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**Original Research Article**

# Effect of J147 on irritable bowel syndrome mouse: Involvement of 5-HT1A-dependent PKA-CREB-BDNF signaling pathway

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## *Abstract*

***Purpose:*** *To determine the effect of J147 on depression, anxiety, and gut malfunction triggered by stress in a mouse model exhibiting symptoms of irritable bowel syndrome (IBS).*
***Methods:*** *An IBS mouse model was established by chronic/acute combined stress and housed individually. The stressed mouse was exposed to 21 consecutive days of random chronic unpredictable stress and received 3 h of acute restraint stress on day 2. Forced swimming test (FST) and elevated plus-maze test were used to evaluate depressive and anxiety behaviour, respectively. The intestinal motility and visceral sensitivity of mouse were measured by abdominal withdrawal reflex test (AWR). Also, 8-OH-DPAT (a 5-HT1A receptor agonist) and NAN-190 hydrobromide (a 5-HT1A receptor antagonist) were used in combination with different doses of J147 (2 and 10 mg/kg) for three weeks and AWR test was done. Furthermore, IBS-related protein expression (PKA, pCREB, BDNF) in the hippocampus, colon and ileum was determined by western blotting.*
***Results:*** *After CACS induction, mice showed depression/anxiety-like behaviour along with intestinal allergy, and altered levels of 5-hydroxytryptamine (5-HT) in both the hippocampus and gut. Furthermore, J147 significantly alleviated depression, anxiety, intestinal motility disorders, and intestinal hypersensitivity. Additionally, it normalized abnormal levels of brain-gut 5-HT neurotransmitters observed in IBS mice. Pre-treatment with NAN-190 reversed the effect of J147, whereas the addition of 8-OH-DPAT, augmented the effect of low dose J147 (2 mg/kg) on behavioural abnormalities associated with CACS. Furthermore, J147 significantly increased expression levels of IBS-related proteins in the hippocampus, and decreased their levels in the ileum and colon.*
***Conclusion:*** *J147 inhibits IBS-like depression, anxiety, and visceral hypersensitivity by modulating the 5-HT1A-dependent PKA-CREB-BDNF signaling pathway. Thus, there is need for further studies on the development of J147 in the treatment of irritable bowel syndrome.*

***Keywords:*** *Irritable bowel syndrome, J147, 5-HT, PKA-CREB-BDNF signaling pathway, Hippocampus, Depression*



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## INTRODUCTION

Irritable bowel syndrome (IBS) manifests as a disorder impacting digestive functions, typically marked by stomach ache or unease, and frequently paired with alterations in bowel routines and activity [1,2]. About 10 – 20 % of the world's population suffers from IBS symptoms.

Exact pathogenesis of IBS remains completely misunderstood, but it is widely believed to involve abnormal intestinal motility, altered intestinal sensation, and psychosocial factors, among which psychological factors play a major role [3]. Depression and associated negative activity are likely to lead to increased gastrointestinal sensitivity and peristaltic disorders, resulting in IBS. Studies have shown that IBS is associated with 30 - 50 % of stress-related psychiatric disorders. In addition, stress may easily induce deterioration and aggravation of IBS, and emotional disorders such as anxiety and depression may lead to poor treatment efficacy [4]. Also, IBS is almost always associated with anxiety, and patients often show that it coexists with other chronic pain and mental disorders [5]. The role of brain-gut axis dysfunction in IBS progression has become a focus of current IBS research. Recent studies have suggested that peripheral hypersensitivity may affect the central nervous system (CNS) leading to depression or anxiety, visceral sensitivity and intestinal motor dysfunction [1,6]. Furthermore, J147 is a small lipid-soluble molecule characterized by low dosage, easy passage through the blood-brain barrier, accumulation in the brain, high bioavailability in the central nervous system, and minimal effect on the peripheral nervous system. As a derivative of the natural polyphenol compound, curcumin, J147 exhibits unique functional activity that combines the properties of curcumin with neuroprotective effects. For the first time, curcumin was found to alleviate depression-like behaviour in stressed mice [7]. However, later studies found that curcumin only penetrated the blood-brain barrier in small amounts and had low bioavailability [8], which limited its application in antidepressant therapy. Another study synthesized CNB-001, a pyrazole derivative of curcumin, using curcumin and cyclohexyl bisphenol A (which has distinct neuroprotective effects) as the parent nucleus and biological effects as the entry point [9]. Initially, CNB-001 was used as the lead compound to optimize its structure, and J147 was selected as the new compound with the best biological activity. Previous studies have found that chronic administration of J147 significantly increases 5-HT levels, suggesting that J147 exerts antidepressant effects by modulating the monoamine system. Therefore, J147 alleviates depressive and anxiety symptoms by altering the concentrations of 5-HT and its downstream associated proteins. Additionally, through the brain-gut axis, J147 regulates 5-HT in the gut and expression levels of its downstream-related proteins, thereby ameliorating peripheral dysfunction in IBS.

