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Exploring Screening Models for Irritable Bowel Syndrome in Drug Research and Development: A Comprehensive Review

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ABSTRACT

Irritable bowel syndrome (IBS) is a widespread digestive condition characterized by visceral hypersensitivity and changes in bowel habits. It is primarily influenced by factors such as smoking, stress, changes in the gut microbiota, and genetic variations. Since no specific cure currently exists for IBS, it is crucial to evaluate the benefits and limitations of current animal models of IBS, to utilize them effectively, and to develop improved models for drug research and development. This paper aims to explore various IBS models designed to replicate the symptoms of the disorder, identify the underlying molecular mechanisms, and leverage these findings for the development of potential treatments. The content of this review was derived from articles and research published between 1981 and 2021, using keywords such as stress, brain-gut axis, trinitrobenzene sulfonic acid, and acetic acid. While IBS lacks a clearly defined cause or treatment, using these models offers the potential for developing effective treatments. The pathogenesis of IBS remains poorly understood, and psychosocial stressors of various kinds are believed to play an important role. Animal models that simulate psychosocial stress, such as neonatal maternal separation, water avoidance stress, and restraint stress, have been developed to mirror IBS symptoms and identify biological pathways associated with the condition. In addition, models that focus on antidiarrheal and anti-inflammatory effects are also employed. Research on these models has led to the development of promising medications for the management of IBS.

Keywords: Stress, Acetic acid, Irritable bowel syndrome, Brain-gut axis

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Introduction

Irritable bowel syndrome (IBS) is a disorder affecting the stomach and intestines, causing symptoms such as abdominal discomfort, irregular bowel movements, and the accumulation of fluid and gas in the stomach. The prevalence of IBS is estimated to range from 1.1% to 45%, making it a highly disruptive condition for those affected [1]. Symptoms can vary from mild to severely debilitating, with women being 1.5 to 2 times more likely to develop the condition than men. While IBS is not associated with mortality [2], its pathophysiology remains poorly understood. However, factors such as increased epithelial permeability, inflammation, visceral hypersensitivity, and disturbances in the brain-gut connection play significant roles in the development of IBS [3]. Despite extensive research, the underlying causes and mechanisms of IBS are still unclear, contributing to the lack of effective treatments. Recently, functional gastrointestinal disorders (FGIDs), including IBS, have been classified as brain-gut interaction disorders **(Figure 1)**, emphasizing the bidirectional communication between the central and peripheral nervous systems, which opens up new avenues for research, particularly using animal models of anxiety and depression [4].

Various stimuli have been shown to play a crucial role in the onset and progression of IBS. Animal models that replicate the pathophysiology and symptoms of IBS are vital for advancing research and could aid in the

development of new therapies. Most IBS animal models are induced using different stress stimuli. These models are categorized into three main types based on the location of the stimulus: central stimulus-induced models, peripheral stimulus-induced models, and complex models that combine both central and peripheral stimuli. Central stimulation affects brain activity, which, in turn, influences gut function through the brain-gut axis. In contrast, peripheral stimulation targets the intestinal nervous system to induce IBS-like symptoms **(Figure 2)**.

