RESEARCH ARTICLE

Evaluation of the Effects of Methanolic Extract of Dates, and Oily and Alcoholic Extract of Phoenix Dactylifera Leaves for Treating Diarrheal in Rats

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Received: 08th March, 2021; Revised: 29th April, 2021; Accepted: 05th May, 2021; Available Online: 25th June, 2021

ABSTRACT

Diarrhea results from an imbalance between the absorptive and secretory mechanisms in the intestinal tract, accompanied by hypermotility, bringing about excess loss of body fluids and electrolytes in feces. Many plant materials of nutritional qualities are being used in folklore medicine owing to their availability and safety; one of these plant materials is Phoenix dactylifera fruit. **Method:** The date methanolic extract at a dose (1000 and 500 mg/kg) leaves methanolic extract at a dose (2000 mg/kg and 1000 mg/kg) and leaves oily extract at a dose (1000 mg/kg and 500 mg/kg) were given orally to six groups of rats (five animals per group) to evaluate the activity of the extract against diarrhea induced by castor oil in the rat. While date methanolic extract at a dose (1500 mg/kg and 1000 mg/kg) and leaves oily and methanol extracts at a dose (2000 mg/kg and 1500 mg/kg) significantly ($p < 0.05$) reduced the frequency of several wet feces (inhibition defecation percentage). Two other groups received normal saline and loperamide (5 mg/kg) as a positive control. The result obtained shows that the methanol date and leaves extract and leaves oily extract at different doses of date and leaves extract. Pharmacologic contain for Phoenix dactylifera was active substances with antidiarrhoeal properties.

Keywords: Diarrhea, Drugs, Extracts, Phoenix dactylifera.
International Journal of Drug Delivery Technology (2021); DOI: 10.25258/ijddt.11.2.41

How to cite this article: Al-Zeiny SSM, Al-Safi SMM, Al-Dawah NKJ, Abbas DA. Evaluation of the Effects of Methanolic Extract of Dates, and Oily and Alcoholic Extract of Phoenix Dactylifera Leaves for Treating Diarrheal in Rats. International Journal of Drug Delivery Technology. 2021;11(2):469-473.

Source of support: Nil.

Conflict of interest: None

INTRODUCTION

Diarrhea is the condition of having three or more loose or liquid stools per day or having more stool than normal for that person.[1] Diarrhea is excess loss of body fluids and electrolytes in feces resulting from an imbalance between secretory mechanisms and the absorptive in the intestinal tract characterized by an increase in stool frequency and alteration inconsistency, accompanied by hypermotility, bringing about that as a signee of the gastrointestinal disorder.[2,3] Developing countries, malnutrition considers one of the major causes leading to mortality.[4] For diarrhea, several non-infectious causes include many medications, hyperthyroidism, lactose intolerance, inflammatory bowel disease, and irritable bowel syndrome, while infection causes the most common cause of diarrhea by virus, bacteria or other parasites contracted through contaminated food or water. Some of the antibiotics used as an antidiarrheal drug, besides their high costs, sometimes show some adverse effects and developing resistance to microorganisms.[2] The studies encourage by World Health Organisation (WHO) has stimulate for protection, prevention, and treatment of diarrhoeal illness.[4] Use of folklore medicines in the treatment of many diseases. Many plant materials of nutritional qualities are being used in folklore medicine owing to their availability and safety; one of these plant materials is Phoenix dactylifera fruit.[2] Dates have widely used various sicknesses, for example, and nervous disorders contain analgesic effects, fever, and inflammation because of its antioxidant component, paralysis, loss of consciousness, and intestinal distress. Since it has a tannin that considers detersive and astringent, and neutralizes alcohol intoxication for toxicity of the liver and abdominal disorders.[5] There are knowlegment, date, and leaves extract rich by contents of antioxidants in flavonoids, sterols, procyanidins, carotenoids, anthocyanins.

Moreover, it contains coumaric acid and ferulic acid, glucose, sucrose, fructose, dietary fibers, vitamins as ascorbic, biotin, riboflavin, thiamine, folic acid etc.