This study therefore investigated the effects of J147 on depression, anxiety, and gut malfunction triggered by stress in a mouse model exhibiting symptoms of irritable bowel syndrome (IBS).

## EXPERIMENTAL

### Animals

A total of 60 male ICR mice, approximately 30 g in weight, were obtained from Shanghai Laboratory Animal Co. Ltd, located in Shanghai, China. Mice were first kept in the animal room for four days in a 12-h light and dark cycle at relative humidity of 45 % and temperature of 24 $^{o}$C in cages (four mice per cage) with sufficient food and water. The procedure adhered to the revised 2011 guidelines for the care and use of laboratory animals issued by the National Institutes of Health [10], and approval for the study was granted by the Institutional Animal Ethical Committee of Wenzhou Medical University (approval no. 00301).

### Drugs and treatment

Fluoxetine, diazepam, 8-OH-DPAT and 5-HT1A were all provided by Sigma, St. Louis, MO, USA, J147 was purchased from MedChem Express, Monmouth Junction, NJ, USA while NAN-190 bromide was provided by Ocris Bioscience, Ellisville, MI, USA. Sodium carboxymethylcellulose was obtained from Shanghai Boyun Biotechnology Co., Ltd, Shanghai, China and protein kinase A (PKA) and brain-derived neurotrophic factor (BDNF) antibodies were obtained from Abcam Bio, Cambridge, MA, USA.

### Chronic-acute combined (CACS) model

The mice were randomly divided into control (containing 10 mice), and study group made up of 50 mice that were further divided into 5 groups containing 10 mice each. The study group underwent random exposure to chronic unpredictable stress (CACS) for 22 days. This included various stressors such as being deprived of food for 24 h, deprived of water for 24 h, forced to swim in water at 4 $^{o}$C for 4 min, subjected to tail pinching for 3 min, exposed to night-time illumination, and housed in a wet cage for 6 h [7]. On the 22$^{nd}$ day, all mice except those in control group were subjected to 3 h of acute restraint stress.

### Forced swimming test

A cylindrical container measuring 21 cm across and 50 cm tall was constructed with a water level

(25 ± 1 ℃) set to 25 cm. The mice underwent a 15-minute introductory swim session within this container, dried with a towel and returned to their respective cages 24 h before the forced swim test. Thereafter, the animals were transferred to a tube identical in conditions for a compulsory swim test and duration of inactivity was recorded.

**Elevated plus-maze**

Individual mice were situated at mid-point of the raised plus-shaped maze (situated 40 cm above the floor), oriented such that the enclosed arm was directly ahead. The duration and frequency of animal entry into the open and closed arms (dimensions 30 × 5 × 15 cm) over a 5-minute interval were recorded with entries being counted once all the limbs had crossed into an arm. Anxiolytic efficacy of the medication was evaluated through an assessment of the frequency and duration of entries into the open arm. Anxiolytic efficacy was calculated using Eq 1 and Eq 2.

E (%) = (Ee/Ne)100 …… (1)

T (%) = (Tn/Nt)100 …… (2)

where E is the entry into open arm, En is the number of entries into open arm, and N is the total number of entries into both open and closed arms, T is time spent in open arm, Tn is the time spent in the open arm while Nt is combined time spent in both open and closed arms.

