diazepam (by 22%, *P < 0.001). The effects YKS were associated with a non-significant decrease in the number of crossings in both plus maze and light/dark transition test, whereas diazepam increased transitions between the compartments (data not shown).

The Light-Dark box test is widely used in the assessment of anxiety and the anxiolytic properties of drugs. The basic measure is the animal’s preference for dark, enclosed places over bright and exposed places. Time spent in the enclosed arm by 45% (compare to 20.2% decrease produced by YKS 1000 mg/kg). On the other hand, DOI significantly decreased the effect of ketanserin (by 78%, *P < 0.01%) and YKS (by 31%, *P < 0.05%) on the time spent in the enclosed arm. This result could indicate that activities besides that displayed on 5-HT2 could be involved in the Effect of YKS extract more than ketanserin.
YKS significantly decreased (P < 0.01) the ratio of locomotion in the outer zone to total locomotion, due to increased activity in the inner zone, without significant change (slight increase) of the total activity, a result similar to that produced by diazepam (data not shown).

**DOI-induced wet-dog shakes**

Head twitches in rats are sometimes referred to as wet-dog shakes because in that species the behavior frequently involves the head, neck, and trunk. Each head shok consists of a rapid sequence of reciprocating, side-to-side head movements. Wet-dog shakes can be counted over the observation period. Wet dog shakes are elicited in the septohippocampal pathway, and mediated by 5-HT_{2A} receptors. Accordingly, inhibition or decreasing wet-dog shakes could be one of the activities reflecting the anxiolytic property. Figure 6 shows that ischemic rats that received distilled water did not exhibit wet dog shakes. DOI (5 mg/kg i.p.) induced characteristic shakes (30.0 ± 3.6/10 min; P < 0.001) in the ischemic rats. YKS (1000 mg/kg) reduced the number of DOI-induced shakes by 45% (P < 0.05), whereas ketanserin abolished the DOI-induced wet-dog shakes in ischemic rats (P < 0.001).

**Brain-derived neurotrophic factor (BDNF)**

BDNF is one of the most abundant growth factors in the brain. It is essential for the neuronal development and plasticity. BDNF is reduced in anxiety disorders. However, the variations in BDNF levels are inconsistent due to variation in anxiety types and the sampling methods. The analysis method applied for BDNF in this study showed that serum of sham rats contains (37.0 ± 6.8) pg/mg protein. This concentration was decreased (P < 0.01) to (12.0 ± 1.8) pg/mg protein after seven days in the ischemic rats. YKS increased (P < 0.01) serum BDNF of the ischemic rats to (36.0 ± 3.0) pg/mg protein, a level almost close to that of the sham rats (Figure 7).

