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Review Article

Preclinical experimental models for assessing laxative activities of substances/products under investigation: a scoping review of the literature

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Abstract: Constipation is a common gastrointestinal problem worldwide. Its impact on health can range from an unpleasant problem to being seriously troublesome. When lifestyle modification fails to deal with constipation, laxatives are the mainstay of therapy. There are several types of laxatives currently available; however, there still remains a need for better laxatives because certain currently available laxatives are not appropriate for or accessible to some patients. Preclinical experiments to study the laxative potential of substances/products of interest are vital to improving that situation. The selection of appropriate experimental models for assessing the laxative activities of substances/products under investigation is crucial to achieving valid and meaningful results. This article provides a scoping review of the literature, outlining, and summarizing models currently being used in preclinical experiments assessing the laxative activities of substances/products under investigation. The review includes both screening models, e.g., the isolated organ bath system, *in vivo* fecal assessment and intestinal transit assay, and confirmation models, e.g., *in vivo* constipation models. Chemical substances/drugs used to induce constipation in *in vivo* constipation models, e.g., loperamide, diphenoxylate, montmorillonite, and clonidine, as well as standard laxative agents used as a positive control in experimental models, e.g., bisacodyl, carbachol, lactulose, sodium picosulfate, castor oil, phenolphthalein, and yohimbine, are described in detail. The purpose of this article is to assist researchers in the design and implementation of preclinical experimental models for assessing laxative activities of substances/products under investigation to achieve valid and meaningful preclinical results prior to experimentation in humans.

Keywords: Laxatives, constipation, preclinical drug evaluations, drug screening

Introduction

Constipation is a common and troublesome condition, with a rising prevalence worldwide in tandem with aging populations [1]. It is characterized by having hard feces and incomplete defecation. The pathophysiology of constipation is complicated and poorly understood. Pain, fever, dehydration, food and fluid intake, toilet training, drugs or supplements, e.g., calcium, and daily behaviors, may all possibly be causes of constipation [2]. Primary constipation is caused by defects in colonic and/or anorectal function [3]. It can be present in functional defecation disorder, slow-transit constipation, and constipation-predominant irritable bowel syndrome [4]. Secondary constipation, i.e., constipation that occurs as a result of an underlying health issue or a side effect of medication use, can be associated with adverse drug reactions, bowel obstruction, metabolic disorders, neurological disorders, systemic disorders, or psychological disorders [5]. Although constipation is not a life-threatening condition, it can significantly affect the patient's quality of life and contribute to substantial economic burden [6]. The World Gastroenterology Organization provides clinical practice guidelines for the management of constipation, including a number of interventions that can be used to

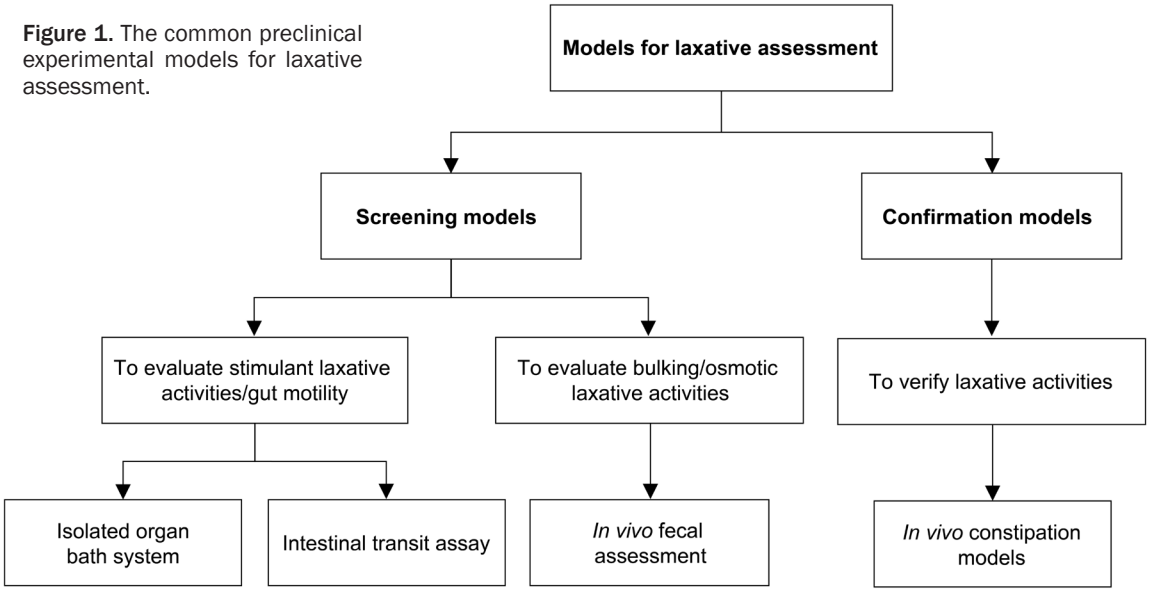

Figure 1. The common preclinical experimental models for laxative assessment.

support defecation through various mechanisms [7].