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The extracts have minerals, protein, and fats, were few amounts example, calcium, iron, copper, cobalt magnesium, fluorine, manganese, phosphorus, potassium, sodium, boron, sulfur, zinc, and selenium.[5] The aims of study: investigate the methanolic extract of dry date and leaves oily and methanolic extract of Phoenix *dactylifera* properties antidiarrheal in rats.

MATERIALS AND METHODS

Collection of Plant and Extract

Fresh dates and leaves of Phoenix dactylifera, were obtained from Kufa cultivar; they were collected during late 2017. The collected material of leave and dry date were washed by tap water, and then the leaves were cut into smaller parts and dried at room temperature. The dried leaves were powder using a mechanical blender while the dry date was cleaned and removed of seed then dried at room temperature after that grinding it with a meat grinder to produce date paste.

Alcoholic Extraction

100 g of dry date of Phoenix dactylifera as paste form or leave powder sample were added to 1000 mL of methanol alcohol diluted 70% mixed thoroughly by magnetic stirrer for 24 hours then were filtered by filter paper whatman No.1 and using a rotary evaporator concentrated under reduced pressure at 40°C, 90 rpm.[6] Extraction yields were weight and calculated by the formula as follow:

Yield (%) = (weight of Dry extract / weight of plant powder or paste) × 100

Leave Oil Extraction

40 g from leaves powder of phoenix dactylifera put in special cellulose thimble placed in soxhlet apparatus the extraction chamber fitted with a condenser. The sample was placed on a 1000-mL distillation flask containing 500 mL of organic solvents hexane. Heating at 45ºC for 5–6 hours under reflex (10–12 cycles/hrs) and evaporated of extraction solvent by heating 40°C with 90 rpm, using a Rotary evaporator at agreeing to the method by Charef, *et al.,*[7] The weight yields of extraction were then calculated by the method as follow:

Yield of oil extraction = (weight of oil extract / weight of leave powder used) × 100%

Experimental Animals

The animals used in the study adult males' rats of Sprague Dawley albino weight about 200–230 g and put rats in the animal house of the veterinary medicine/ university of Kufa, under slander condition, light and dark cycles of 12 hours with given diet and water to drinking.

Drugs

- Castor oil (maleizian)
- loperamide (5 mg/kg) (Egabtian)
- activated charcoal and gum acacia (market).

Antidiarrhoeal Activity

According to one designated method[8] induced diarrhea by Castor oil in rats. A total of 40 adult males' rats weighing (200–230 g) with each group having five rats.

- Group 1: control received distal water
- Group2: as a standard drug (loperamide 5 mg/kg) orally
- Group 3: methanolic date extract was treated at a dose (1000 mg/kg, orally)
- Group 4: methanolic date extract was treated at a dose (500 mg/kg, orally)
- Group 5: methanolic leaves extract was treated at a dose (2000 mg/kg, orally)
- Group 6: methanolic leaves extract was treated at a dose (1000 mg/kg orally)
- Group 7: oily leaves extract was treated at a dose (1000 mg/kg orally)
- Group 8: oily leaves extract was treated at a dose (500 mg/kg orally)

Given 1 mL of castor oil orally to each rat, and that induced diarrhea after two days of treated by date alcoholic and leaves alcoholic and oily extract or loperamide and give for all the animals that not eat for period 18 hours (1–8 groups) before the 3 day of treatment. After 1 hour, usage dates methanolic and leaves methanolic and oily extracts or standard drug on the third day. Each animal put in a single cage contained floor is covered with spongy paper and saw for 4 hours the frequencies diarrhoeal droppings were noted on it. After that calculated the percentage inhibition as follows[9,10]:

% of inhibition = 100 – ((fne/ fnc) x 100)

fne = mean fecal number of each treatment group

fnc = Mean fecal number of the control group

Gastrointestinal Transit Test for Treated Diarrhea Induced by Castor Oil

According to the charcoal method[11] using to induce tested gastrointestinal transit in rats. Used 40 rats in weighted (200–250 g) were distributed into each group with five rats for three days a period.