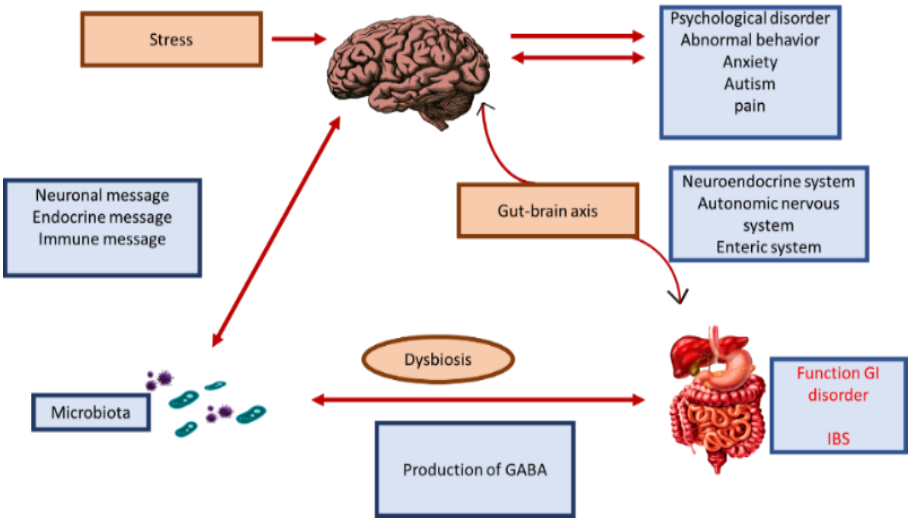

Figure 1. Stress alters brain-gut function and plays a vital role in the development of irritable bowel syndrome

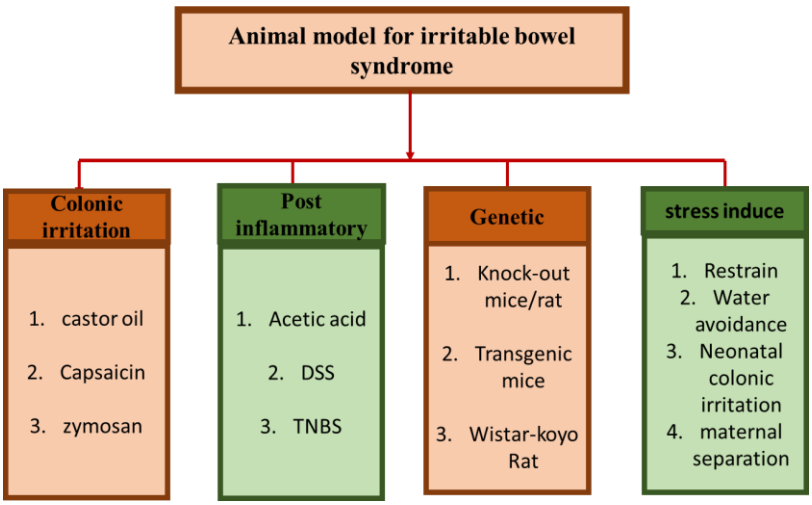

Figure 2. The different Animal models used in the development of irritable bowel syndrome

This paper aims to explore various IBS models designed to replicate the symptoms of the disorder, identify the underlying molecular mechanisms, and leverage these findings for the development of potential treatments.

Results and Discussion

Stress-related IBS model: water avoidance stress (WAS)

Mental stress is a known trigger for IBS, with prolonged or intense stress leading to long-lasting changes in the central nervous system (CNS) that can exacerbate IBS symptoms [5]. A widely used method to induce IBS-like conditions in animals is the water avoidance stress (WAS) model. In this approach, the animal is placed in a Plexiglas tank containing clean, room-temperature water, with a block positioned in the center, raised 1 cm above the water's surface. The animal is kept on the block for one hour daily over ten consecutive days. Research has shown that this procedure can increase visceral hypersensitivity, a hallmark of IBS, in animals **(Figure 3)** [6-8].

The WAS model was initially developed by Bradesi *et al.* [9], who observed a brief somatic pain relief response accompanied by prolonged visceral sensitivity. Later, Da Silva *et al.* [10] refined the method by placing the animals on the block for 4 hours daily over four consecutive days. They found that this extended WAS protocol could induce both visceral hypersensitivity and alterations in gut microbiota, including a reduction in *Lactobacillus farciminis* growth, similar to what is seen in IBS patients. Additionally, a study by Myers and Greenwood-Van Meerveld [11] found that chronic stress, when applied for an hour each day for seven days, resulted in sustained visceral hypersensitivity in rats. This effect was mitigated with the use of glucocorticoid receptor blockers and mineralocorticoid receptor antagonists.