Laxatives are the mainstay of therapy for constipation when lifestyle modifications, e.g., changes in diet and increased physical activity, are not sufficient to relieve symptoms. Laxatives can be classified into several types based on modes of action, including bulking laxatives, osmotic laxatives, and stimulant laxatives [8]. Bulking laxatives generally contain a high amount of unabsorbable fiber which provides a supplementary source of fecal mass, whereas osmotic laxatives are a type of stool softener that works by increasing water-holding capacity to produce stools that are softer and easier-to-pass [9]. Stimulant laxatives, on the other hand, induce intestinal motility via stimulation of intestinal nerves and muscles or through activation of ion channels of the intestinal epithelium resulting in an influx of fluid and electrolytes into the intestinal lumen [9].

Not all laxatives are safe for long-term use and not all patients respond to the currently available laxatives [10]. For instance, abdominal discomfort is one of the most prevalent adverse effects of bisacodyl, a stimulant laxative [8]. The development of new laxative agents is needed to provide alternative options that offer better efficacy, fewer adverse effects, or even lower cost [11]. Preclinical experiments which provide information on the laxative potential of substances/products under investigation prior to testing in humans are essential. At present, several models have been developed and are being used in the assessment of laxative activities of substances/products under investigation [12, 13]. However, many experiments are costly, time-consuming and may result in unnecessary use of experimental animals. A selection of potentially appropriate preclinical experimental models for assessing laxative activities of substances/products under investigation could help assure valid and meaningful preclinical results prior to experimentation in humans.

This article offers a scoping review of the literature to provide a map and summary of preclinical experimental models used to examine laxative activities of substances/products under investigation.

Preclinical experimental models for determining laxative activities

An overview of preclinical experimental models commonly used to investigate laxative activities of the substances/products is provided in **Figure 1**. The models can be classified into two groups based on their main purpose: screening or verification [12, 14]. Screening models principally aim to explore the laxative potential of substances/products of possible interest for further investigation, while confirmation models are designed to verify the laxative activity of substances/products [15]. The screening mod-

els involve experiments conducted under physiological conditions which are used to make an initial determination of the laxative potential of the substances/products under investigation, i.e., whether or not they have a laxative effect [12]. In regard to ethical aspects, screening models usually provide preliminary data that can help justify further experimentation and minimize the number of animals necessary in confirmation experiments [16].

Isolated organ bath system

The isolated organ bath system is a classical model used primarily for screening purposes to identify substances/products with stimulant laxative activities [17]. With the organ bath system, intestinal smooth muscle and intestinal cells function like an intact intestine in the body [18]. In an excised section of intestine, basal contraction occurs through the activity of the interstitial cells of Cajal, pacemaker cells located within circular and longitudinal muscles, via the production of spontaneous bioelectrical slow waves [19]. This basal contractile activity is modulated by the enteric nervous system (ENS), which can be induced by the excitatory neurotransmitters [20]. Stimulant laxatives can directly stimulate the enteric nerve plexus, leading to peristaltic contraction [21]. This intestinal contraction can be observed when substances/products with stimulant laxative activity are applied. Because this system is simple, convenient, and reproducible and because it operates in the absence of external influences, e.g., circulating hormones or extrinsic nervous system, it can effectively respond to the substances/products under investigation [22].

The main instrumental setup of an isolated organ bath system is comprised of an isolated section of intestine (approximately 3 cm long), an organ bath, and a tension transducer connected to an analog-to-digital converter and display systems (**Figure 2**). Measured concentration-tension curves are increased in the presence of contractile inducers or substances/products that have stimulant laxative activity.

Beyond the basic principles of contractile evaluation, this technique can be modified to enhance its versatility. For instance, a perfusion system has been developed to measure the net fluid absorption and to maintain tissue viability so that the section of intestine can be used over a prolonged period [23]. Data analysis software integrated with computer-based data recording and/or an image sensor/processing algorithm can help improve contractility analysis [24]. However, an isolated organ system can only be used with substances/products that are hydrophilic and can dissolve well in water to form aqueous solutions because the isolated intestine sections are entirely submerged under a fluidic organ bath system.

The rate of intestinal propulsion differs in the various different parts of the gastrointestinal (GI) tract due partly to the uneven distribution of the various enteric regulatory mechanisms, including myogenic and neural control systems [25]. Although feces are largely formed in the large intestine, the isolated small intestine is a preferable model for screening laxative activities of substances/products under investigation [26, 27]. The rationale behind this is that the spontaneous contractile activity of this segment is dominant, so contraction of the small intestine can be readily observed if the substances/products have stimulant laxative activities [26, 27]. Furthermore, intestinal peristalsis in the small and large intestines shares a similar mechanism, that is, stimulation of the intestinal epithelium causes enterochromaffin cells to release serotonin, which, in turn, drives a cascade of muscle contractions via neurotransmitters, e.g., acetylcholine [28].

