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Potential Laxative Effects of Dried Plums (Prunes) and Red Beetroot on Loperamide-Induced Constipation in Adult Rats

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ABSTRACT:

Constipation is a prevalent gastrointestinal problem affecting individuals' life quality. Prunes and red beetroot are traditional remedies for constipation. This study investigated the potential laxative effects of prune powder (PP), beetroot powder (BP), and their mixture (PBM) on constipation in rats. Forty-two adult rats were divided into two main groups. Group (1, n=6), the normal control, was fed the basal diet (BD), and group (2, n=36) was given loperamide (LOP) to induce constipation and subdivided into six equal subgroups. Group (I), the constipated control, was fed BD. The remaining constipated subgroups fed experimental diets containing PP (groups II&III) or BP (groups IV&V) at 5.0 and 10.0% w/w of the BD, respectively. While group (VI) was given an experimental diet containing PBM (in equal ratios) at 10.0% of the BD. LOP administration resulted in a significant ($p \leq 0.05$) reduction in water intake by -30.46%, fecal parameters (number of pellets, wet weight, dry weight, and water content by -44.81%, -48.06%, -31.82%, and -32.89%, respectively), gastrointestinal motility by -35.91%, and markedly increased gastrointestinal transit time by 35.20%. Additionally, it caused disruptions in serum lipid profile, liver and kidney functions in constipated control rats compared to normal rats. Feeding the constipated rats with the experimental diets significantly ($p \leq 0.05$) restored these parameters toward normal levels without causing diarrhea, most effectively with the PBM diet. In conclusion, PP and BP relieved LOP-induced constipation in rats, providing a scientific justification for their traditional use.

Keywords: *Constipation, prunes, red beetroot, fecal parameters, gastrointestinal motility, gastrointestinal transit time*

1. INTRODUCTION

Constipation is a prevalent issue in public health that significantly affects individuals' overall quality of life and healthcare systems [1]. It ranks as the sixth most frequent gastrointestinal (GI) disorder worldwide [2]. The global prevalence of constipation is estimated between 12% and 19% [3], while critically ill patients experience a higher incidence rate ranging from 16% to 83% [4]. Furthermore, it is prevalent in 50% of

community-dwelling elderly individuals and rises to 74% among residents in nursing homes [5]. Constipation is defined as a reduction in defecation frequency to less than three times per week, accompanied by symptoms such as discomfort during bowel movements, incomplete elimination, abdominal bloating, prolonged or unsuccessful attempts to defecate, hardened stools, and post-defecation discontent [6]. Its pathophysiology is complicated and involves various factors such as dietary habits, disruptions in colon movement, genetic predisposition, certain medications, lifestyle habits, and psychological stress [7].

Constipation is not only discomforting but can lead to restlessness, vomiting, abdominal bloating, blockages in the intestines, and even perforation. Additionally, it may also be associated with aspiration or fatal pulmonary embolism [8]. Moreover, critically ill patients who experience constipation may encounter several hazards, including the excessive growth of bacteria in the digestive system, which has detrimental effects on the colon mucosa and disrupts the ability to tolerate an enteral diet. These complications can lead to increased mortality rates due to delayed weaning from mechanical ventilation, intestinal obstruction, aspiration pneumonia, and prolonged hospitalization [9]. Furthermore, the prolonged accumulation of toxic substances in the body can contribute to the development of various intestinal disorders, including irritable bowel syndrome and colorectal cancer [10]

Laxatives are the most commonly prescribed medications for constipation management when lifestyle modifications, such as dietary changes, fiber-rich foods, and increased physical activity and fluids, prove insufficient to alleviate symptoms [11]. It can be categorized into numerous classifications according to their mechanisms of action, including osmotic laxatives, bulking laxatives, and stimulant laxatives [12]. Osmotic laxatives function as a stool softener by improving the water-holding capacity, producing softer stools that are easier to excrete. While bulking laxatives typically consist of a substantial quantity of non-absorbable fiber, which serves as an additional source of fecal mass. On the other hand, stimulant laxatives stimulate intestinal motility by either stimulating intestinal nerves and muscles or activating ion channels in the intestinal epithelium, leading to an inflow of electrolytes and fluid into the intestinal lumen [13].

Long-term use of laxatives may not be safe, and not all patients respond positively to existing laxatives [14]. It frequently causes a variety of adverse effects, such as stomach upset, stomach cramping, vomiting, and diarrhea. Specifically, osmotic laxatives containing ions with poor absorbability, like phosphate or magnesium, can induce metabolic disturbances, especially in

cases of renal dysfunction [15]. Also, Tegaserod, a medication for chronic constipation, has side effects like coronary artery constriction, coronary spasms, and, in some cases, myocardial infarction [16]. Furthermore, Zhao et al. [17] reported that the repeated use of laxatives containing anthraquinone glycosides may lead to the development of melanosis coli, a risk factor for colorectal neoplasms. As a result, there is a need to develop new laxative substances to offer choices that offer better effectiveness, fewer side effects, or lower costs [18]. Several traditional remedy approaches for constipation are available, such as osmotic laxatives present in fiber-rich foods, the use of bulking agents like psyllium, stimulant laxatives like senna, and stool softeners found in prunes, grapes, plums, apricots, and peaches [19].

Dried plums, commonly referred to as prunes, are cultivar fruits of Prunus domestica L. Prunes are considered a nutritious food option due to their reduced fat content and significant presence of essential nutrients such as carbohydrates, minerals, and vitamins [20]. Prunus species, in addition to their nutritional content, contain significant concentrations of bioactive dietary compounds, including phytosterols such as **β**-sitosterol and stigmasterol, along with polyphenols like phenolic acids, flavonols, and flavan-3-ols [21]. Furthermore, plums contain a high concentration of phenolic compounds, particularly neochlorogenic and chlorogenic acids. These compounds may contribute to a laxative effect and delay glucose absorption. Anthocyanins and flavonoids are predominantly found in the fruit's skin and exhibit powerful antioxidant, anti-inflammatory, anticarcinogenic, and antidiabetic effects. Consequently, they play a crucial role in addressing issues related to blood circulation, obesity, diabetes, osteoporosis, digestive disorders, and measles, in addition to preventing cardiovascular disease and possessing neuroprotective properties [20, 22].

Beetroot, scientifically known as *Beta vulgaris L.*, is a biennial herbaceous plant belonging to the *Chenopodiaceae* family. Its taproot is available in two colors: yellow pulp and red [23], with the red root commonly used in juices, salads, food coloring, and medicinal applications [24]. Moreover, across different food cultures, it is usually consumed in various forms, including as supplemental juice, pickled, oven-dried, powdered, pureed, boiled, or jam-processed [25]. Recent research has strongly suggested that beetroot consumption has favorable physiological impacts on improving certain clinical conditions like hypertension, atherosclerosis, type 2 diabetes, and dementia [26]. Beetroots are recognized as highly beneficial vegetables as a rich source of phytochemicals and bioactive compounds, containing betalain pigments such as betacyanins (red-violet

color) and betaxanthins (yellow-orange color) along with phenolic acids like syringic, gallic, and caffeic acids, as well as flavonoids, and inorganic nitrate (NO3). These components contribute to their antioxidant and anti-inflammatory properties, effectively scavenging free radicals, contributing to cancer prevention, aiding in the expulsion of kidney stones, controlling diabetes and insulin hemostasis, lowering the risk of cardiovascular diseases, and lowering systolic and diastolic blood pressure [27, 28].

There is growing attention to natural dietary effective substances that have the potential to relieve constipation with minimal adverse effects. Prunes and red beetroot are traditional remedies for constipation. However, to our knowledge, there is limited data on their effectiveness, and no studies have investigated their synergistic laxative effects. Therefore, the current study was designed to explore the potential laxative properties of prunes, red beetroot, and their mixture on constipation induced by loperamide (LOP) in adult male albino rats.