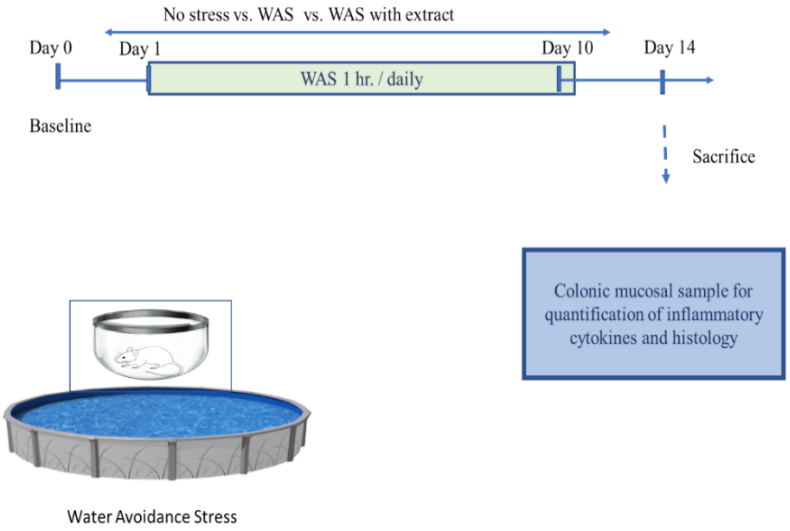

Figure 3. Water avoidance stress (WAS) induced model

Restraint stress-induced animal model

The initial restraint stress model was proposed by Williams *et al.* [12], who confined the upper body of rats for 24 hours. This model demonstrated a decrease in intestinal transport and an increase in fecal output, without the formation of ulcers, making it a commonly used IBS model. Over time, more refined versions of this model have emerged and are widely used for IBS research **(Figure 4)**.

In an alternative approach, Lv *et al.* utilized ether anesthesia to restrain the upper body, including the shoulders, upper arms, and chest, with paper tape for 1 hour, while allowing other body movements. This modified model resulted in enhanced colonic motility, increased frequency of defecation with loose stools, and increased visceral sensitivity, suggesting its effectiveness in studying motility and visceral sensitivity.

Sun *et al.* [13] found that brief restraint stress could cause a temporary increase in nociceptive response, without influencing muscle contraction. Although restraint stress can provoke gastrointestinal changes characteristic of IBS, its suitability for long-term studies is limited, as it can result in somatic injury to the animal.

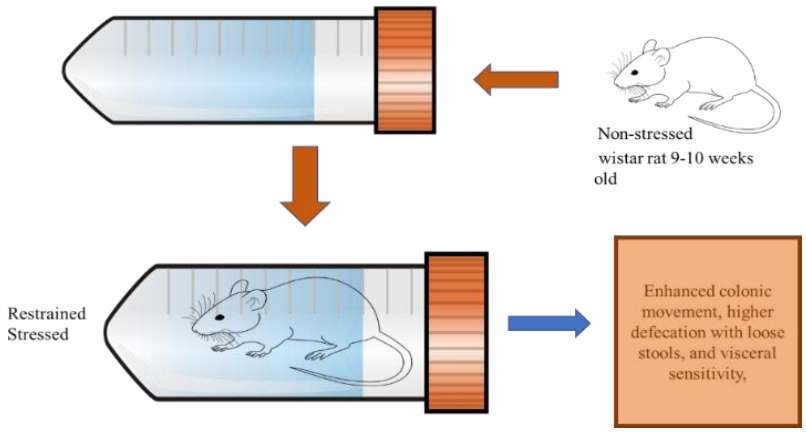

Figure 4. Restraint stress-induced animal model

Neonatal maternal separation (NMS) induced animal model

The neonatal maternal separation (NMS) model involves isolating newborn rats from their mothers for 3 hours daily between postnatal days 2 and 14. This practice leads to the development of visceral hypersensitivity and disrupts the regulation of the hypothalamic-pituitary-adrenal (HPA) axis [14-17]. Barouei *et al.* [15] discovered that NMS increased plasma adrenocorticotropic hormone (ACTH) levels and altered gut microbiota composition, raising the numbers of aerobes, anaerobes, enterococci, clostridia, and *E. coli*, while lowering plasma IgA levels. Further investigations by Miquel *et al.* [16] showed that NMS reduced the population of *Faecalibacterium prausnitzii*, a bacterium crucial for gut health, in C57Bl/AJ mice. Additionally, Zhou *et al.* [17] observed a decrease in *Fusobacterium* abundance, a microbiome change that may contribute to increased visceral hypersensitivity, underlining the microbial shifts linked to stress and their potential role in IBS-like symptom development.