Prior to testing substances/products, a reference level of the maximum intestinal contraction in the system should be obtained. The isolated intestinal segments should initially be tested against known chemical substances for induction of maximum intestinal contraction. Acetylcholine, a major neurotransmitter of ENS, is a standard reference which is commonly used for this purpose in an isolated organ bath system [26]. It acts by binding to muscarinic acetylcholine receptors, including M1 and M3 subtypes of the intestinal smooth muscles, leading to G protein-coupled receptor-mediated activation of the cascade involving phospholipase C [29]. In addition to acetylcholine, other drugs that act on muscarinic or serotonin receptors, e.g., bethanechol, may also be used for the induction of maximum intestinal contraction to obtain a reference for each experiment [30]. With the isolated organ bath system, a single intestinal segment can be used in mul-

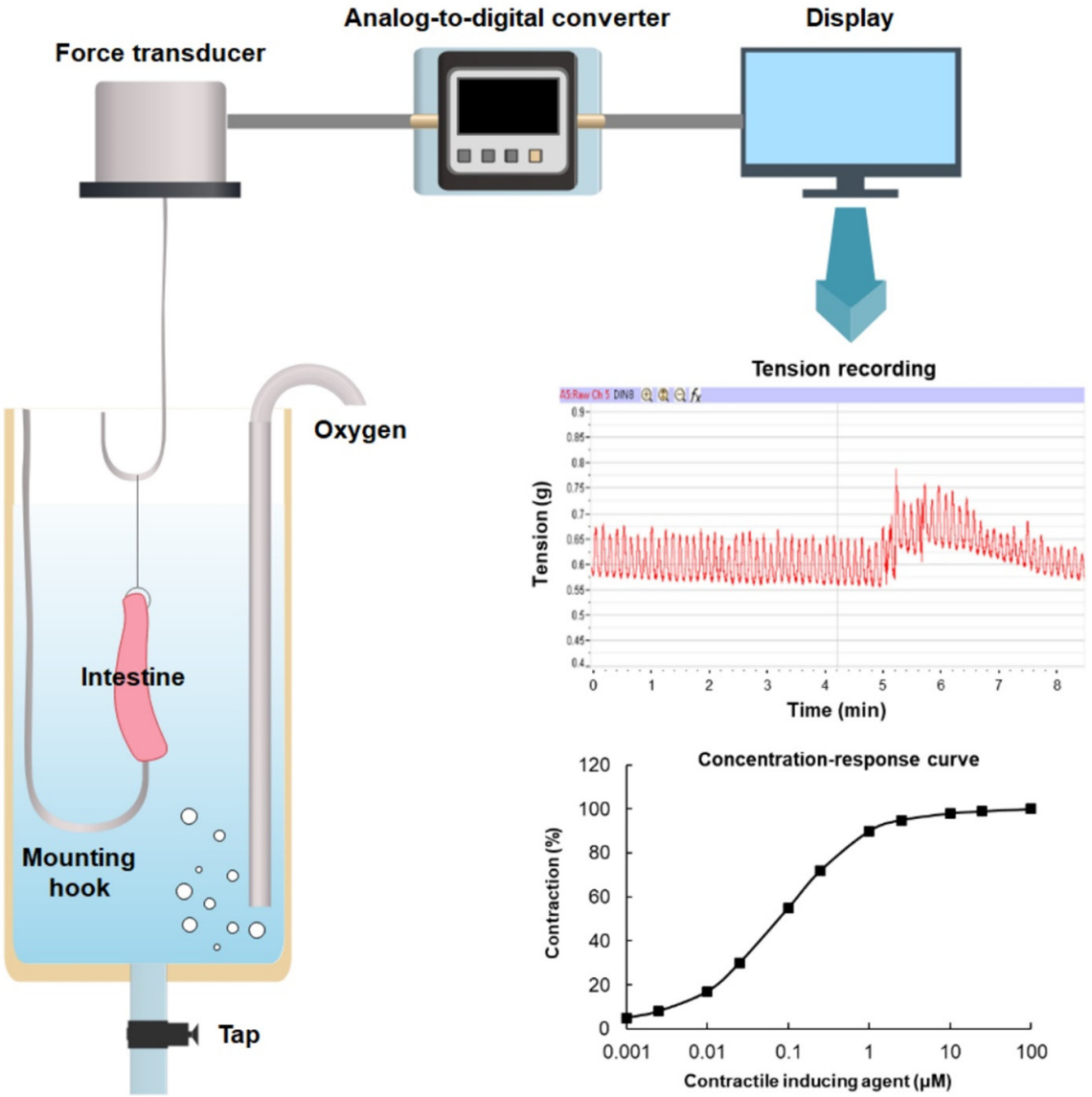

Figure 2. Instrumental diagram and the concentration-response curve of the isolated organ bath system.

tiple experiments until the tissues lose their response to the contractile inducer.

It is noteworthy that the isolated organ bath system can also be used to define some underlying mechanisms of the substances/products under investigation [31, 32]. GI motility is partly regulated by the ENS and some hormones [33]. Therefore, pretreatment with known intestinal receptor agonists or antagonists can potentially help researchers identify additional specific mechanisms responsible for stimulant laxative activities of the substances/products under investigation. For example, following pretreatment with atropine (a muscarinic antagonist) [26], mepyramine (a histamine H1 receptor antagonist) [31, 32], methysergide (a serotonin receptor antagonist) [32], or ondansetron (also a serotonin receptor antagonist) [31], the isolated organ bath system can be used to determine whether or not the laxative effects of the substances/products are attributable to that receptor-mediated response. Another example is calcium channel blockers such as verapamil and nifedipine which can be used as a pretreatment in the isolated organ bath system to determine whether or not the substances/products under investigation act through stimula-

tion of calcium channels resulting in intestinal contraction [29, 31]. It should be noted, however, that some chemical agents with broad-spectrum agonistic/antagonistic effects, e.g., cyproheptadine, should not to be used to pretreat a model because it may not be possible to differentiate the specific receptors attributable to the laxative effects of the substances/products under investigation [34].