**Abdominal withdrawal reflex (AWR)**

A balloon 5 cm long was made with a rubber finger sleeve and attached to an angiogram catheter (1.67 mm). After the mouse was anesthetized by inhalational isoflurane, the balloon was inserted into the anus at a depth of 6 cm, and 1 cm from the anus. After 30 min adaptation, the mice were injected with gas at 20, 40, 60 and 80 mmHg successively for 5 min each.

Abdominal wall withdrawal reflex scores were recorded by assessors (unaware of the experiment), and the scores were classified as follows: No response (0), slight head movement (1), abdominal muscle contraction (2), abdominal muscle contraction and lifting (3), strong contraction of abdominal muscles, abdominal lift and body arch (4).

**Western blot**

Brains from 5 - 6 mice per group were lysed using radioimmunoprecipitation assay (RIPA) buffer, which contained inhibitors for proteases and phosphatases (Sigma Chemical Co. in St. Louis, MO, USA). Subsequently, the lysates were centrifuged at 12,000 rpm for 20 min at 4 $^{o}$C. Protein samples ranging from 30 to 90 µg were then denatured by mixing with a five-fold sample buffer and heating at 90 – 95 $^{o}$C. The samples were first subjected to 60 V constant pressure electrophoresis to separate the glue, and to 110 V electrophoresis for a total of 80 min. After a 70-minute transfer using constant flow wet method at 300 mA, the polyvinylidene fluoride (PVDF) membrane (Millipore, Billerica, MA, USA) was removed.

Subsequently, the membrane was blocked with a sealing solution (prepared with 5 % milk and tris-buffered saline with 0.1 % tween 20 (TBST)) for 90 min at room temperature. The membrane was then washed three times with TBST for 7 min each. Primary antibody was incubated overnight at 4 $^{o}$C after which they were recovered, and the washing cycle was repeated.

The membrane was kept at room temperature for 60 min, where it was incubated with secondary antibody and washed thrice with TBST for 7 min each. Primary and secondary antibodies were diluted with TBST according to the corresponding dilution ratio as follows: anti-PKA (1:50000), anti-CREB (Millipore Bio, Billerica, MA, USA) (1:500), anti-p-CREB (Millipore Bio, Billerica, MA, USA) (1:1000), anti-BDNF (1:2500) and anti-β-actin (Bioworld Biotechnology, USA) (1:5000). The strips were exposed utilizing the Bio-Rad Gel Doc XR + gel imaging system (Bio-Rad, Hercules, California, United States).

Subsequently, the PVDF membranes were incubated with the secondary horseradish peroxidase-conjugated antibody at a dilution of 1:5,000 for 1 h at 25 $^{o}$C. Detection of labeled protein bands was carried out using the enhanced chemiluminescence (ECL) method, and band intensity was quantified using ImageJ software [10].

**Statistical analysis**

Data analysis was done using Statistical Packages for Social Sciences (SPSS, SPSS version 22.0, IBM, Armonk, NY, USA). The data were expressed as mean ± standard error of the mean (SEM) and compared using independent sample t-test. Multiple comparisons were done using one-way analysis of variance (ANOVA). Furthermore, Dunnett's multiple comparison test was conducted to evaluate group variations. $P < 0.05$ was considered statistically significant.

## RESULTS

### J147 alleviates depression/anxiety-like behavior

Study group exhibited significant prolongation in the duration of immobility during the forced swim test ($P$ < 0.01, Figure 1 A). There was a significant dose-dependent reduction in FST inactivity duration among the CACS-afflicted mice with the most significant change occurring at 10 mg/kg ($p$ < 0.001, Figure 1 A).

Study group exhibited significantly shorter latency to enter the open arm compared to control group ($p$ < 0.001, Figure 1 B). After J147 and diazepam administration, the time to enter the open arm was significantly prolonged, reversing the anxiety-like behavior induced by CACS. At 10 mg/kg, J147 showed the most significant improvement effect ($p$ < 0.001, Figure 1 B). In comparison with the normal group, study group exhibited a significant decrease in the frequency of entering the open arms ($p$ < 0.001, Figure 1 C) which was reversed after treatment with J147 or diazepam, with the high-dose group of J147 showing the most significant improvement effect ($p$ < 0.001, Figure 1 C). These results indicated that J147 has antianxiety-like effects in IBS mice within the CACS model.