2. Materials and methods

2.1 Materials

2.1.1 Prunes and red beetroot samples

Dried plums (prunes) and fresh red beetroot (*Beta Vulgaris L.*) root samples were purchased from the local market in Sheibin El-Kom city, Menoufia governorate, Egypt. The prune samples were in sealed packets with over six months of shelf life. The B. vulgaris root samples underwent authentication by the Botany Department, Faculty of Sciences, Menoufia University.

2.1.2 Chemicals and reagents

Loperamide hydrochloride, Coomassie blue dye, and carmine were obtained from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). Casein, serving as the primary protein source for rats' diet preparation, was supplied by Morgan Company for Chemicals in Cairo, Egypt. Vitamins, salt mixtures, choline chloride, cellulose, and L-methionine were procured from El-Ghomhorya Company for Trading Drugs, Chemicals, and Medical Requirements, Cairo, Egypt. Biochemical assay kits were sourced from Alkan Medical Company in St. El Doky, Giza, Egypt. All other chemicals and reagents used in this study were acquired from El-Ghomhorya Company for Trading Drugs, Chemicals, and Medical Requirements, Cairo, Egypt, and were of analytical grade or the highest commercially available purity.

2.1.3 Experimental animals

Healthy forty-two adult male albino rats of the Sprague–Dawley strain, aged 8 weeks and weighing 155 - 165 g, were procured from the Laboratory Animal Unit at the College of Veterinary Medicine, Cairo University, Egypt. The rats were housed individually in stainless steel cages placed in a well-ventilated laboratory under typical controlled conditions:

Temperature range of 20–23 °C, humidity levels of 50–60%, and a light/dark cycle of 12/12 hours. The animals underwent a one-week acclimatization before the commencement of the experiments, during which they were allowed free access to a basal diet and water ad libitum.

2.2 Methods

2.2.1 Preparation of prunes and beetroot powders

Prunes and red beetroots were carefully cleaned and washed, sliced into small pieces, and subjected to a drying process at a temperature of 50°C in an Alab Tech oven under vacuum conditions (Model No. Lvo-2040-Korea) until a constant weight of dehydrated samples was achieved. The dried prunes and red beetroot were finely crushed using an electric mill (Moulinex Egypt, ElAraby Co., Benha, Egypt) and sifted through 80 mesh screens (British Standard Sieve). The obtained fine powders were transferred to polyvinyl chloride containers, sealed, and kept away from moisture and light till used in experimental diet preparation.

2.2.2 Approximate nutritional composition

The nutritional composition of the prunes and red beetroot samples was analyzed using standard methods to determine moisture (oven drying), protein (total nitrogen), fat (Soxhlet), ash (Muffle furnace), and fiber (Enzymatic-gravimetric, AOAC 991.43), following the appropriate methods outlined in AOAC [29]. The difference method was used to calculate total carbohydrates: Total carbohydrates (%) = 100 - (%moisture + %protein + %fat + %ash). The available carbohydrates were determined by subtracting crude fiber from total carbohydrates, while energy values were calculated based on the obtained macronutrient data, multiplying macronutrients by Atwater factors.

2.2.3 Total phenolic content (TPC)

The TPC of both prune and beetroot samples was assessed using the standard spectrophotometric Folin–Ciocalteu reagent, following the procedures outlined by Jaćimović et al. [30], with minor modifications. The TPC was presented as milligrams of gallic acid equivalents per 100 grams of fresh weight samples (mg GAE/100g).

2.2.4 Total flavonoid content (TFC)

The TFC of prune and beetroot samples was determined spectrophotometrically using the standard aluminium chloride method, as outlined by Jaćimović et al. [30], with minor modifications. Results were expressed as milligrams of rutin equivalents per 100 grams of fresh weight samples (mg RE/100g).

2.2.5 Basal diet (BD) and experimental diets

The BD was prepared according to the method described by Reeves [31], using the AIN-93M diet specifically designed for adult rodents. The experimental diets were formulated using either prune powder (PP), beetroot powder (BP) at concentrations of 5.0% and 10.0% (w/w) of the BD, respectively, or their mixture

(PBM) in a (1:1, w/w ratio) at a concentration of 10.0% (w/w) of the BD. The selected tested concentrations were based on previous studies by Gallaher and Gallaher [32] and Johnson et al. [33]. According to the approximate estimated nutrient composition of prunes and red beetroot (as shown in Table 1), the protein, carbohydrates, and fat contents in the experimental diets were carefully adjusted to match those of the BD. Additionally, all experimental diets were approximately isocaloric and had the same energy distribution from carbohydrates, protein, and fat as the BD.

2.2.6 Constipation induction

The experimental animals were orally administered a 1 ml dose of LOP (3 mg/kg BW) dissolved in normal saline (0.9% sodium chloride solution) using a metal oropharyngeal cannula to induce constipation. This administration was performed once a day for three consecutive days, as prescribed by Wintola et al. [34]. On the other hand, normal rats received only a saline solution. The passage of hardened and dry feces and the decrease in the total weight and number of fecal pellets after the LOP treatment course compared with those in normal rats were considered indicators of the successful establishment of the constipation model in rats.

2.2.7 Experimental design

Following a one-week adaptation period, the rats were divided randomly into two main groups: the normal group (6 rats) and the constipation model group (36 rats). Rats in the normal group received a vehicle treatment of 1 ml of saline solution once/three days and fed the BD. The constipation model group was treated with LOP and subsequently subdivided into six subgroups, each comprising six rats, and subjected to the following treatments:

Group (I): served as the constipated control rats and remained on the BD.

Groups (II and III): received experimental diets containing PP at 5.0% and 10.0% w/w of the BD, respectively.

Groups (IV and V): were provided with experimental diets comprising BP at 5.0% and 10.0% w/w of the BD, respectively.

Group (VI): was fed an experimental diet consisting of a mixture of prune and beetroot powders (PBM) 1:1, w/w at 10.0% w/w of the BD.

During the seven days of experimental dietary interventions, the feed intake, water consumption, body weight gain, and fecal parameters of each rat in all experimental groups were recorded. Additionally, following the 7-day treatment with the experimental diets, the gastrointestinal transit GIT time (indicating defecation time), GIT ratio (reflecting GI motility), and serum biochemical assays were evaluated, as illustrated in Figure (1).

2.2.8 Changes in water intake, feed intake, and body weight

During the seven-day treatment period, each rat's daily water volume intake, feed consumption, and body weight were recorded at 10:00 a.m. Feed intake and

body weight were measured using an electronic balance, and water volume was quantified using a measuring cylinder. The average intake of the feed and water was then computed. Furthermore, the body weight gain (BWG) was calculated by subtracting the body weight at the initiation of the experimental dietary intervention from the body weight at the end of the experiment period.

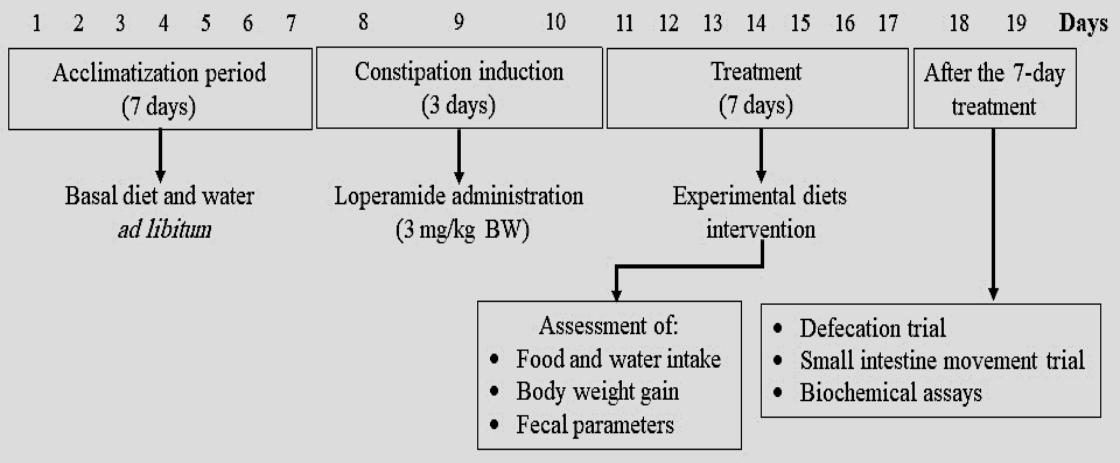


Figure 1. The schedule of experimental trials conducted in the study

2.2.9 Assessment of fecal parameters

Subsequently, the fecal water content was calculated using the following formula:

$$\text{Fecal water content (\%)} = \left(\frac{(\text{Fecal wet weight} - \text{Fecal dry weight})}{\text{Fecal wet weight}}\right) \text{x}100$$

2.2.10 Defecation trial: Assessment of GIT time

The GIT time was assessed using the method outlined by Lee et al. [35], with slight modifications. Following a 12-hour fasting period on day 18, the rats were given unrestricted access to diets blended with 1 g of 10% Coomassie blue dye and water. The duration starting from the initiation of feeding the rats with the Coomassie-mixed diet until the first excretion of blue-colored fecal pellets within 6 hours was recorded as GIT time.