In vivo fecal assessment

In vivo fecal assessment in mice or rats under physiological conditions can primarily be used to determine the bulking and/or osmotic laxative activities of substances/products under investigation [9, 12]. This involves an examination of fecal parameters, including the amount of feces, fecal weight, and fecal water content. An increase in any of these parameters following administration of substances/products under investigation may be considered a signal indicative of the laxative potential of such substances/products. The average transit time from ingestion to excretion in rodents is approximately 6-7 hours [35]. The Bristol stool scale scoring system is one of the most widely used and well-accepted tools for rapid evaluation of fecal appearance in rodents and in other species whose feces resemble human feces [15, 36].

For an *in vivo* fecal assessment to be as valid as possible, a metabolic cage is recommended for qualitative and quantitative examination of fluid intake, fecal parameters, and urine output of mice/rats. The feces collected from this type of cage can be assumed to be free of environmental contamination, making it suitable for valid fecal assessment and for further advanced analytical techniques, such as microbiota analysis and metabolomics analysis [37, 38]. However, on occasion some mice/rats may not adapt well to a metabolic cage which may result in stress that could interfere with their normal physiology [39]. To avoid that possibility, mice/rats should first be acclimatized in a metabolic cage for a period of time prior to experimentation.

Intestinal transit assay

The intestinal transit assay is a validated model used primarily to determine the GI motility and peristaltic response following administration of substances/products under investigation [40]. In this assay, oral administration of detectable and unabsorbable tracers is required, followed by direct observation of their first appearance in the feces or postmortem measurement of their movement speed in the GI tract. Tracers commonly used in intestinal transit assays include charcoal [41], phenol red [42], India ink [43], radiopaque tracers, e.g., barium sulfate or chromium 51 [44, 45], carmine red [46], fluorescent tracers [47], and steel beads [48]. These tracers can be used to directly track the distance of gut motility at a specific time following euthanasia. For a particular experiment, tracers are selected depending on the availability of equipment as well as the simplicity, sensitivity, and visibility for detection of the different options.

There are numerous types of intestinal transit assays, most of which are based primarily on the specific area in the GI tract to be observed [12, 49]. A whole gut transit assay may best represent the overall GI motility along the GI tract, but it may require a long observation period [42, 50]. The small intestine transit assay may be a preferable option in certain circumstances as it requires a shorter period of time to conduct one experiment [49, 51]. Thanks to the long length of the small intestine, this type of assay can be used to assess substances/products causing excessive GI motility since the tracers may remain in the small intestine for up to 5 hours [52]. The small intestine transit assay is highly accurate and precise in estimating the rate of GI motility at a specific time point. The transit ratio can be calculated using the distance traveled by the tracer and the total length of the intestine. The intestinal transit assay is illustrated in **Figure 3**.

In vivo constipation models

To verify the laxative activities of substances/products under investigation, *in vivo* constipation models should be used as a confirmation model. A mouse/rat constipation model involves inducing constipation in experimental mice or rats and evaluating the therapeutic effects of the substances/products under investigation [14, 15]. There are a number of chemical substances/drugs that can be used to induce constipation in mice/rats (see Section 3). Changes in phenotypic characteristics of experimental mice/rats following administration of a constipation inducer can be observed

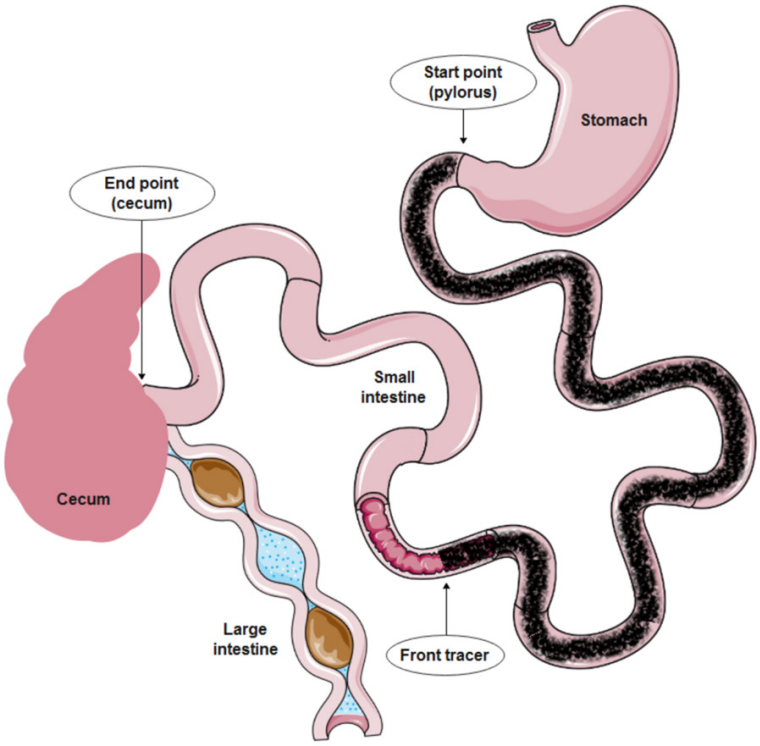

Figure 3. Schematic diagram of intestinal transit assay.

through alterations in the amount of feces, fecal weight, and/or fecal water content of the animals [43, 53]. Histological alterations in drug-induced constipated mice/rats can be observed under a microscope. The changes may include, e.g., a decrease in the thickness of the mucosa and the muscular layers and mucin secretion in the transverse colon [13, 44]. The molecular patterns of mRNA and/or protein expression as well as any downstream signaling pathways associated with constipation can also be explored to identify mechanisms associated with the action of the substances/products [54].