Colonic irritation model of IBS

Castor-oil induced diarrhea

In the castor-oil-induced diarrhea model, rats weighing between 150-250 g are deprived of food for 18 hours and then divided into four groups (n = 6). The first group receives a control treatment of saline (2 mL/kg), while the second group is given Loperamide (2 mg/kg), a standard treatment for diarrhea. The third and fourth groups receive test compounds at doses of 100 mg/kg and 200 mg/kg, respectively. One hour after treatment, all rats are administered 1 ml of castor oil. Over the following 4 hours, researchers measure the frequency of bowel movements and the liquid content of the feces using transparent containers lined with filter paper. The weight of the paper before and after defecation is carefully recorded to assess the impact of each treatment on the diarrhea symptoms and overall bowel activity **(Figure 5)** [18].

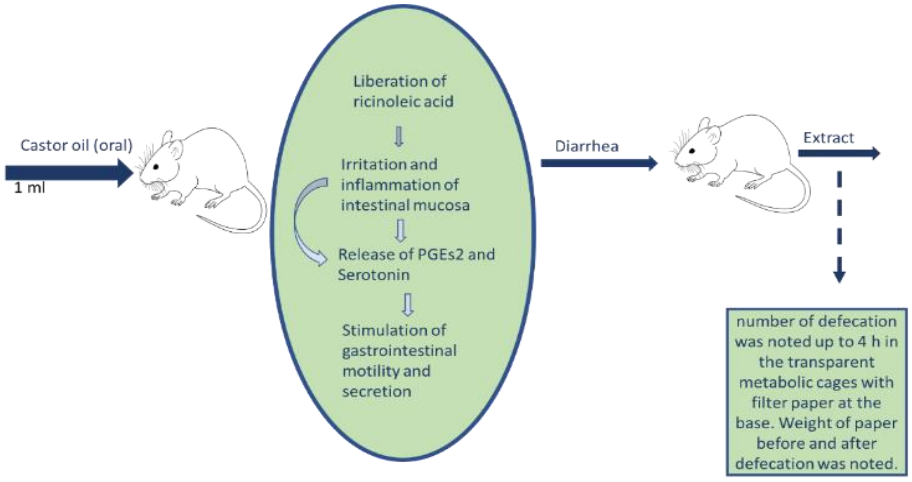

Figure 5. Castor-oil induces colonic irritation that leads to the diarrheal condition

Zymosan-induced generalized inflammation (ZIGI) model

Zymosan is derived from the *Saccharomyces cerevisiae* yeast cell wall and is composed mainly of polysaccharides (73%), along with proteins (15%), lipids, and inorganic substances (7%) [19]. When administered to animals, zymosan activates a wide range of inflammatory responses [20]. In rodents, a dose of 0.8–1.0 mg/g body weight of zymosan injected intraperitoneally triggers a three-phase inflammatory illness. Initially, rats develop acute peritonitis, displaying symptoms such as ruffled fur, increased defecation, sluggishness, and reduced muscle mass. During this phase, the animals exhibit leukopenia, elevated oxygen consumption, and increased myeloperoxidase levels, indicating neutrophil activity in the lungs and peritoneum [21-23], along with heightened endothelial permeability [24].

Inflammatory IBS model

NSAIDs are often used to treat inflammation but can cause gastrointestinal side effects like irritation, ulcers, and bleeding. This has led to increased research into natural anti-inflammatory agents. For instance, poncirin has

demonstrated significant pain relief in various inflammatory pain models, showing its promise as a potential analgesic for inflammatory conditions [25, 26].