2.2.11 Small intestine movement trial: Assessment of GIT ratio

The GIT ratio was evaluated using the method described by Nafiu et al. [36] and Zhai et al. [37] with minor modifications. On the 19th day, the rats again underwent an overnight fasting period. Subsequently, the following morning, all rats received 1 ml of carmine (60 mg/ml) dissolved in normal saline as a marker via oral administration. After one hour of marker administration, all rats were humanely euthanized. The entire length of the small intestine, extending from the pyloric sphincter to the cecum, was determined. Furthermore, the distance from the pylorus to the carmine frontier was measured as the carmine movement

distance. The GIT ratio was calculated using the following formula:

$$\text{GIT ratio (\%)} = \left(\frac{\text{Transit distance of carmine (cm)}}{\text{Whole length of the small intestine (cm)}}\right) \text{x}100$$

2.2.12 Blood sampling

Blood samples were drawn from the portal vein during the small intestine movement trial while the rats were under light anesthesia and placed in sterile and dry centrifuge tubes. After clotting for 30 minutes at room temperature, the samples were centrifuged at 3000 rpm for 15 minutes for serum separation. The serum was then carefully extracted and transferred to sterile Eppendorf tubes, which were stored at -20°C for further biochemical assays.

2.2.13 Biochemical assays

Liver function markers: Liver enzyme activities were assessed spectrophotometrically using commercially available kits. The measurement of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) was conducted following the method outlined by Moss and Henderson [38]. While alkaline phosphatase (ALP) activity was estimated using the method described by Bergmeyer and Harder [39].

Kidney markers: Serum creatinine and urea levels were assessed following the procedures outlined by Houot [40].

Serum lipid profile: Total cholesterol (TC), triglycerides (TG), and high-density lipoprotein cholesterol (HDL-c) were assessed following the methods described by Allain et al. [41], Fossati and Prencipe [42], and Demacker et al. [43], respectively. Additionally, as outlined by Lee and Nieman [44], very low-density lipoprotein cholesterol (VLDL-c) and low-density lipoprotein cholesterol (LDL-c) were calculated using the following formulas:

VLDL-c = TG/5

LDL-c = Total Cholesterol - (HDL-c + VLDL-c)

2.2.14 Statistical analysis

The Statistical Package for Social Sciences (SPSS, version 22) was used to analyze the obtained data. Results were expressed as means ± standard deviation (SD). Differences between experimental groups were determined by a one-way analysis of variance (ANOVA) and Duncan's multiple-range test. Values were considered statistically significant at p-value ≤ 0.05.

The percentage (rate) of changes between constipated control rats and normal control rats was determined to assess the severity of spastic constipation caused by LOP treatment. Likewise, to further evaluate the laxative effects of the tested experimental diets, the rate of changes between the constipated rats treated with experimental diets and constipated control rats was calculated as follows:

$$\text{\% of change compared with normal control (\%)} = \left(\frac{(\text{Constipated control rats} - \text{Normal control rats})}{\text{Normal control rats}}\right) \text{x}100$$

$$\text{\% of change compared with constipated control (\%)} = \left(\frac{(\text{Constipated rats treated with experimental diets} - \text{Constipated control rats})}{\text{Constipated control rats}}\right) \text{x}100$$

2.2.15 Ethical approval

All the experimental and animal care protocols were ethically approved by the Institutional Animal Care and Use Committee (IACUC) at Menoufia University, Sheibin El-Kom, Egypt (Approval No. MUFHE/F/NFS/20/23). All biological experiments were performed in compliance with the policies of the IACUC for the use and care of laboratory animals.

3. Results

The approximate nutritional composition, TPC, and TFC of prune and red beetroot samples in wet and dry weight is shown in Table (1). Regarding the nutritional composition of prunes sample per 100 g wet weight, the results indicated that prunes are a nutritious food characterized by elevated levels of carbohydrates (56.47 ±5.45 g) and protein (2.31 ±0.24 g), along with low-fat content (0.3 ±0.04 g). This combination positions prunes as a moderately calorie-dense source, providing 237.8 ±15.63 kcal per 100 grams. Furthermore, prunes boast a high crude fiber content (7.8 ±1.03 g), TPC (184.56 ±14.77 mg GAE/100g), and TFC (147.21 ±6.9 mg RE/100g). Concerning the nutritional value of fresh red beetroot, it was clear that beetroot is a good source of carbohydrates (8.7 ±1.15 g) and protein (1.5 ±0.12 g) while being low in fat (0.15 ±0.02 g) and high fiber content (2.4 ±0.14 g), making it a potentially low-calorie source (42.15 ±4.22 kcal/100 g). The TPC and TFC were 262.81 ±17.29 mg GAE/100g and 275.63 ±18.9 mg RE/100g, respectively. Moreover, the water and ash content constituted 86.2 ±9.63% and 1.05 ±0.14%, respectively.

Table 1. Approximate nutritional composition, TPC, and TFC of prunes and red beetroot (per 100 g)

Components	Prunes		Red beetroot	
	Wet weight	Dry weight *	Wet weight	Dry weight *
Water content (g)	31.7 ±3.2	----	86.2 ±9.63	----
Ash content (g)	1.42 ±0.12	2.08	1.05 ±0.14	7.61
Calorie (kcal/100 g)	237.8 ±15.63	348.17	42.15 ±4.22	305.43
Total carbohydrates (g)	64.27 ±7.81	94.10	11.1 ±1.87	80.43
Available carbohydrate (g)	56.47 ±5.45	82.68	8.7 ±1.15	63.04
Protein (g)	2.31 ±0.24	3.38	1.5 ±0.12	10.87
Total fat (g)	0.3 ±0.04	0.44	0.15 ±0.02	1.09
Crude fiber (g)	7.8 ±1.03	11.42	2.4 ±0.14	17.39
Total phenolic content (TPC)**	184.56 ±14.77	270.22	262.81 ±17.29	1904.42
Total flavonoid content (TFC)#	147.21 ±6.9	215.53	275.63 ±18.9	1997.32

Data represent the mean values of three replicates ± SD. Available carbohydrates were calculated by difference; Energy (kcal) was calculated by multiplying macronutrients by Atwater factors. Dry weight = component per wet weight / (100% – water content %)×100. ** mg gallic acid equivalent (GAE)/ 100 g sample; # mg rutin equivalent (RE)/100 g sample.*

The effect of PP, BP, and PBM diets on body weight gain (BWG), feed intake (FI), and water intake (WI) in constipated rats is presented in Table (2). The data clearly showed that administering LOP (3 mg/kg BW) orally for three days resulted in a

significant ($p \leq 0.05$) increase in BWG by 61.06% and a notable ($p \leq 0.05$) decrease in WI by -30.46% in the constipated control group compared to the normal control group. However, there were no significant differences regarding FI between all experimental groups. Interestingly, treating constipated rats with the experimental diets that contained PP, BP at concentrations of 5.0 and 10.0%, and PBM at 10.0% exhibited a significant ($p \leq 0.05$) decrease in BWG compared to the constipated control rats with rates of decrease by -25.99, -35.70, -28.64, -33.10, and -37.47%, respectively. Conversely, WI notably ($p \leq 0.05$) increased by 27.38, 29.34, and 32.46% with PB, BP, and PBM diets at 10.0%, respectively, compared to the constipated control group. Nonetheless, diets containing PP and BP at 5.0% did not show significant differences compared to the constipated control group. Additionally, there were no significant differences in BWG and WI among all constipated groups treated with the various experimental diets.