In vivo constipation models can also be developed using techniques other than the drug-induced technique, e.g., low-fiber diet-induced constipation and intestinal irritation with ice-cold saline [15, 55-58]. The latter technique can cause GI dysfunction, delayed intestinal transit time, and reduced fecal weight because ice-cold saline can cause an intestinal irritation which interferes with the ENS in the small and large intestines [15]. This alteration subsequently increases the level of intestinal nitric oxide, one of the gut inhibitory neurotransmitters, which leads to a decrease in GI motility. Such models may be considered as an alternative for confirmation of the *in vivo* laxative activities of the substances/products, including their laxative potential.

In a recent publication, Kim *et al.* observed obesity-induced constipation in CRISPR-Cas9-mediated leptin knockout mice [13]. The knockout mice displayed distinct constipation phenotypes in GI motility, histopathological changes, and protein expression associated with constipation in the transverse colon. This observation suggests the possibility for *in vivo* investigation of laxative activities of substances/products against obesity-induced constipation using CRISPR-Cas9 techniques. However, the link between leptin deficiency and the molecular mechanism of constipation needs to be elucidated and further validation studies of this novel model are needed.

Summary of the preclinical experimental models with examples of implementation

The models mentioned above are useful for screening or verifying the laxative potential of substances/products under investigation. To facilitate the selection of optimal models, the advantages, disadvantages, and limitations of each model are summarized in **Table 1**.

Previously, development of laxative drugs regularly required preclinical models for determining laxative mechanisms prior to conducting clinical trials. For instance, in 1998 a novel prokinetic drug R093877 (prucalopride) developed by the Janssen Research Foundation was tested using an isolated organ bath assay in guinea pig and rat colonic segments [59]. This demonstrated a stimulant effect of prucalopride on the intestinal contraction and verified its selective targeting of the 5-HT_4 receptor when the selective 5-HT_4 antagonist GR 113808A was added. The experiment was expanded to include testing of isolated guinea-pig, canine, and human intestinal sections,

Table 1. Advantages, disadvantages, and limitations of laxative models

Model	Advantages	Disadvantages and limitations
Isolated organ bath system	Uses very small amounts of substances/products	Requires many instrument setups
	Can be used to test many substances/products in multiple experiments until the tissues become unresponsive	May show no contractile response when spontaneous contraction of the intestines is greater than that induced by the substances/products
	Can be modified to evaluate receptor-mediated intestinal contraction	Cannot test substances/products that do not dissolve in water
	Mimics an intact intestine in the body without interfering with external influences	Cannot evaluate bulking and osmotic activities
	Can use several intestinal sections from one animal	
In vivo fecal assessment	Simple to observe fecal parameters	Requires many animal cages per experiment (one animal per cage)
	Affordable instruments	Requires to keep track of the amount of food and water consumption
	Can be used in multiple experiments using the same set of animals	Requires workforce to observe wet feces as soon as possible to avoid water evaporation
	Not necessary to euthanize animals which can be transferred to other experiments after a washout period of 7 days	
Intestinal transit assay	Easy to track an ingested tracer in the GI tract	Provides only one result from each animal
	Requires a short period of time for one experiment to be done	Can only be performed on euthanized animals (except when using radiopaque or fluorescent tracers, which require advanced imaging techniques for live animals)
		Should be done within a specific time before the tracer moves into the large intestine
In vivo constipation model	Verifies the overall laxative effects on constipation	Mimics secondary constipation but not primary constipation
	Appropriate for experiments in various dimensions, e.g., fecal parameters, molecular assays, signaling pathways, and pathology	Takes time to induce constipation which may result in different symptom levels of constipation in each animal
		Requires model optimization to avoid the strong symptoms of constipation that cannot be relieved by substances or products

which confirmed the role of prucalopride as the selective 5-HT_4 receptor agonist [60, 61]. An intestinal transit assay in rats was conducted by tracking the distance moved by an activated charcoal suspension in the small intestine and showed a significant acceleration of gut motility [62]. These preclinical findings propelled prucalopride research into clinical trials ranging from healthy volunteers to patients with constipation [63, 64]. In 2018, prucalopride was approved by the US FDA for chronic constipation treatment.

Notably, there was no *in vivo* constipation model used in the early research on prucalopride. Similar to the study of the novel selective 5-HT_4 receptor agonist YH12852, the preclinical experiment was conducted with intestinal transit assays in guinea pigs involving tracking of a charcoal mixture in the small intestine to assess the upper GI transit [65]. The lower GI transit was also evaluated by assessment of fecal parameters. A second publication confirmed YH12852 as a selective 5-HT_4 receptor agonist by using organ bath assay in guinea pig isolated distal colons [66]. Later, clinical trial experiments were conducted after preclinical assays [67, 68]. In summary, the intestinal transit assay is currently one of the best available models and provides sufficient preliminary information about the laxative potential of substances/products under investigation prior to conducting further research in clinical trials. In addition, the organ bath assay is a confirmation method for determining the specific target receptor of a novel drug.