### J147 alleviates symptoms of intestinal visceral nerve hypersensitivity

At 20, 40, 60 and 80 mmHg, the response score of study group was significantly increased compared to control group ($p$ < 0.05). At 40, 60 and 80 mmHg dilation intensity, J147 had a significant effect on reducing AWR score, and the positive drug fluoxetine also significantly reduced AWR score of IBS mice ($p$ < 0.05). These results suggest that J147 alleviated CACS-induced visceral hypersensitivity and raise visceral pain threshold in IBS mice (Figure 2).

### Effect of J147 on protein expression of PKA, pCREB, and BDNF

The results showed that concentrations of PKA, pCREB/CREB, and BDNF in the hippocampus of CACS mice were significantly decreased compared to control group ($p$ < 0.05) (Figure 3 A). After chronic administration of J147, expression levels of relevant proteins in the hippocampus of CACS mice were increased to varying degrees with 10 mg/kg exerting the most significant effect. Furthermore, fluoxetine enhanced protein levels of PKA, pCREB/CREB, and BDNF, which had been modulated by CACS.

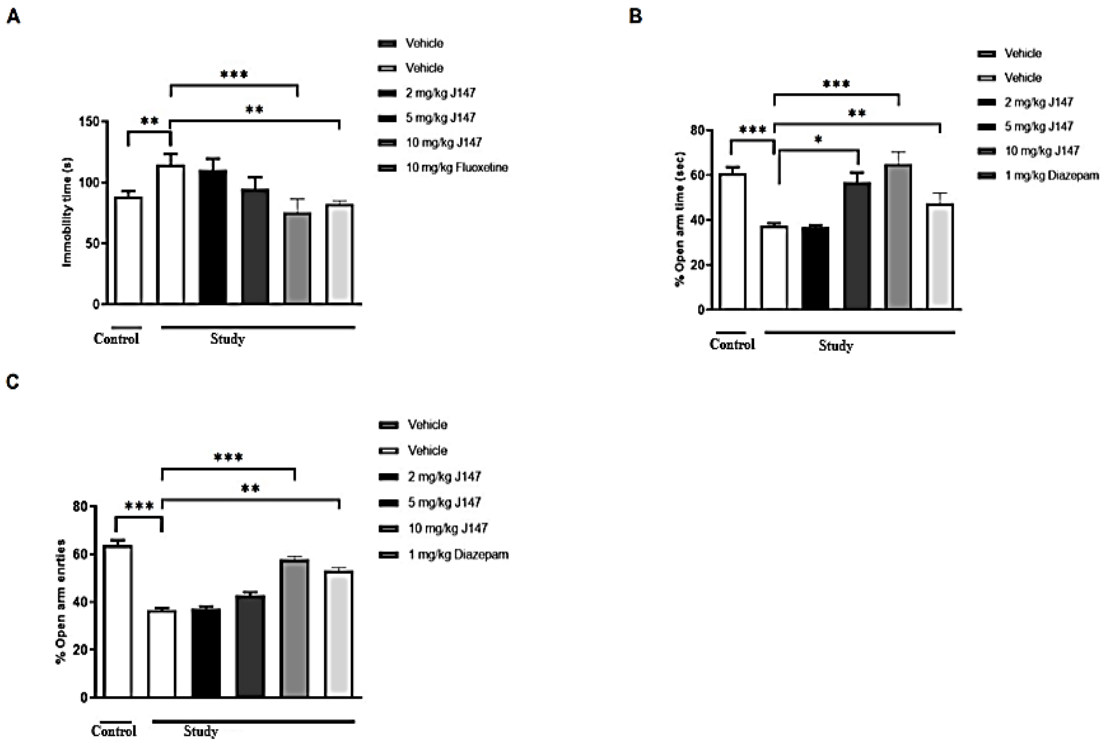


**Figure 1:** Forced swimming test (A), depressive-like behavior (B), elevated plus-maze (C). Results are presented as mean ± SEM (n = 8 - 10, per group)