- Group 1: Control received distal water
- Group 2: as a standard drug (loperamide 5 mg/kg) orally
- Groups 3: was received date methanolic extract orally at doses of 1000 mg/kg
- Group 4: was received date methanolic extract orally at doses of 1500 mg/kg
- Group 5: was received leaves methanolic extract orally at doses of 2000 mg/kg
- Group 6: was received leaves methanolic extract orally at doses of 1500 mg/kg
- Group 7: was received leaves oily extract orally at doses of 2000 mg/kg
- Group 8: was received leaves oily extract orally at doses of 1500 mg/kg

All Animals in (1–8) groups didn't eat for 18 hrs after 3 day treatment, then allowed to drinking water. Each rat in groups was administered 1 mL of castor oil, then after 1 hour, given were1 mL of marker mixing of (10% charcoal suspension in 5% gum acacia) to all rats in groups orally.

After 1 hour of marker administration, all the animals were sacrificed and rapidly were separated the small intestine of them and put on a clean surface. The distance traveled of

the intestine was measured from the pylorus to the caecum, saw by charcoal meal mass. The length of the whole intestine also was measured. Expressed the percentage was calculated of the distance travelled according to some previously found methods.[12-15]

Percentage of inhibition: by using the below calculation[11,16] results were determined compared with the control group

$$IP\ \% = (lm/\ lsi) \times 100$$

$$\%\ Inhibition = IP\ \%\ (control) - IP\%\ (treatment)/\ IP$$

PI = Peristaltic index
lm = Length of charcoal meal
lsi = Length of small intestine

Statistical

Results were expressed as mean ±S.E.M and were analyzed using the statistical software, by the programme (SPSS version 18.0) and determined significant variances between means of the groups using the method statically of one-way ANOVA.

RESULTS

Antidiarrheal Activity of Date Alcoholic Extract in Rats

The date alcoholic extract at does 1000 mg/kg significantly ($p < 0.05$) more increase in the inhibited number of pouring feces (frequency of defecation) at a percentage (63.71 %) from group treated with dose (500 mg/kg) decreased significantly at percentage (47.59%) compartment with the group treated standard drug loperamide 0.5 mg/kg and also with the control group (Table 1).

Gastrointestinal Transit of Date Alcoholic Extract Induced by Castor Oil in Rats

Rats were treated with date methanolic extract at a dose (1500 mg/kg) equal in decelerated the force of charcoal meal ($p < 0.05$) by the GIT to the decelerated force of charcoal meal by loperamide (5 mg/kg) as a standard drug at percentage 23%. While the group treated at dose 1000 mg/kg less activity ($p < 0.05$) in the forced movement at percentage 20% when compared to group 1 (Table 2).

Antidiarrhea Activity of Leaves Alcoholic and Oily Extract Induced by Castor Oil in Rats

Leaves alcoholic extract of *P. dactylifera* at dose 2000 mg/kg and leaves oily extract at dose 1000 mg/kg in percentage (87.5 and 82.15%) respectively were increased significantly ($p < 0.05$) inhibited the frequency of defecation than the group treated loperamide (0.5 mg/kg) standard drug at a percentage (78.58%) while the group treated with leaves oily extract at dose 500 mg/kg) was in percentage 75% nearly to standard drug. The group treated with leaves alcoholic extract at dose 1000 mg/kg was less effective in percentage (57.15 %) when compared with standard drug and the control. (group 1) (Table 3).

Table 1: Antidiarrhoea activity of date alcoholic extract in rats

Animal groups	*Number of pouring faeces*	*% Inhibition of defecation*
Control D. W		-
Loperamide 0.5 mg/kg	3 ± 0.4	78.58 %
Date alcoholic 1000 mg/kg	4.5 ± 1.5	63.71 %
Date alcoholic 500 mg/kg	6.5 ± 0.7	47.59 %

Gastrointestinal Transit of Leaves Alcoholic and Oily Extract Induced by Castor Oil in Rats

Treatment of leaves alcoholic extract at dose 1500 mg/kg significantly showed was decelerated the force of charcoal meal by the gastrointestinal tract in percentage 22.7% nearly from percentage 23.57% of standard drug loperamide 0.5 mg/kg while the two groups of leaves alcoholic and oily extracts at dose 2000 mg/kg were significantly ($p < 0.05$) in percentage 20.12% and 20.22%. Where group treated with leaves oily extract at a dose (1500 mg/kg) statistically ($p < 0.05$) appearance was a decrease in the forced movement and the intestinal length traveled by charcoal meal in percentage 19.57% when compared with standard drug and control group. (Table 4).