Acetic acid-induced colitis

The acetic acid-induced colitis model is a widely used method to simulate IBS, resembling many aspects of clinical inflammatory bowel disease (IBD) [27-29]. It involves introducing a dilute acetic acid solution into the rectum, causing a non-transmural inflammatory response. This includes neutrophil infiltration, cell death in the mucosal and submucosal layers, blood vessel dilation, edema, and ulcer formation, all of which are symptoms commonly seen in human colitis **(Figure 6)** [30-38].

In this model, acetic acid induces severe epithelial damage due to acidification. The procedure involves anesthetizing the rodents and fasting them for 24 hours. Afterward, 12 mL of a 3-4% acetic acid solution is infused 5-6 cm into the rectum. The acid is retained for a brief period before being removed, and the animals are sacrificed. Both blood and colon samples are collected for subsequent histological and biochemical analysis after 24–48 hours [37, 39-43]. Some studies also use a dose of 4 mL of 4% acetic acid at 5 mL/kg to induce colitis in rats [44-48].

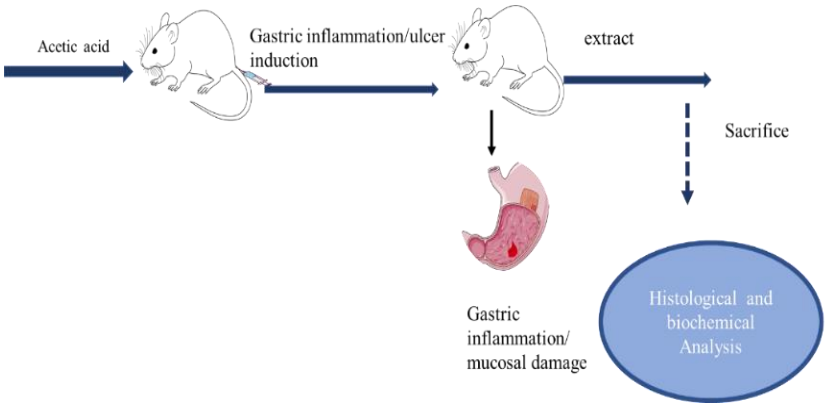

Figure 6. Acetic acid produces inflammation and gastric mucosal damage

Acetic acid–induced writhing test

The acetic acid–induced writhing test is used in mice to evaluate the peripheral pain response. In this method, acetic acid injection induces abdominal contractions. After administering the final dose of medication, 10 mL/kg of a 0.6% acetic acid solution is injected intravenously. The animals are then allowed to rest for 5 minutes, after which the number of writhing episodes is recorded over 10 minutes [49].

2, 4, 6-Trinitrobenzene sulfonic acid (TNBS)

TNBS induces transmural inflammation in the gut and triggers immune responses that resemble those seen in IBD patients [50, 51]. A common technique involves administering 10 mg of TNBS mixed with 0.25 mL of 50% ethanol via a catheter to male and female Wistar rats at a site 8 cm from the anal margin. Rats are held in a face-down position for 13 minutes after the TNBS infusion to ensure even distribution and minimize leakage. The rats are sacrificed 2–6 days later for histological and immunohistochemical analysis of colonic inflammation [51, 52]. Some studies modify the procedure by adjusting the TNBS dose and alcohol content to vary the severity of IBD in rats.

Miscellaneous models of IBS

Various combined animal models have been created to better mimic the complex and multifactorial nature of IBS. For example, Zhuang *et al.* [53] combined acetic acid administration with restraint stress to induce gut visceral hypersensitivity, increased IL-4 and IL-9 levels, and enhanced mast cell degranulation. Additionally, Spreadbury *et al.* [54] found that chronic WAS stress combined with *C. rodentium* infection significantly increased dorsal root ganglion (DRG) excitability. Due to the complex etiology of IBS, there is an increasing need to develop integrated animal models that incorporate multiple stimuli for pathophysiological and therapeutic research.

Non-rodent models of IBS

While most IBS research focuses on rodents, other species have also been utilized to study the pathophysiology of functional gastrointestinal disorders (FGID) like IBS. Guinea pigs are frequently used to study gut motility and the enteric nervous system. Their models are similar to those in rodents, but include additional stress techniques such as water avoidance and CRH injection [55, 56]. Pharmacological approaches like mustard oil gavage and serotonin or TRH injection have also been used in guinea pigs to simulate altered gastrointestinal transit. Mustard oil increases upper GI transit time (e.g., esophagus) and decreases lower GI transit time (e.g., colon) when administered orally [57].