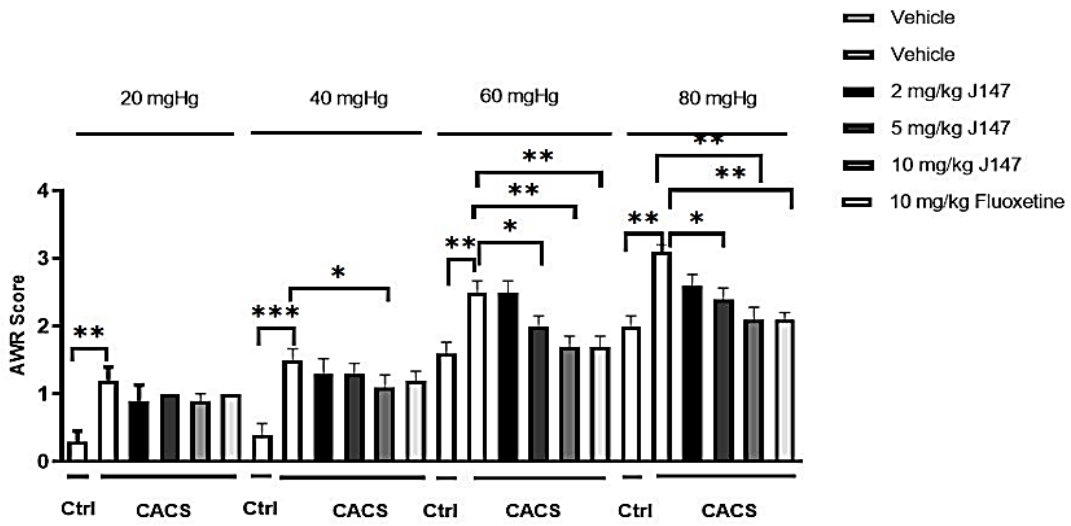


**Figure 2:** AWR score. The results are presented as the mean ± SEM (n = 8 - 10, per group)

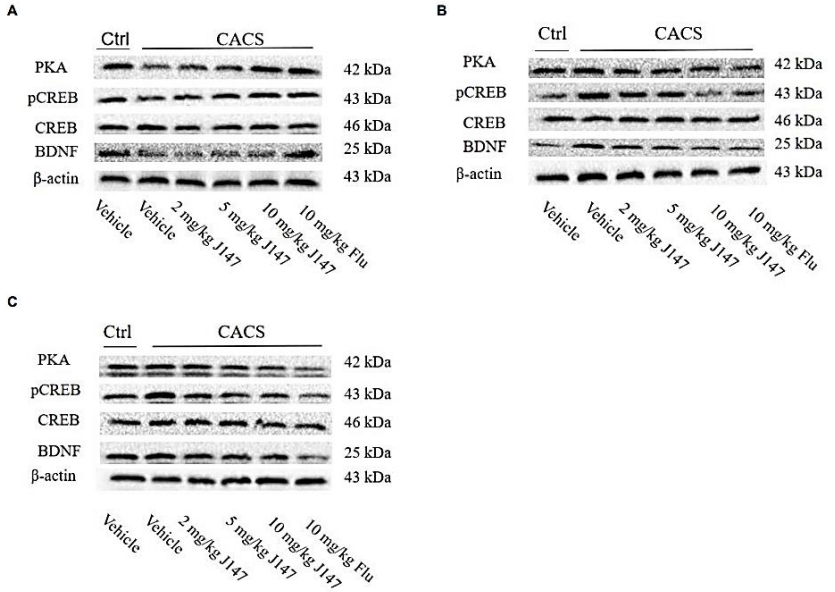


**Figure 3:** Expression of PKA, pCREB and BDNF proteins in the hippocampus (A), ileum (B) and colon (C) by western blot analysis. Results are presented as the mean ± SEM (n = 5 - 6, per group)