The development of preclinical models for laxative assessment has recently been growing. *In vivo* constipation models have been developed to determine the potential of substances/products in counteracting constipation. For instance, *in vivo* drug-induced constipation models were initially characterized in 2009 [69]. Since then, the CRISPR-Cas9-mediated leptin knockout mice model has been able to create constipation characteristics [13]. It is noteworthy that the most recent methodology to investigate laxative activities of substances/products regularly includes constipation models together with intestinal transit assay to obtain various dimensions of results, including gut motility and underlying mechanisms [70-73]. As a result, future preclinical constipation models will be able to imitate any aspect of constipation in a way that is close to the condition of human in both phenotypic and genotypic characteristics and are expected to become a critical tool for gathering information prior to conducting research in humans.

Constipation-inducing substances

Chemical substances/drugs commonly used to induce constipation in animal experiments include loperamide, diphenoxylate, montmorillonite, and clonidine [53, 54, 72, 74-77]. Details of each chemical substance/drug and representative examples of their use in animal experiments are summarized in **Table 2**. It should be noted that such chemical substances/drugs can be applied not only in a drug-induced constipation model but also in intestinal transit assays for the purpose of slowing gut motility [78-80].

Loperamide, a potent μ-opioid receptor agonist, is the most common drug used to induce constipation in animal models [72, 74-78, 81-92]. It acts to decrease GI motility and peristalsis, and reduce intestinal fluid, resulting in a reduction in the amount of feces, fecal weight, and fecal water content [93, 94]. Histopathological changes in the transverse colon, i.e., a decrease in the length of both mucous membrane and muscular layers, are also observed following loperamide administration [54].

Diphenoxylate is another opioid receptor agonist that is often used to induce constipation in experimental animals [79, 95-100]. It stimulates μ-opioid receptors in the GI tract, resulting in a decrease in GI motility, fecal parameters, and intestinal fluid as well as a delay in intestinal transit time [101]. It has been observed that rats treated with diphenoxylate have decreased fecal parameters and delayed intestinal transit time, even at a hundred days post-treatment [79]. Another feature of diphenoxylate-induced constipation is that the drug can contribute to a decrease in short-chain fatty acid levels in the colon [102]. For that reason, this model may be preferable for experiments searching for bulking laxatives such as substances/products with high dietary fiber content [96].

Montmorillonite, a naturally adsorbent clay mineral isolated from bentonite, is an antidiar-

Table 2. Summary of chemical substances/drugs commonly used to induce constipation in experimental animals

Agent	Animal strain	Route of administration	Dose and period of usage	References
Loperamide	Sprague-Dawley rats	s.c.	3-4 mg/kg BW, twice daily, for 3-7 days	[53, 54, 72, 74-77, 109, 111, 133-135]
			16 mg/kg BW, twice daily, for 1 week followed by once daily, for 1 week	[136]
		p.o.	3-5 mg/kg BW, once daily, for 6-7 days	[81-83]
			2-4 mg/kg BW, twice daily, for 1-2 weeks	[44, 84, 85]
		i.g.	1.5 mg/kg BW, twice daily, for 7 days	[110]
	Wistar rats	p.o.	5-10 mg/kg BW, single dose	[107, 137, 138]
			3 mg/kg BW, once daily, for 3 days	[80]
			3 mg/kg BW, twice daily, for 5 days	[89]
		i.p.	1-4 mg/kg BW, twice daily, for 3-6 days	[88, 90, 139]
		i.g.	5 mg/kg BW, single dose	[140]
	Unspecified rat strains	p.o.	4 mg/kg BW, once daily, for 2 weeks	[141]
		i.p.	5 mg/kg BW, twice daily, for 5 days	[142]
	BALB/c mice	s.c.	10 mg/kg BW, twice daily, for 14 days	[87]
		p.o.	5-10 mg/kg BW, single dose	[78, 86]
		i.p.	5 mg/kg BW, single dose	[32]
		i.g.	10 mg/kg BW, once daily, for 17 days	[43]
	ICR mice	s.c.	4 mg/kg BW, twice daily, for 4 days followed by 8 mg/kg BW, twice daily, for 4 days	[92]
		p.o.	10 mg/kg BW, single dose	[143]
			5 mg/kg BW, once daily, for 3 days	[144]
	Kunming mice	i.g.	1.5 mg/kg BW, once daily, for 15 days	[145]
	Swiss albino mice	p.o.	5 mg/kg BW, single dose	[146]
			3-5 mg/kg BW, once daily, for 3-6 days	[93, 94, 147]
Diphenoxylate	Sprague-Dawley rats	p.o.	5 mg/kg BW, single dose	[95]
		i.g.	10 mg/kg BW, once daily, for 20 days	[148]
	Wister rats	i.g.	8 mg/kg BW, single dose	[79]
	C57BL/6J mice	i.g.	5 mg/kg BW, for 1 week	[96]
	ICR mice	p.o.	ICR mice (6-8-week-old; 18-22 g): 50 mg/kg BW, single dose	[149]
		i.g.	ICR mice (18-22 g): 50 mg/kg BW, once daily, for 7 days	[100]
	Kunming mice	p.o.	30 mg/kg BW, once daily, for 3 days	[99]
		i.g.	10 mg/kg BW, once daily, for 5-14 days	[97, 98]
			10 mg/kg BW, twice daily, for 2 weeks	[150]
Montmorillonite	Kunming mice	Unspecified	30 mg/kg BW, once daily, for 3 days	[103, 104]
Clonidine	Wistar rats	i.p.	2 mg/kg BW, single dose	[107]
	Slc:ddY mice	s.c.	0.03 mg/kg BW, single dose	[106]

Abbreviations: BW, body weight; i.g., intragastric administration; i.p., intraperitoneal injection; p.o., per oral; s.c., subcutaneous injection.

rheal agent that is sometimes used to induce constipation in animal experiments [103]. A high dosage of montmorillonite can coat the surface of the gut lumen and interact with the mucosal proteins, thereby lessening the moisture content in the GI tract. This feature leads to a decrease in GI motility and an increase in stool hardness [104].