Table 2. Effect of PP, BP, and PBM diets on body weight gain (BWG), feed intake (FI), and water intake (WI) in constipated rats

Experimental groups		BWG (g/rat)		FI (g/rat/day)		WI (ml/rat/day)	
		Mean ± SD	% of change	Mean ± SD	% of change	Mean ± SD	% of change
Normal control		12.66b ±2.57	0.00	16.64a ±2.02	0.00	22.06a ±3.25	0.00
Constipated control		20.39a ±2.07	61.06	14.49a ±1.08	-12.92	15.34c ±1.83	-30.46
Constipated treated groups	PP 5.0%	15.09b ±1.83	-25.99	15.09 a ±2.97	4.14	18.85abc ±1.17	22.88
	PP 10.0%	13.11b ±1.35	-35.70	15.28a ±2.61	5.45	19.54ab ±2.02	27.38
	BP 5.0%	14.55b ±2.15	-28.64	15.82a ±2.87	9.18	17.83bc ±1.44	16.23
	BP 10.0%	13.64b ±1.39	-33.10	16.73a ±2.19	15.46	19.84ab ±1.48	29.34
	PBM 10.0%	12.75b ±2.04	-37.47	16.59a ±1.52	14.49	20.32ab ±1.96	32.46

Values are expressed as mean ± SD. Different superscript letters in the same column represent statistically significant differences ($p \leq 0.05$). PP: prune powder; BP: beetroot powder; PBM: prune and beetroot mixture (1:1, w/w). BWG: body weight gain; FI: feed intake; WI: water intake.

Fecal parameters, such as the number of fecal pellets, wet and dry weight of feces, and fecal water content, are commonly utilized indicators to assess the effectiveness of laxative agents against constipation. Table (3) illustrates the effect of PP, BP, and PBM diets on fecal parameters in the experimental groups. Following the LOP treatment course, it was evident that the parameters of the collected fecal pellets, including the total number, wet weight, dry weight, and water content in the constipated control rats decreased significantly ($p \leq 0.05$) by -44.81, -48.06, -31.82, and -32.89%, respectively, compared to the normal control rats. On the contrary, feeding constipated rats with the tested experimental diets markedly restored these parameters toward normal levels.

Diets containing either 5.0% or 10.0% of PP, BP, or PBM (10.0%) significantly ($p \leq 0.05$) increased the total number of excreted fecal pellets by 25.91, 65.46, 20.44, 63.53, and 73.21%, and the wet weight of feces by 27.85, 71.40, 31.78, 83.93, and 88.04%, respectively, compared to the constipated control group. Also, the same trend was observed concerning the dry weight and water content of excreted fecal pellets, except for experimental diets that contained 5.0% PP or BP.

Table 3. Effect of PP, BP, and PBM diets on the fecal parameters in constipated rats

Experimental groups		Fecal parameters							
		Pellet number (n/rat/24h)		Wet weight (g/rat/24h)		Dry weight (g/rat/24h)		Water content (%)	
		Mean±SD	% of change	Mean±SD	% of change	Mean± SD	% of change	Mean± SD	% of change
Normal control		55.39a±4.84	0.00	10.30a±1.03	0.00	5.28a±0.38	0.00	48.74a ±5.54	0.00
Constipated control		30.57c±3.36	-44.81	5.35c ±0.30	-48.06	3.60d±0.13	-31.82	32.7c±4.05	-32.89
Constipated treated groups	PP 5.0%	38.49b±2.52	25.91	6.84b±0.28	27.85	4.25cd±0.1	18.06	37.87bc±1.0	15.76
	PP 10.0%	50.58a±2.10	65.46	9.17a ±0.69	71.40	5.0abc±0.4	39.17	45.37ab±4.9	38.69
	BP 5.0%	36.82b±3.05	20.44	7.05b±0.69	31.78	4.3bcd±0.6	20.00	38.7bc±5.95	18.38
	BP 10.0%	49.99a±1.98	63.53	9.84a ±1.07	83.93	5.08ab±0.5	41.11	48.37a±4.17	47.89
	PBM 10.0%	52.95a±2.96	73.21	10.06a±0.80	88.04	5.20a±0.27	44.44	48.31a±6.51	47.69

Values are expressed as mean ± SD. Different superscript letters in the same column represent statistically significant differences ($p \leq 0.05$). PP: prune powder; BP: beetroot powder; PBM: prune and beetroot mixture (1:1, w/w).

The effect of PP, BP, and PBM diets on GIT time in constipated rats is shown in Table (4). Oral administration of LOP resulted in a significantly prolonged GIT time ($p \leq 0.05$) compared to the normal control group, evident in a 35.20% increase in the time taken for the first blue fecal pellets to excreted and a -45.98% decrease in the number of blue fecal pellets. On the other hand, feeding constipated rats with the experimental diets containing PP, BP, and PBM at the tested concentrations markedly reversed these indices. The defecation time of the first blue fecal pellets was decreased significantly by -17.98, -27.74, -13.50, -21.59, -29.83% in constipated rats treated with 5.0% and 10.0% PP and BP or 10.0% PBM, respectively. Meanwhile, the number of excreted blue fecal pellets increased significantly ($p \leq 0.05$) in all constipated rats fed with the

experimental diets, except those containing 5.0% BP. Moreover, the PBM diet at 10.0% was far superior to other diets and resulted in an 88.89 % increase in execrated blue fecal pellets compared to the constipated control rats.

Table 4. Effect of PP, BP, and PBM diets on gastrointestinal transit (GIT) time in constipated rats*

Experimental groups		Defecation time of first blue fecal pellets (min)		Number of blue fecal pellets (n/rat/6h)	
		Mean ± SD	% of change	Mean ± SD	% of change
Normal control		235.67bc ±19.01	0.00	8.33a ±1.53	0.00
Constipated control		318.63a ±32.5	35.20	4.50b ±0.50	-45.98
Constipated treated groups	PP 5.0%	261.34bc ±18.04	-17.98	6.75a ±1.09	50.00
	PP 10.0%	230.25c ±12.66	-27.74	8.17a ±1.76	81.56
	BP 5.0%	275.6b ±12.06	-13.50	6.50ab ±0.87	44.44
	BP 10.0%	249.85bc ±14.19	-21.59	7.75a ±0.43	72.22
	PBM 10.0%	223.57c ±30.01	-29.83	8.50a ±1.32	88.89

*Values are expressed as mean ± SD. Different superscript letters in the same column represent statistically significant differences ($p \leq 0.05$). PP: prune powder; BP: beetroot powder; PBM: prune and beetroot mixture (1:1, w/w). * The parameters mentioned were assessed within a 6-hour starting from the initiation of feeding the rats with the Coomassie-mixed diets.*

Carmine was used as a marker to assess gastrointestinal motility (GIT ratio) in this study. As shown in Table (5), oral administration of LOP led to a statistically significant ($p \leq 0.05$) reduction in GIT ratio in the constipated control rats (45.81 ±8.50%) compared to the normal rats (71.48 ±1.77%), with a rate of change of -35.91% compared to the normal group. However, treating constipated rats with the experimental diets containing PP (5.0 & 10.0%), BP (10.0%), or PBM (10.0%) for a continuous seven days markedly increased the transit distance of carmine travelled through the GI tract.