### Effect of J147 on protein expression of PKA, pCREB and BDNF in colon and ileum

Concentrations of IBS-related proteins within the colon of CACS group significantly increased compared to control group ($p < 0.05$). Conversely, mice subjected to J147 treatment exhibited significantly lower levels of IBS-related proteins in the colon compared to CACS group ($p < 0.05$) (Figure 3 B). Additionally, at 10 mg/kg, J147 and fluoxetine significantly reduced PKA, pCREB and BDNF protein levels in colon induced by CACS ($p < 0.05$). Expression of related proteins in the ileum of CACS mice resembled those observed in the colon. After 22 consecutive days of exposure to CACS, protein expression levels in the ileum were significantly increased compared to control group ($p < 0.05$) (Figure 3 C). After chronic treatment with different doses of J147, an abnormal increase in PKA, pCREB and BDNF protein expression induced by CACS was significantly reduced, and fluoxetine, also significantly normalized overexpression of PKA and pCREB in the ileum of CACS mice ($p < 0.05$).

### Anti-IBS effect of J147 on visceral hypersensitivity

Compared to CACS mice, there was no significant difference in AWR scores when 8-OH-DPAT or 2 mg/kg J147 was used alone ($p >$

0.05). However, the combination of 8-OH-DPAT and 2 mg/kg J147 significantly reduced AWR scores of IBS mice ($p < 0.05$) (Figure 4 A). These results suggest that the pretreatment group with 8-OH-DPAT enhanced the anti-IBS-like effect of 2 mg/kg J147 in the AWR test. Also, AWR scores of the NAN-190 and 10 mg/kg J147 group were significantly increased (Figure 4 B). The findings indicate that the group treated with NAN-190 is capable of counteracting the anti-IBS-like effect observed in the 10 mg/kg dose group during the AWR test. These results imply that the improved effect of J147 on visceral hypersensitivity in IBS mice is related to the 5-HT1A receptor.

## DISCUSSION

Irritable bowel syndrome (IBS) is a disease that affects intestinal function, which often manifest as abdominal discomfort, pain, altered bowel habits, and psychological problems such as depression and anxiety in many patients, and the exact mechanism behind the development of IBS remains completely misunderstood. Currently, it is postulated that IBS involves gastrointestinal motility disturbances, hypersensitivity of the visceral organs, intestinal infections, brain-gut interactions, as well as various other contributory factors [11]. Recent studies have suggested that pain and visceral hypersensitivity associated with IBS may be caused by increased primary sensory afference in the colorectal area [6,12]. In addition, affective disorders in IBS patients may be the result of enhanced peripheral hypersensitivity to primary sensory afference in the central nervous system, resulting in long-term brain damage [13].

In this study, the IBS mouse model was employed based on CACS, to investigate the potential effects of J147 on IBS. The behavioral results of forced swimming and elevated cross maze showed that the mice developed significant depressive and anxiety-like behavior after 22 days of CACS stress process, and the CACS mice simultaneously developed visceral hypersensitivity and intestinal movement disorders.