Clonidine, an α2 adrenergic receptor agonist, affects the sympathetic nervous system and leads to relaxation of intestinal smooth muscle [105]. Mice/rats treated with clonidine manifest signs of constipation such as a diminished amount of feces, decreased fecal weight, and delayed intestinal transit time [91, 106, 107]. Due to its mechanism of action which involves the nervous system, clonidine is sometimes the drug of choice to mimic constipation associated with neurological diseases [108].

Positive controls for laxative assessment

The use of standard laxative drugs as a positive control in any experimental models is critical in light of the fact that any model should demonstrate that it is valid. Drugs commonly used for this purpose include bisacodyl, carbachol, lactulose, sodium picosulfate, castor oil, phenolphthalein, and yohimbine (**Table 3**). Nevertheless, it is not uncommon to see experiments in which no standard laxative agent was applied as a positive control [54, 72, 97, 103]. In those cases, it might be acceptable for the study to substantiate the laxative activity of certain substances/products under investigation if those substances/products demonstrate potent laxative effects against constipation [104, 109-111].

Bisacodyl is a stimulant laxative drug which works by stimulating the ENS to enhance the peristaltic contractions of the GI tract [112]. It also has an osmotic laxative property as the drug can decrease the expression of aquaporin-3 water channels in the colon, resulting in a decrease in water absorption and, as a result, an increase in fecal water content [113]. Notably, bisacodyl is the drug most commonly used as a positive control in experimental models for laxative assessment [56, 84, 85, 114, 115].

Carbachol is a parasympathomimetic agent which acts as an agonist on intestinal muscarinic receptors, particularly M2 and M3, and on nicotinic receptors [116-118]. Carbachol can be used in an intestinal transit assay as a positive control to enhance GI motility [78]. It is also considered an alternative drug for use as a positive control in *in vivo* laxative models [86].

Lactulose is a type of osmotic laxative. It helps to soften the stool and pass the stool out during a bowel movement [119]. Lactulose is a galactose- and fructose-based synthetic disaccharide, so it can be metabolized by colonic bacteria [120]. This results in an increase in GI motility through gas formation and osmolality, which activates intestinal osmoreceptors in the intestinal lumen leading to an increase in intestinal motor activity [121]. Recent studies have shown that lactulose is effective against loperamide-induced constipation and that it can restore normal fecal parameters and the intestinal transit rate in a few weeks [87, 93].

Sodium picosulfate is known as a colorectal cleansing agent [122]. It is an unabsorbable prodrug and requires colonic gut microbiota to metabolize it into the active compound *bis*-(p-hydroxyphenyl)-pyridyl-2-methane (BHPM) which can stimulate peristalsis in the GI tract [123]. However, the stimulant laxative effect of sodium picosulfate is sometimes unpredictable because the conversion of sodium picosulfate into its active metabolite depends largely on the gut flora of experimental animals [8].

Castor oil is effective at relieving constipation and is regarded as a stimulant and irritant laxative [124]. The laxative activity of castor oil is primarily mediated by ricinoleic acid, the main fatty acid in castor oil [125]. Evidence has shown that ricinoleic acid can activate E-type prostanoid receptors and stimulate intestinal peristalsis [124]. Owing to its potent effect on gut motility, castor oil is also commonly used as a diarrhea inducer in animal experiments, and castor oil-induced diarrhea is one of the standard methods used to investigate anti-diarrheal activities of substances/products of interest [126-128].

Phenolphthalein had been used to treat chronic constipation due to its stimulant laxative activity [129]. The drug affects production of eicosanoids and acts on the Na^+/K^+-ATPase pump on the surface of enterocytes leading to a reduction of fluid absorption in the GI tract [8].

Table 3. Summary of standard laxative drugs commonly used as a positive control in constipation models