**DISCUSSION**

BPSD represents a burden for patients, relatives, caregivers and health authorities. Accordingly, treatment of BPSD may have an equal importance as treating the core symptoms of dementia. The polymorphism of 5-HT transport plays a role in the development of BPSD in dementia. Drugs that regulate the serotonergic activity are effective in the treatment of anxiety. Despite the verified anxiolytic effects of YKS, both clinically and in experimentally induced ischemia, its mechanisms of action are not yet fully clarified, and no previous study in a CI + Aβ 42-ischemia model is available. The pathogenic mechanisms of CI and Aβ not only overlap but are also highly interactive, especially the pathogenesis of CI is closely linked with Alzheimer’s disease. Moreover, a synergistic memory impairment had also been reported between permanent common carotid occlusion and single Aβ, possibly mediated by neuroinflammation. Animal studies have shown that CI stimulates synthesis and parenchymal deposition of Aβ, and the latter causes greater hemodynamic dysfunction characterized by disrupted cerebral perfusion and exacerbated postsischemic injury. In the combination group, the activity of protein phosphatase 2A
is reduced, the activity of glycogen synthase kinase 3β and the level of tau protein phosphorylation is increased, the loss of neurons appeared in both the CA1 and CA2 regions, compared to CA1 in CI alone.\textsuperscript{30} We previously reported that hypoxia, which is a key feature of ischemia, enhances Aβ-induced apoptosis in rat cultured hippocampal neurons.\textsuperscript{31} We had reported that CI + Aβ, but not either treatment alone, impaired spatial memory, induced apoptosis of pyramidal neurons in the CA1 region, and decreased high K\textsuperscript{+}-evoked acetylcholine release in the dorsal hippocampus.\textsuperscript{32} Clinically, it has been reported that anxiety symptoms could increase the effect of Aβ, resulting in more rapid decline in several cognitive domains.\textsuperscript{33} In this study, CI + Aβ produced an anxiety state that was characterized by the preference for dark ambiance and thigmotaxis accompanied by decreased BDNF, without affecting total locomotor activity. YKS at 1000 mg/kg ameliorated the anxiety exhibited by the ischemic rats, an effect characterized by decreased dark/closed area preference, decreased thigmotaxis whereas increasing the activity in the center opened zone of the open field. The fact that YKS displayed a significant effect at 1000 mg/kg is reminiscent of the activity reported for YKS at the same dose in other studies: maximum neuroprotective effect, and improvement of cognitive deficits, an amelioration of schizophrenia-like symptoms\textsuperscript{34} and aggressive behavior.\textsuperscript{35} Our results revealed that the effects of YKS extract 1000 mg/kg were comparable to diazepam 20 mg/kg in both elevated plus arm and light-dark transition tests. Considering that Aβ oligomers together with ischemia can induce neurodegeneration and disturb neurotransmission, it is logical to suggest that the neuroprotection provided by YKS\textsuperscript{36} may contribute to its anxiolytic activity in ischemic rats. 5-HT is one of the neurotransmitters whose activity is disturbed by Aβ and CI. YKS exerts multiple potential actions on a variety of the neurotransmitter systems via different mechanisms: decreased glutamate release but increased its uptake; increased acetylcholine release, dopaminergic activity, positive modulates GABA activity and inhibition of α2A-adrenoceptors.\textsuperscript{37} The effect of YKS extract on the serotonergic system is one of the possible mechanisms by which it exerts anxiolytic activity. YKS increases both serotonergic transmission\textsuperscript{38} and the density of 5-HT receptors in the prefrontal cortex.\textsuperscript{39} In addition, YKS is a partial agonist of 5-HT\textsubscript{1A},\textsuperscript{40} while it antagonizes 5-HT\textsubscript{1B} and increases 5-HT\textsuperscript{1D} release in the PFC which might be associated with the anxiolytic effects of YKS.\textsuperscript{16} In this study, the Effect of YKS extract in the elevated plus maze test was decreased by DOI, whereas YKS decreased DOI-induced wet-dog shakes, a result in accordance with previous reports.\textsuperscript{24} On the other hand, ketanserin also decreased the time spent in the enclosed arm and abolished the DOI-induced head shakes, albeit more than YKS. These results coincide with those of other groups and suggest that YKS produces anxiolytic activity by antagonizing 5-HT\textsubscript{1A}.\textsuperscript{16} The activation of 5-HT\textsubscript{1A} and GABA receptors by YKS could be involved in YKS activity in open-field/ light-dark transition and elevated plus maze respectively, because drugs that lack effects on GABA receptors but possess 5-HT\textsubscript{1A} agonistic activity (like buspirone) display anxiolytic activity in the contextual models but not the elevated plus maze.\textsuperscript{23} It is noteworthy to mention that although ketanserin produced a greater effect than YKS, ketanserin has several drug interactions such as with diuretics and antiarrhythmics. In addition to sedation, fatigue, lightheadedness, dizziness, headache, dry mouth, and gastrointestinal disturbances, ketanserin use has been associated with ventricular arrhythmias, especially in patients with predisposing factors such as QT prolongation, because ketanserin itself causes prolongation of QT interval.\textsuperscript{30} No such adverse or side effects had been reported for YKS, at least up to now. The salient finding of this study is that CI + Aβ decreased BDNF concentration in serum. It is rational to measure BDNF concentration in serum because a positive correlation between peripheral and brain BDNF protein levels have been reported in rodents, suggesting that peripheral BDNF level may reflect BDNF level in the brain.\textsuperscript{37} BDNF plays an important regulatory role in emotional disorders. It has been reported that BDNF signaling improves short- and long-term memory, a phenomenon that may also contribute to learned social avoidance.\textsuperscript{38} Moreover, there is evidence for a possible correlation between serum BDNF and psychiatric disorders such as major depressive disorders\textsuperscript{39} and contextual fear conditioning.\textsuperscript{40} BDNF modulates the serotonergic system. Of note, elevated BDNF increases 5-HT\textsubscript{1A}, which may also be involved in the anxiolytic effects of YKS\textsuperscript{36} and desensitization of 5-HT\textsubscript{1A} receptors.\textsuperscript{16} Moreover, it has been reported that BDNF downregulates 5-HT\textsubscript{1A} via a partial agonistic activity on the receptor.\textsuperscript{41,42} Accordingly, it is possible to suggest that YKS reduces 5-HT\textsubscript{1A} activity both directly and via BDNF. It is plausible to suggest that the Effect of YKS extract on BDNF plays an important role in the reduction of DOI-induced wet-dog shakes and amelioration of anxiety. Furthermore, our results indicate that YKS may have an indirect effect on 5-HT\textsubscript{1A} receptor expression via increasing BDNF. Overall, disruption by CI + Aβ of activities regulated by 5-HT\textsubscript{1A}, GABA and BDNF are involved in the anxiogenic behavior, whereas improvement of the disrupted function is involved the ameliorating Effect of YKS extract.

In conclusion, YKS extract ameliorated the anxiogenic state of CI + Aβ-ischemic rats via antagonizing 5-HT\textsubscript{1A} receptors and increasing BDNF.

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