Table 5. Effect of PP, BP, and PBM diets on gastrointestinal transit (GIT) ratio in constipated rats

Experimental groups		Total small intestine length (cm)	Transit distance of carmine (cm)	GIT ratio (%)	
		Mean ± SD	Mean ± SD	Mean ± SD	% of change
Normal control		108.00a ±5.29	77.2a ±3.82	71.48a ±1.77	0.00
Constipated control		105.50a ±9.26	48.33d ±6.51	45.81b ±8.50	-35.91
Constipated treated groups	PP 5.0%	111.17a ±11.62	66.67bc ±3.21	59.97a ±3.75	30.91
	PP 10.0%	104.00a ±8.54	72.17ab ±5.20	69.39a ±7.82	51.48
	BP 5.0%	104.42a ±8.00	61.33c ±5.20	58.73ab ±7.96	28.21
	BP 10.0%	110.00a ±10.58	68.50abc ±4.09	62.27a ±8.33	35.94
	PBM 10.0%	107.67a ±10.02	77.25a ±5.75	71.75a ±10.73	56.62

Values are expressed as mean ± SD. Different superscript letters in the same column represent statistically significant differences ($p \leq 0.05$). PP: prune powder; BP: beetroot powder; PBM: prune and beetroot mixture (1:1, w/w).

Furthermore, it was observed that there was a significant ($p \leq 0.05$) increase in GIT ratio by 30.91, 51.48, 35.94, and 56.62%, respectively, compared to the constipated group fed the BD. Although the BP diet with a concentration of 5.0% increased the GIT ratio by 28.21% compared to the constipated control group, the difference was statistically insignificant.

Serum biochemical markers were assessed to investigate further the effect of dietary interventions on constipated experimental animals (Tables 6 & 7). As shown in Table (6), compared to the normal control rats, the mean values of liver enzyme activities (ALT, AST, and ALP) in the constipated control animals significantly ($p \leq 0.05$) increased, with values of 68.37 ±3.70 vs. 41.50 ±4.82 for ALT, 82.50 ±5.57 vs. 69.80 ±6.04 for AST, and 174.10 ±7.40 vs. 138.53 ±8.17 (U/L) for ALP. Likewise, renal function markers (urea and creatinine) showed a remarkable increase (59.20 ±2.88 vs. 41.23 ±4.40 and 1.36 ±0.16 vs. 0.71 ±0.07 (mg/dl), respectively). Moreover, as illustrated in Table (7), the mean values of serum lipid and lipoprotein profiles (TC, TG, LDL-c, and VLDL-c) in the constipated rats were significantly ($p \leq 0.05$) higher compared to the normal rats (97.50 ±7.09 vs. 85.92 ± 4.40 for TC), (87.97 ±3.07 vs. 68.67 ±7.55 for TG), (48.68 ±4.96 vs. 29.29 ±4.26 for LDL-c), and (17.59 ±0.61 vs. 13.73 ±0.71 (mg/dl) for VLDL-c). On the other hand, HDL-c levels decreased significantly, with values of 31.23 ±2.99 vs. 42.90 ±3.42 (mg/dl).

Table 6. Effect of PP, BP, and PBM diets on liver and kidney functions in constipated rats

Experimental groups		ALT (U/L)	AST (U/L)	ALP (U/L)	Urea (mg/dl)	Creatinine (mg/dl)
Normal control		41.50c ±4.82	69.80b ±6.04	138.53d ±8.17	41.23d ±4.40	0.71c ±0.07
Constipated control		68.37a ±3.70	82.50a ±5.57	174.10a ±7.40	59.20a ±2.88	1.36a ±0.16
Constipated treated groups	PP 5.0%	49.83b ±4.25	75.47ab ±4.39	159.57b ±2.85	50.63b ±5.48	0.89b ±0.05
	PP 10.0%	43.25bc ±5.13	70.87b ±7.53	145.53cd ±6.10	42.80cd ±2.88	0.77bc ±0.05
	BP 5.0%	47.25bc ±2.61	73.83ab ±3.75	155.27bc ±4.50	49.30bc ±3.74	0.87b ±0.04
	BP 10.0%	44.13bc ±4.01	69.25b ±2.46	143.97d ±5.48	42.93cd ±3.39	0.76bc ±0.03
	PBM 10.0%	41.93bc ±4.85	65.90b ±9.21	140.40d ±4.51	40.73d ±4.02	0.72c ±0.04

Values are expressed as mean ± SD. Different superscript letters in the same column represent statistically significant differences ($p \leq 0.05$). PP: prune powder; BP: beetroot powder; PBM: prune and beetroot mixture (1:1, w/w). ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; ALP: Alkaline phosphatase.

In contrast, feeding the experimental diets to the constipated rats showed significant ($p \leq 0.05$) ameliorative effects on liver enzyme activities and renal function markers compared to the constipated control rats, except diets containing PP and BP at 5.0% regarding AST. The constipated group receiving the PBM diet at 10.0% showed the most notable effect, exhibiting a reduction in ALT, AST, and ALP levels by -38.67%, -20.12%, and -19.36%, along with a decrease of -31.20% and -47.06% in urea and creatinine concentrations,

respectively, compared to the constipated control rats. Furthermore, it is worth mentioning that dietary interventions restored the elevated serum lipids and lipoproteins in constipated animals to normal levels. Compared with the constipated control group, there was a marked increase in HDL-c levels and a significant ($p \leq 0.05$) decrease in TC, TG, LDL-c, and VLDL-c levels in the constipated rats fed the experimental diets, except PP at 5.0% concerning TC, HDL-c, and LDL-c. Among the experimental diets, the one containing PBM at 10.0% exhibited the most effect on normalizing serum lipid profiles. Interestingly, no significant differences in these indices were observed between the normal control and constipated groups treated with PP 10.0%, BP (5.0 & 10.0%), or PBM 10.0%.

Table 7. Effect of PP, BP, and PBM diets on serum lipid and lipoprotein profiles in constipated rats

Experimental groups		TC (mg/dl)	TGs (mg/dl)	HDL-c (mg/dl)	LDL-c (mg/dl)	VLDL-c (mg/dl)
Normal control		85.92b ±4.40	68.67cd ±7.55	42.90a ±3.42	29.29bc ±4.26	13.73cd ±0.71
Constipated control		97.50a ±7.09	87.97a ±3.07	31.23c ±2.99	48.68a ±4.96	17.59a ±0.61
Constipated treated groups	PP 5.0%	91.50a ±4.00	79.13b ±4.09	35.83bc ±3.75	39.84ab ±6.01	15.83b ±0.82
	PP 10.0%	85.97b ±5.22	68.53cd ±5.61	39.00ab ±3.28	33.26bc ±7.66	13.71cd ±1.12
	BP 5.0%	88.20b ±6.41	76.33bc ±3.69	38.70ab ±1.57	34.23bc ±3.50	15.27bc ±0.74
	BP 10.0%	84.20b ±4.52	66.80d ±5.52	41.17ab ±3.01	29.67bc ±1.56	13.36d ±1.10
	PBM 10.0%	83.00b ±5.29	65.60d ±4.75	41.63ab ±4.05	28.25c ±4.92	13.12d ±0.95

Values are expressed as mean ± SD. Different superscript letters in the same column represent statistically significant differences ($p \leq 0.05$). PP: prune powder; BP: beetroot powder; PBM: prune and beetroot mixture (1:1, w/w), TC: Total cholesterol, TG: Triglycerides, HDL-c: High-density lipoprotein cholesterol, LDL-c: Low-density lipoprotein cholesterol, VLDL-c: Very low-density lipoprotein cholesterol.

4. Discussion

The current study evaluated the potential laxative effects of experimental diets containing either PP or BP at 5.0 and 10.0% or their mixture (PBM) at 10.0% of the BD on constipation induced by LOP in adult male albino rats. The approximate nutritional composition of prunes and red beetroot was determined. In addition, the effects of the experimental diets on changes in BWG, FI, WI, fecal parameters, GIT ratio (reflecting motility), GIT time (indicating defecation time), and serum biochemical parameters in constipated rats were evaluated.