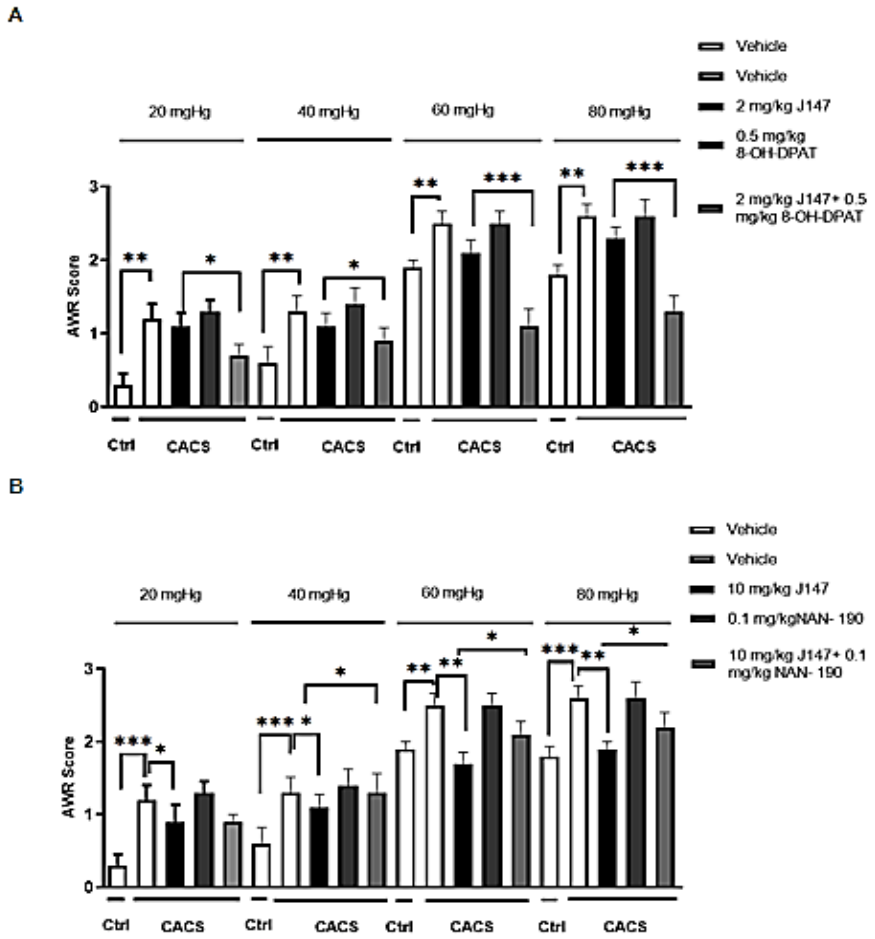


**Figure 4:** The results of AWR score by Co-administration of 8-OH-DPAT or NAN-190 with J147. The results are presented as the mean ± SEM (n = 8-10, per group)

The present study demonstrated that J147 therapy has the potential to reverse brain-gut axis-related behavioral abnormalities induced by CACS. More specifically, it not only alleviates depression and anxiety-related behaviour associated with IBS, it also enhances intestinal function. Furthermore, 5-HT is one of the key neurotransmitters that mediate bidirectional communication between the brain and the intestines. It plays a crucial role not only in emotional regulation within the central nervous system but also in the pathophysiological processes underlying IBS. Multiple studies have revealed that 5-HT plays a pivotal role in gastrointestinal motility and visceral sensitivity among individuals suffering from IBS-D [14,15]. Among the receptors involved in regulating rectal movement, the 5-HT1AR receptor is strongly associated with depression and anxiety [16,17]. Hence, targeting 5-HT and its 5-HT1AR may be a potentially effective approach in treating IBS-like symptoms such as psychiatric disorders and gut dysfunctions.

The study revealed that J147 significantly reduced level of 5-HT in brains of CACS rats, while simultaneously increasing 5-HT in the intestines. This suggests that J147 exerts differential regulation of 5-hydroxytryptamine (5-HT) levels in the brain and intestine. However, the inhibitory effect was observed upon administration of NAN-190 (a hydrobromide antagonist of the 5-HT1A receptor), indicating that 5-HT1A-associated signalling plays a role in regulating brain-gut axis dysfunction. These results suggest that J147 maintains homeostasis by reversing 5-HT content and metabolic abnormalities in the brain-gut axis of IBS. This outcome is in tandem with the behavioral response induced by fluoxetine, suggesting that J147 may improve depression-like symptoms in IBS mice.

## CONCLUSION

In CACS-induced IBS mice, J147 exhibits dual regulatory effect on both the brain and the intestine. Through the modulation of IBS-related proteins, including 5-HT, PKA, pCREB, and BDNF levels in the brain, J147 alleviates depressive and anxiety-related behavior in mice. Furthermore, J147 targets 5-HT1A receptors in the gastrointestinal tract, thereby regulating intestinal 5-HT levels and PKA-pCREB-BDNF signaling pathway.

This modulation ameliorates intestinal hypersensitivity induced by IBS, indicating a promising therapeutic potential for J147 in treating IBS-like symptoms.

## DECLARATIONS

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### *Funding*

None provided.

### *Ethical approval*

None provided.

### *Availability of data and materials*

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

### *Conflict of Interest*

No conflict of interest associated with this work.