In rabbits, intracolonic zymosan infusion causes intestinal irritation, which is alleviated by NK2 tachykinin receptor antagonists [58, 59]. Pigs, which share a more similar gastrointestinal system with humans, are increasingly used for studies of the GI response to early life stress. Unlike rodents, pigs have a more complex enteric nervous system and a sophisticated central nervous system (CNS) that allows them to better replicate human reactions to psychosocial stimuli [60, 61]. The stressful experience of weaning in pigs, which causes both physical and psychological stress, leads to gut barrier dysfunction and is often used in IBS studies [62, 63].

Behavioural testing

To assess the behavioral responses in animals, a sequence of psychological tests is conducted, including the Y maze, elevated-plus maze, and forced swim test **(Figure 7)**. These tests are commonly used to evaluate anxiety, stress, and other psychological responses related to IBS.

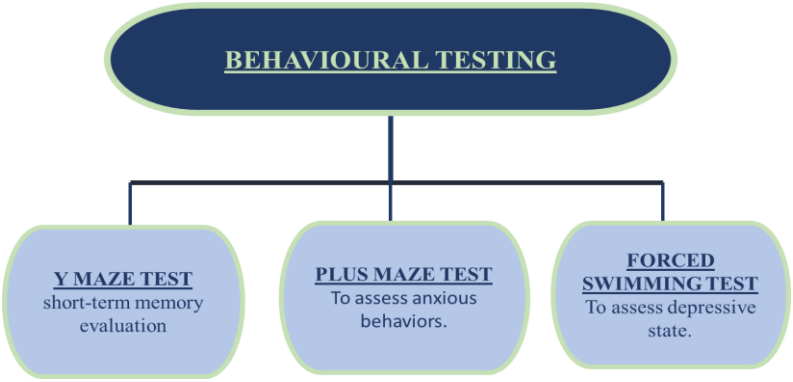

Figure 7. Psychological testing evaluated anxiety and depressive states in rodents

Y-Maze

The Y-maze test is used to assess short-term memory by evaluating the exploration activity of animals within a Y-shaped apparatus. The maze consists of three sections (40 cm in length, 8 cm in diameter, and 15 cm tall) connected at 120-degree angles, with an equilateral triangular center. During the test, the rat is placed at the end of one section and allowed to explore freely for 8 minutes. The test measures spontaneous alternation as an indicator of short-term memory, where the animal's ability to alternate between different sections is tracked [64].

Elevated-plus maze

The elevated-plus maze (EPM) is used to examine anxiety-like behaviors. This maze consists of a cross-shaped apparatus raised 50 cm above the ground. Two of the four sections are enclosed by 30 cm high fences, while the other two are open. The rat is placed at the intersection of the open and closed sections and given 5 minutes to explore. During this time, the time spent in each section, the number of entries, and the overall movement are recorded to assess anxiety-like behavior [65].

Forced swim test

The forced swim test (FST) is an altered version of Porsolt's method used to evaluate behavioral distress in rodents. In this test, the animal is placed in a transparent cylindrical glass container (30 cm in diameter, 59 cm tall) filled with water at 26 °C, with the water level about 15 cm high. The animal is left in the container for 6 minutes, with the first 2 minutes for acclimatization and the remaining 4 minutes used to observe behavioral responses, including swimming, floating (inactivity), and struggling behavior. These actions are indicative of depression-like behavior in rodents [66].

Conclusion

The pathophysiology of IBS remains complex and not entirely understood due to its multifactorial nature. Human intervention studies are not possible for many aspects of IBS research, making animal models a valuable tool in studying IBS without the ethical and practical challenges of human studies. Each animal model has its advantages and limitations, and the choice of model often depends on specific research needs, such as pharmacological requirements and the desired outcomes. These models have been crucial in the development of potential therapeutic options for IBS management.

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Ethics Statement: None

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