The approximate nutritional composition and TPC of prunes on the wet-weight basis in this study were somewhat consistent with those reported by Gill *et al*. [45], who analyzed and compared prune composition (per 100 g wet weight) from major countries of origin, including the USA, Chile, France, and Argentina. Their findings revealed that the total fiber content was (8.2, 7.7, 8.3, and 8.1 g/100 g), respectively. Additionally, their results suggested that

the energy values (kcals) for prunes seem to be within the range of 230 to 240 kcals/100 g, aligning with both their analytical data (230 kcals/100 g) and the USDA database (240 kcals/100 g). Additionally, Stacewicz-Sapuntzakis *et al*. [46] reported that prunes are rich in phenolic compounds, with concentrations reaching 184 mg per 100 g. The majority of the total phenolics are neochlorogenic (71%) and chlorogenic acids (24%), which are thought to contribute to laxative effects, in addition to a significant quantity of soluble fiber in the form of pectin, with a minimum of 3 g of pectin/100 grams of fruit.

Our data demonstrated that the estimated nutritional composition, TPC, and TFC of red beetroot aligned with the results of Neelwarne [47]. However, there were some partial differences compared to the findings reported by El-Dreny *et al*. [48], who noted that red beetroot, on a wet basis, contained available carbohydrates, protein, ether extract, and crude fiber of (8.24, 1.45, 0.38, and 1.70 %), respectively. Furthermore, a recent study conducted by Anuska *et al*. [26] revealed that the nutritional value of 100 g red beetroot was 1.61 g protein, 0.17 g lipids, 9.56 g carbohydrates, 2.8 g total dietary fiber, and energy 43 kcal. The TPC and TFC of red beetroot samples used in this work were relatively similar to those found by Guldiken *et al*. [25], who reported that the total phenolic and total flavonoid contents in fresh red beetroot were 255 mg GAE/100g and 260 mg RE/100g samples, respectively. The differences observed in the nutrient composition of prunes and red beetroot between the findings of the mentioned studies could be attributed to several factors, such as the utilization of different analytical methods, variations in the cultivars/varieties studied, differences in the degree of ripeness, dehydration or storage conditions, diverse soil properties, nitrogen fertilization practices, and other environmental factors. According to these data, prunes and beetroot qualify as functional foods due to their considerable nutrient compositions and bioactive constituents.

The findings indicated that, although WI experienced a significant ($p \leq 0.05$) decrease, BWG showed a notable increase in the constipated control group compared to the normal control group, with no significant differences observed in FI. These findings align with those of Liu and Zhi [49], who showed that a three-day subcutaneous injection of LOP (3 mg/kg BW) had no significant differences in FI between the constipated and normal groups. Nafiu *et al*. [36], also demonstrated that BWG was higher in LOP-induced constipated rats than in untreated groups. In addition, Lee *et al*. [35] found that WI significantly decreased by 13.5% in the constipation group after induction of LOP-induced constipation compared to the normal group. In contrast, Inatomi and Honma [50] found that oral administration of LOP showed no significant differences in BWG in the

constipation group compared to the placebo group. This variation can be attributed to differences in LOP dosage and treatment duration.

In the present study, various parameters of fecal pellets, including total number, wet weight, dry weight, and water content, along with GIT ratio and frequency of fecal excretion, were significantly ($p \leq 0.05$) decreased in constipated control rats compared with normal control rats. While, GIT time showed a significant ($p \leq 0.05$) increase. These findings suggest that LOP administration led to a reduction in fecal water content and hardened feces. These effects are likely to exacerbate the difficulty of defecation, similar to the clinical symptoms of constipation, as evidenced by a significant ($p \leq 0.05$) reduction in the number of fecal pellets excreted by the constipated control rats compared to the normal rats, leading to the retention of fecal pellets in the large intestinal lumen. Although there were no significant differences in FI between the normal and constipated control groups, the increased BWG in the constipated control rats might be due to the accumulation of fecal pellets in their intestines. This accumulation may have resulted in additional weight in their bodies. In addition, this prolonged contact leads to excessive water absorption from the fecal pellets, resulting in a significant ($p \leq 0.05$) decrease in the water content of the excreted fecal pellets. These results are consistent with earlier studies conducted by Wintola *et al*. [34], Lee *et al*. [35], Han *et al*. [51], and Jabri *et al*. [52]. Moreover, consistent with our results, Choi *et al*. [53] noted that the GIT ratio decreased by 20.87% in the LOP control group compared to the normal control group.

LOP is the most frequent medication employed for constipation induction in experimental animal models [49 and 54]. Its mechanism involves diminishing GI motility and peristalsis by lowering spontaneous contractions of the intestinal muscles while decreasing fluid content in the intestine, leading to a decrease in stool volume, fecal weight, and fecal water content [55]. By inhibiting the secretion of water in the intestines and the peristaltic movements in the colon, it prolongs the time required for fecal evacuation and delays the transit of intestinal luminal contents [56]. Consequently, LOP-induced constipation is considered a model for spastic constipation. The study showed that oral administration of LOP (3 mg/kg) over three days resulted in a significant reduction in water consumption, fecal pellet counts, fecal wet weight, fecal dry weight, and fecal water content in the constipated control rats compared to those in the normal rats, whilst there were no significant differences in feed intake between experimental groups. These changes suggest the successful induction of a constipation model in rats.

Dietary interventions using the experimental diets containing PP, BP, and PBM in the constipated rats showed significant ($p \leq 0.05$) improvements in the fecal pellet counts, fecal wet and dry weight of the feces, and fecal water content compared to the constipated control rats fed the BD. In addition, these experimental diets led to a noticeable reduction in GIT time while significantly ($p \leq 0.05$) increasing the GIT ratio. The observed rise in the gastrointestinal carmine transit ratio among constipated rats fed the experimental diets suggests an enhancement in intestinal motility. This improvement may promote better intestinal peristalsis, facilitating defecation in rats afflicted with LOP-induced constipation. Consistent with our findings, in a randomized controlled trial (RCT) conducted by Lever *et al*. [57], the results indicated that a four-week consumption of 80 g/d or 120 g/d prunes resulted in a significant increase in stool weight and stool frequency among healthy individuals with infrequent stool habits, the increase in stool weight amounted to approximately 27.5 g/d per 100g of prunes. Additionally, the consumption of prunes led to an enhanced relative abundance of Bifidobacteria in the participants, which was attributed to the fiber content, sorbitol, or phytochemicals in prunes. Furthermore, Koyama et al. [58] observed that prune juice consumption led to a notable decline in hard and lumpy stools among Japanese subjects with chronic constipation, in addition to increasing the frequency of normal stools without increasing loose and watery stools. Unfortunately, to our knowledge, no studies have investigated the laxative properties of beetroot or its synergistic effect with prunes.

Our findings indicated that fresh prunes and red beetroots contain a significant amount of crude fibers, 7.8% and 2.4% (equivalent to 11.42% and 17.39% on a dry weight basis), respectively. Stacewicz-Sapuntzakis et al. [46] reported that prunes exhibit high fiber content, approximately 6 g, composed of hemicellulose (3.0 g), pectin (2.1 g), and cellulose (0.9 g), per 100 g. While, beetroot fiber typically consists of 22–24% cellulose, 30% hemicellulose, and 25% pectin, with an approximate ratio of insoluble to soluble fiber of 2:1, as reported by Harland *et al*. [59]. The mechanisms by which fiber influences fecal weight, fecal water content, fecal frequency, GIT time, and GIT ratio are thought to be mediated by multiple factors. Fiber encompasses a range of molecules with differences in solubility, viscosity, and fermentability. In this context, Buttriss and Stokes [60] (2008) and Jha *et al*. [61] reported that certain fibers are resistant to fermentation in the colon (poorly fermented fibers), such as cellulose. On the other hand, other types undergo rapid fermentation by the colonic microbiota (fermentable fibers), such as pectin. The non-fermentable fibers remain intact as they reach the

lower gut, whereas the viscous fibers have a higher capacity to bind water. Consequently, both types contribute significantly to stool bulk, causing luminal expansion and stimulating peristalsis. In addition, fermentable fibers promote the proliferation of gut microbiota, leading to increased fecal biomass and the production of short-chain fatty acids (SCFAs). This, in turn, increases the colonic osmotic load, resulting in higher water content in the feces and softer stools. Additionally, consumption of high-fiber foods is associated with a reduction in GIT time [57, 62, and 63].