Experimental model	Positive control	Animal strain	Dose and period of usage	References
Screening models				
Isolated organ bath system	None			[31, 32, 151-154]
	Bisacodyl	Isolated rat colon	0.025 mg/ml	[56]
	Carbachol	Isolated rat ileum	0.001-100 μM	[94, 155, 156]
		Isolated rat colon	10 μM	[142]
		Isolated mouse colon	1 μM	[144]
		Isolated rabbit jejunum	0.3 μM	[29]
In vivo fecal assessment	None			[74, 109, 137, 142, 145]
	Bisacodyl	Wister rats	0.25 mg/kg BW, i.g.	[114]
		Swiss albino mice	5 mg/kg BW, p.o., single dose	[115]
	Carbachol	BALB/c mice	1 mg/kg BW, p.o., single dose	[29, 78]
			1 mg/kg BW, i.p., single dose	[86]
		Swiss albino mice	1 mg/kg BW, i.p., single dose	[157]
	Lactulose	BALB/c mice	30 mg/kg BW, p.o., single dose	[158]
	Sodium picosulfate	Sprague-Dawley rats	25 mg/kg BW, p.o., single dose	[159]
		Swiss albino mice	5 mg/kg BW, p.o., single dose	[146]
	Castor oil	Swiss albino mice	0.3 ml/animal, p.o., single dose	[160]
		Unspecified mouse strain	10 ml/kg BW, i.g., single dose	[154]
Intestinal transit assay	None			[54, 72, 74, 77, 81, 94-97, 103, 104, 109, 111, 142, 143, 145, 161, 162]
	Bisacodyl	Sprague-Dawley rats	5 mg/kg BW, p.o., single dose	[56]
			3.3-5.5 mg/kg BW, p.o., once daily, for 2-4 weeks	[44, 82, 84, 85]
		Wister rats	0.21 mg/kg BW, p.o., once daily, for 7 days	[80]
			20 mg/kg BW, p.o., once daily, for 30 days	[79]
		Kunming mice	100 mg/kg BW, p.o., once daily, for 14 days	[163]
		Swiss albino mice	5 mg/kg BW, p.o., single dose	[115]
	Carbachol	BALB/c mice	1 mg/kg BW, p.o., single dose	[78]
			1 mg/kg BW, i.p., single dose	[29]
		Swiss albino mice	1 mg/kg BW, i.p., single dose	[157]
	Lactulose	Sprague-Dawley rats	4-8% in drinking water for 4 weeks	[134]
		BALB/c mice	10 mg/ml in drinking water for 2 weeks	[87]
		ICR mice	500 mg/kg BW, p.o., once daily, for 14 days	[144]
	Sodium picosulfate	Sprague-Dawley rats	5 mg/kg BW, p.o., once daily, for 6 days	[83]
	Castor oil	Swiss albino mice	0.3 ml/animal, p.o., single dose	[146, 160]
	Phenolphthalein	BALB/c mice	70 mg/kg BW, i.g., once daily, for 17 days	[43]
		Kunming mice	70 mg/kg BW, i.g., once daily, for 14 days	[98]
	Yohimbine	Wistar rats	1-2 mg/kg BW, i.p., single dose	[89, 90, 107, 138]

Confirmation models				
Loperamide-induced constipation	None			[32, 53, 54, 72, 74-77, 81, 86, 88, 92, 94, 109-111, 138, 141, 143, 161]
	Bisacodyl	Sprague-Dawley rats	3.3-5.5 mg/kg BW, p.o., once daily, for 2-4 weeks	[44, 82, 84, 85]
		Wistar rats	0.21 mg/kg BW, p.o., once daily, for 7 days	[80]
	Lactulose	Sprague-Dawley rats	4-8% in drinking water for 4 weeks	[134]
		BALB/c mice	10 mg/ml in drinking water for 2 weeks	[87]
		ICR mice	500 mg/kg BW, p.o., once daily, for 14 days	[144]
	Sodium picosulfate	Sprague-Dawley rats	5 mg/kg BW, p.o., once daily, for 6 days	[83]
	Castor oil	Wistar rats	2 ml/kg BW, i.g., once for 9 hours	[140]
	Phenolphthalein	BALB/c mice	70 mg/kg BW, i.g., once daily, for 17 days	[43]
	Yohimbine	Wistar rats	2 mg/kg BW, i.p., once daily, for 5 days	[89, 90]
Diphenoxylate-induced constipation	None			[95-97]
	Bisacodyl	Wister rats	20 mg/kg BW, p.o., once daily, for 30 days	[79]
		Kunming mice	100 mg/kg BW, i.g., once daily, for 14 days	[99]
	Phenolphthalein	Kunming mice	70 mg/kg BW, i.g., once daily, for 14 days	[98]
			400 mg/kg BW, i.g., twice daily, for 14 days	[150]
Montmorillonite-induced constipation	None			[103, 104]
Clonidine-induced constipation	None			[91]
Low-fiber diet-induced constipation	None			[55]
	Bisacodyl	Sprague-Dawley rats	2.5 mg/kg BW, p.o., once daily, for 3 days	[56]
Ice-cold saline-induced constipation	None			[15, 57, 58]

Abbreviations: BW, body weight; i.g., intragastric administration; i.p., intraperitoneal injection; p.o., per oral; s.c., subcutaneous injection.

However, phenolphthalein is no longer used as a laxative agent in humans due to its carcinogenic potential; presently, its use is limited to animal experiments [8, 130].

Yohimbine, an alkaloid found in numerous botanic sources, is an α2 adrenergic receptor antagonist [131]. It increases the release of acetylcholine by the presynaptic neurons, leading to an increase in colonic contraction [132]. Yohimbine can be used as a positive control in loperamide-induced constipation as it can increase GI motility and stool amounts in experimental rats [89, 90].

Conclusions

This article provides a scoping review of preclinical experimental models commonly used for assessing laxative activities of substances/products under investigation. The isolated organ bath system, *in vivo* fecal assessment, and/or intestinal transit assays can aid in the selection of substances/products of interest for further investigation in confirmation models. Substances/products with laxative potential should be further investigated in *in vivo* constipation models, where each substance/product under investigation can be tested against constipation in an entire living organism. Exploration of the mechanisms and/or pathways responsible for laxative activities can provide support for further development of laxative candidates in clinical trials.

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Disclosure of conflict of interest

None.

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