### *Contribution of Authors*

The authors declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by them.



## REFERENCES

1. *Enck P, Aziz Q, Barbara G, Farmer AD, Fukudo S, Mayer EA, Niesler B, Quigley EM, Rajilic-Stojanovic M, Schemann M, et al. Irritable bowel syndrome. Nat Rev Dis Primers 2016; 2: 16014.*
2. *Jizhong S, Qiaomin W, Chao W, Yanqing L. Corticotropin-releasing factor and toll-like receptor gene expression is associated with low-grade inflammation in irritable bowel syndrome patients with depression. Gastroent Res Pract 2016; 2016: 7394924.*

3. Ford AC, Sperber AD, Corsetti M, Camilleri M. Irritable bowel syndrome. Lancet 2020; 396(10263): 1675-1688.
4. Bengtson MB, Aamodt G, Vatn MH, Harris JR. Co-occurrence of IBS and symptoms of anxiety or depression, among Norwegian twins, is influenced by both heredity and intrauterine growth. BMC Gastroenterol 2015; 15: 9.
5. Guthrie E, Creed F, Fernandes L, Ratcliffe J, Van Der Jagt J, Martin J, Howlett S, Read N, Barlow J, Thompson D, et al. Cluster analysis of symptoms and health seeking behaviour differentiates subgroups of patients with severe irritable bowel syndrome. Gut 2003; 52(11): 1616-1622.
6. Park BK, Chun E, Choi JJ, Shin Y, Kho YT, Oh SH, Kim SY, Lee TH, Kim TW, Shin E, et al. Administration of Wasabia koreana ameliorates irritable bowel syndrome-like symptoms in a zymosan-induced mouse model. J Med Food 2017; 20(5): 474-484.
7. Xu Y, Ku BS, Yao HY, Lin YH, Ma X, Zhang YH, Li XJ. The effects of curcumin on depressive-like behaviors in mice. Eur J Pharmacol 2005; 518(1): 40-46
8. Hu G, Wang D, Jiang L, Xu L, Zhao L, Zhou M. Curcumin protects hepatocytes from sepsis by regulating inflammatory response and hepatocyte apoptosis. Trop J Pharm Res 2022; 21(1):67-71 doi: 10.4314/tjpr.v21i1.11
9. Liu Y, Dargusch R, Maher P, Schubert D. A broadly neuroprotective derivative of curcumin. J Neurochem 2008; 105(4): 1336-1345.
10. National Research Council. Guide for the care and use of laboratory animals, National Academies Press. Washington, DC; 2010.
11. Cervero F, Laird JM. Understanding the signaling and transmission of visceral nociceptive events. J Neurobiol 2004; 61(1): 45-54.
12. Feng B, La JH, Schwartz ES, Gebhart GF. Irritable bowel syndrome: methods, mechanisms, and pathophysiology. Neural and neuro-immune mechanisms of visceral hypersensitivity in irritable bowel syndrome. Am J Physiol-Gastr L 2012; 302(10): G1085-G1098.
13. Greenwood-van MB. Importance of 5-hydroxytryptamine receptors on intestinal afferents in the regulation of visceral sensitivity. Neurogastroent Motil 2007; 19(2): 13-18.
14. Kennedy PJ, Clarke G, O'Neill A, Groeger JA, Quigley EM, Shanahan F, Cryan JF, Dinan TG. Cognitive performance in irritable bowel syndrome: evidence of a stress-related impairment in visuospatial memory. Psychol Med 2014; 44(7): 1553-1566.
15. Grenham S, Clarke G, Cryan JF, Dinan TG. Brain-gut-microbe communication in health and disease. Front Physiol 2011; 2: 94.
16. Siegert F, Nieber K. New therapeutical approaches for treatment of irritable bowel syndrome. Med Monatsschr Pharm 2010; 33(8): 285-292, 293-294.
17. Dunlop SP, Coleman NS, Blackshaw E, Perkins AC, Singh G, Marsden CA, Spiller RC. Abnormalities of 5-hydroxytryptamine metabolism in irritable bowel syndrome. Clin Gastroenterol H 2005; 3(4): 349-357.