The laxative effect of prunes, in addition to the mentioned above, can also be attributed to the high sorbitol content and phenolic compounds, particularly neochlorogenic and chlorogenic acids. In this regard, sorbitol, an indigestible and unabsorbable sugar alcohol in the small intestine, can retain water within its molecular structure, leading to an increase in luminal water content in the gut, potentially leading to the softening of stools and facilitating defecation [58 and 64]. In an RCT conducted by McRorie *et al*. [65], the administration of 40 g/day of sorbitol for six days demonstrated a significant increase in fecal water and fecal weight compared to a placebo. Furthermore, chlorogenic acid has been found to stimulate colonic serotonin production, enhance GI motility, and ultimately improve the defecation process [66]. In addition, chlorogenic acid helps to maintain gut health and integrity by repairing intestinal barriers and preventing colonization by parasitic bacteria [67]. Han *et al*. [51] documented that phytochemicals exhibit laxative properties by acting on the acetylcholine receptor in constipated animal models. In a recent study examining the impact of postoperative prune consumption on the timing of the first bowel movement following gynecologic surgery, it was found that women who consumed 12 prunes along with 100 g of docusate sodium (a laxative medication) twice daily vs. those who used docusate alone for three days, had an increase in bowel movements and were discharged from the hospital earlier than the control group [68]. The above findings suggest that the laxative effect of PP could stem from its nutritional composition, which includes fiber, notably pectin, as well as sorbitol and phenolic compounds, particularly neochlorogenic and chlorogenic acids.

To our knowledge, this study is the first to explore the laxative properties of beetroot or its synergistic effect with prunes. Nevertheless, the laxative effect of beetroot can be ascribed not solely to its rich fiber content, as previously noted, but also to its elevated levels of bioactive compounds. Several studies have documented that various medicinal plants and foods rich in bioactive compounds have recently emerged as innovative therapeutic agents for treating or preventing constipation. This emergence is attributed to their capacity

to enhance the fecal frequency of feces, intestinal tension, fecal volume, and intestinal motility [35, 52, and 69]. In addition, Lee *et al*. [35] reported that antioxidant properties might potentially play a role in relieving constipation, and they proposed that polyphenolic compounds could be accountable for this effect. In this study, the estimated nutritional composition of beetroot on a wet basis revealed that it contains significant levels of TPC (262.81 mg GAE/100g) and TFC (275.63 mg RE/100g). According to Lechner and Stoner [70], phenolic acid components such as **ρ**-coumaric acid, syringic acid, ferulic acid, vanillic acid, and caffeic acid, along with flavonoids (like kaempferol, rhamnocitrin, astragalin, rutin, and rhamnetin), betanines, carotenoids (including **β**-carotene and lutein), triterpenes, and saponins are the bioactive compounds responsible for beetroot's chemopreventative effects.

Providing the constipated rats with the experimental diet containing BP at 10.0% of the BD led to a significant ($p \leq 0.05$) increase in the GIT ratio by 35.94% and a -21.59% decrease in GIT time compared to the constipated control group. These effects may be attributed to, in addition to fiber content, phenolic compounds such as chlorogenic acid (as in prunes), caffeic acid, and ferulic acid in beetroot, which could promote the GIT ratio. Badary *et al*. [71] reported that caffeic acid increases intestinal motility by decreasing the expression of nitric oxide synthase genes, leading to a decrease in nitric oxide production. Nitric oxide plays a crucial role in regulating gastrointestinal motility by relaxing gastrointestinal smooth muscles and exerting vasodilatory effects. Therefore, the suppression of nitric oxide production would lead to promoting intestinal peristalsis and improving gastrointestinal motility. In addition, ferulic acid noticeably increased the GI transit and gastric emptying in rats in a manner that depended on the dosage administered [71]. These phenolic compounds have been shown to regulate the gut microbiota by promoting the colonization of beneficial bacteria while suppressing the habitation of pathogenic flora. This collective action promotes the GI motility [72].

A study conducted by Wang *et al*. [73] showed that consuming red beetroot for 14 days among healthy adults has the potential to influence gut microbial populations and their associated catabolites, indicating its potential benefits for both intestinal and systemic health. Additionally, beetroot consumption increased the SCFAs production, particularly (iso) butyric acid. The production of SCFAs, including butyric acid, has been shown to enhance propulsive contractions in the ileum by inducing prolonged and discrete clustered contractions [49]. In addition, SCFAs can directly activate smooth muscle contractility in both the ileum and colon, promoting GIT motility [74]. This

particular effect may be related to the release of intestinal serotonin from activated enterochromaffin cells of the colon, which in turn stimulate secretion and propulsive motility and ultimately promote fecal evacuation [75]. The potential mechanisms mentioned previously suggest that experimental diets containing BP could alleviate LOP-induced constipation in rats and may offer scientific support for the traditional use of beetroot for relieving constipation. However, further clinical trials are needed for a thorough understanding of the underlying mechanisms of action.

Serum biochemical markers were assessed to investigate further the effect of the experimental diets on the constipated rats. According to our results, the activities of liver enzymes (ALT, AST, and ALP), renal functions (urea and creatinine), and serum lipid profile (TC, TG, LDL-c, and VLDL-c) were significantly ($p \leq 0.05$) higher in the constipated control rats than in the normal rats, with a marked decrease in HDL-c levels. These findings are consistent with those of Sabiu and Ashafa [76]. A study conducted by Nafiu *et al*. [36] concluded that oral administration of LOP resulted in a significant increase ($p \leq 0.05$) in ALP and AST in constipated rats compared to normal rats. Lee *et al*. [77] illustrated that HDL-c, albumin, total protein, ALT, ALP, and glucose exhibited significant differences between the LOP group and control group. Furthermore, Jabri *et al*. [52] demonstrated that LOP-induced inhibition of intestinal motility and peristalsis was associated with colic oxidative stress, characterized by an increase in reactive oxygen species (ROS) formation, lipid peroxidation, and a reduction in both enzymatic and non-enzymatic antioxidants. Similarly, it has been shown that oxidative stress is involved in constipation by increasing the concentration of ROS, which damages proteins, lipids, and DNA [35 and 78]. According to Jabri *et al*. [52] and Lee *et al*. [35], the administration of LOP significantly increased the production of malondialdehyde and hydrogen peroxide (H2O2) in the intestinal mucosa compared to the normal group, resulting in an increase in lipid peroxidation and a reduction in the activities of antioxidant enzymes. Additionally, LOP resulted in disturbances of the serum lipid profile in experimental constipated rats.

Oxidative stress, if not tightly controlled, is regarded as a pathogenic mechanism that triggers the initiation and development of numerous degenerative and chronic diseases [79]. ALP is commonly utilized as a marker enzyme to evaluate the integrity of the plasma membrane and endoplasmic reticulum [80]. An elevation in ALP activity could indicate damage to the structural integrity of the liver. This increase is likely a result of leakage from altered membrane permeability [81]. Hence, the rise in ALP activity in constipated rats may signify damage to the plasma membrane,

resulting in compromised membrane integrity. According to Sabiu and Ashafa [76], the elevated serum levels of ALT and AST in constipated rats suggest potential damage to the hepatocytes caused by changes in membrane permeability. It is feasible that constipation could contribute to an increased risk of the development of kidney dysfunction. However, as far as we know, limited studies have investigated the relationship between constipation and the risk of kidney disease. Sumida *et al*. [82] revealed that patients with constipation exhibited higher incidence rates of chronic kidney disease, end-stage renal disease, and a decline in glomerular filtration rate when compared to those without constipation and concluded that more severe constipation is associated with progressively higher risks for various kidney-related outcomes. Moreover, constipation is a clinical manifestation that alters the gut environment and may be a contributing factor to the progression of kidney disease. The disturbance of the gut microbiota is associated with the accumulation of uremic toxins that originate from the gut, like indoxyl sulfate and p-cresyl sulfate [83]. These toxins appear to expedite the progression of kidney disease by inducing oxidative stress, inflammation, and renal fibrosis [84]. Another study by Choi *et al*. [85] confirmed that the injection of LOP induces stress and has been shown to alter the serum lipid profile. According to their results, the LOP group had a 43% lower HDL-c/TC ratio than the normal group. These findings may explain the observed alterations in serum biochemical markers among the constipated rats in this study.

Interestingly, feeding constipated rats with the experimental diets at 5.0% and 10.0% of PP, BP, or PBM significantly ($p \leq 0.05$) attenuated the observed changes in serum biochemical markers dose-dependently, which may be considered indirect proof of their potential to alleviate constipation through improving fecal parameters, enhancing GI motility, and reducing GIT time, or potential scavenging ROS-induced by LOP. Prunes and red beetroot contain significant bioactive compounds in combination with their high fiber contents, especially soluble fibers. These compounds may contribute to reversing these changes due to their potent antioxidant and free radical scavenging properties. Several studies have well-documented the hepatoprotective, nephroprotective, hypolipidemic, and antioxidative effects of prunes and beetroot [86-93].

It is worth mentioning that the diet containing PBM at 10% of the BD outperformed the other experimental diets in relieving constipation symptoms in rats afflicted by constipation compared to the constipated control rats without inducing diarrhea. These favorable effects are likely due to the interactions between various potent bioactive compounds found in prunes and beetroot, as well as their fiber

contents. However, further clinical trials are required to clarify the precise synergistic mechanisms. In conclusion, the current study may provide notable proof supporting the effectiveness of prunes and red beetroot as natural laxatives for preventing or treating constipation.

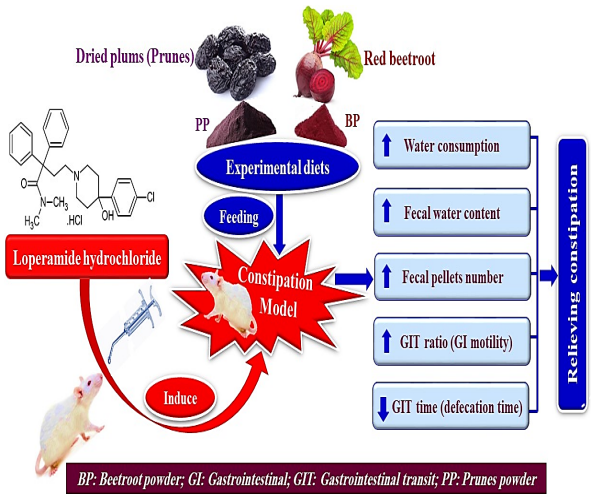
5. Conclusion

Constipation is a common health problem that affects people of all ages. The long-term use of anti-constipation medications can lead to serious health consequences. This study showed that the experimental diets containing PP and BP at 5.0 and 10.0%, or PBM at 10.0% of the BD, exhibited marked laxative effects in rats afflicted with constipation induced by LOP. These effects were concentration-dependent and involved increasing fecal pellet count, enhancement of fecal water content, improvement in GI motility, reduction of GIT time, and normalization of changes in serum biochemical markers induced by constipation, all without causing diarrhea. These effects could be attributed to the phytoconstituents of PP and BP, such as dietary fiber, polyphenols, or flavonoids. These findings could provide scientific support for the traditional use of prunes and red beetroot in preventing and treating constipation. However, further studies are needed to explore the underlying physiological mechanisms behind the laxative effect of red beetroot, as well as its synergistic effect with prunes.

Abbreviations

ALP: Alkaline phosphatase; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; BD: Basal diet; BP: Beetroot powder; BWG: Body weight gain; DNA: Deoxyribonucleic acid; FI: Feed intake; GI: Gastrointestinal; GIT: Gastrointestinal transit; HDL-c: High-density lipoprotein cholesterol; LDL-c: Low-density lipoprotein cholesterol; LOP: Loperamide; PBM: Prune and beetroot mixture; PP: Prune powder; RCT: Randomized controlled trial; ROS: Reactive oxygen species; SCFAs: Short-chain fatty acids; SD: Standard deviation; TC: Total cholesterol; TFC: Total flavonoid content; TG: Triglycerides; TPC: Total phenolic content; USDA: United States Department of Agriculture; VLDL-c: Very low-density lipoprotein cholesterol; WI: Water intake

Graphical Abstract



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الترقيم الدولي للطباعة الترقيم الدولي اون لاين

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التغذية وعلوم الاطعمة

التأثيرات الملينة المحتملة للبرقوق المجفف (القراصيا) والبنجر الأحمر على الإمساك الناجم عن اللوبراميد في الفئران البالغة

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الملخص العربي:

الإمساك مشكلة مَعِدِيّة معوية منتشرة تؤثر على نوعية حياة الأفراد. البرقوق والبنجر الأحمر من العلاجات التقليدية للإمساك. استكشفت هذه الدراسة التأثيرات الملينة المحتملة لمسحوق البرقوق (القراصيا)، البنجر وخليطهما على الإمساك الناجم عن اللوبيراميد في الفئران. تم تقسيم 42 فأرا بالغا لمجموعتين رئيسيتين. المجموعة الأولى (ن=6)، المجموعة الضابطة الطبيعية، تم تغذيتها على الوجبة الأساسية، وتم إعطاء المجموعة الثانية (ن=36) اللوبراميد للحث على الإمساك وتم تقسيمها إلى ست مجموعات فرعية متساوية. تم تغذية المجموعة (I)، المجموعة الضابطة المصابة بالإمساك، على الوجبة الأساسية. تم تغذية المجموعات الفرعية المتبقية المصابة بالإمساك بأنظمة غذائية تجريبية تحتوي على مسحوق القراصيا للمجموعات (II&III) أو مسحوق البنجرللمجموعات (IV&V) بنسبة 5.0 و10.0٪ (وزن/وزن من الوجبة الأساسية)، على التوالي، بينما تم إعطاء المجموعة (VI) نظاما غذائيا تجريبيا يحتوي على خليط بنسب متساوية من مسحوق القراصيا والبنجر بنسبة 10.0% من الوجبة الأساسية. أدى إعطاء اللوبيراميد إلى انخفاض معنوي ($p\leq0.05$) في استهلاك الماء -30.46%، ومؤشرات البراز (عدد الكريات، الوزن الرطب، الوزن الجاف، ومحتوى الماء بنسبة -44.81%، -48.06%، -31.82%، و -32.89% على التوالي)، وحركية الجهاز الهضمي بنسبة -35.91%، وزيادة ملحوظة في زمن العبور الهضمي بنسبة 35.20%. بالإضافة إلى ذلك، فقد تسبب في حدوث اضطرابات في مستوى دهون الدم ووظائف الكبد والكلى في المجموعة الضابطة المصابة بالإمساك مقارنة بالفئران الطبيعية. أدى التدخل الغذائي بالأنظمة الغذائية التجريبية في الفئران المصابة بالإمساك الى استعادة هذه المؤشرات بشكل ملحوظ ($p\leq0.05$) نحو المستويات الطبيعية دون التسبب في الإسهال، وبشكل أكثر فاعلية مع النظام الغذائي التجريبي المحتوي على خليط المساحيق. في الختام، القراصيا والبنجر خففا بشكل فعال الإمساك الناجم عن اللوبراميد في الفئران، مما يوفر مبررا علميا لاستخدامهما التقليدي.

الكلمات الكاشفة: الإمساك، البرقوق، البنجر، مؤشرات البراز، حركية الجهاز الهضمي، زمن العبور الهضمي