DISCUSSION

Castor oil is a laxative with a harsh action when used in long-term therapy.[17] because the castor oil contains active component is ricinoleic acid, which stimulates the making of prostaglandins as mediator substances by colonic cells, nitric oxide, platelet-activating factor, cAMP, and tachykinins.[18]

Table 2: Date alcoholic extract effect of on gastrointestinal transit induced by castor oil in rats

Group treatment	*Peristaltic index (PI) (%)*	*Inhibition (%)*
Control D. W	23.9 %	-
Loperamide 0.5 mg/kg	7.89 %	23.57 %
Date alcoholic 1000 mg/kg	92.59 %	20.3 %
Date alcoholic 1500 mg/kg	1.1%	23.85 %

Table 3: Antidiarrhoea activity of leaves alcoholic and oily extract in rats

Animal groups	*Number of pouring faeces*	*% Inhibition of defecation*
Control (D.W)	14 ± 1.2	-
Loperamide 0.5 mg	3 ± 0.4	78.58 %
leaves alcoholic extract 2000 mg/mL	1.75 ± 0.7	87.5 %
leaves alcoholic extract 1000 mg/mL	6 ± 1.6	57.15 %
Leaves oily extract 1000 mg/mL	2.5 ± 0.9	82.15 %
Leaves oily extract 500 mg/mL	3.5 ± 0.78	75 %

Table 4: Effect of leaves alcoholic and oily extract on gastrointestinal transit induced by castor oil rats

Group treatment	*Peristaltic index (PI) (%)*	*Inhibition (%)*
Control	23.9 %	-
Loperamide 0.5 mg	7.89 %	23.57 %
leaves alcoholic extract 2000 mg/mL	90.47 %	20.12 %
leaves alcoholic extract 1500 mg/mL	24.44 %	22.7 %
Leaves oily extract 2000 mg/mL	88.04 %	20.22 %
Leaves oily extract 1500 mg/mL	91.4 %	19.57 %

Castor oil (*oleum ricini*) is a triglyceride hydrolyzed in the small intestine to release glycerol and ricinoleic acid. This causes a laxative effect by irritating the intestinal mucosa.[17,19] Also, castor oil is an older agent that both inhibits glucose and sodium absorption and enhances electrolyte and water secretion in the bowel lumen.[1] The fact of the oil leads to permeability changes in the intestinal mucosal membrane to water and electrolytes, therefore its yield watery luminal content that flows quickly through the small and large intestines.[18]

This study showed thqat the administration of charcoal meal and from the aqueous date extracts, significantly increased GIT, because the date methanolic extract, and oily and methanolic extract of *P. dactylifera* leaves contained components in phytochemical analysis known for its richness of tannins and other component Tannins[5] active with denaturingd protein to form the protein tannate complex. Tannins present in many plants may be accountable for inhibiting the the gastrointestinal tract, which makes the intestinal mucosa more resistant and reduces secretion.[18] In addition, their antioxidant activity and phenolic compounds and isolated compounds are flavonoids, saponins, terpenoids, steroids, cumarine, and alkaloids; these extracts are associated with regulation of GIT by scavenging free radicals.[5]

Flavonoids and saponins inhibit the release of prostaglandins, autocoids, and contractions caused by spasmogens and motility and hydro electrolytic secretions, while saponins may prevent the release of histamine. Polyphenols and tannins provide strength to the intestinal mucosa, decrease intestinal secretion, intestinal transit, and promote balance in water transport across the mucosal cells; these recorded in the report by Ashish *et al*.[20]

Results of aqueous fruit extract of *P. dactylifera* (1000 mg/kg) exhibited antidiarrheal activity and inhibition of frequency of defecation and wetness of the fecal. It is possible that the extract was able to inhibit electrolyte permeability in the intestine due to castor oil and through inhibition of prostaglandins release.

It can be supposed that the prostaglandin, nitric oxide, and platelet-activating factor, cAMP, and tachykinins synthesis inhibition might be involved in the mechanism of action.[18]

The aqueous extract significantly reduced both castor-oil induced intestinal transit and frequency of diarrhea effects according to the report by Rajeev.[21]

Bright[2] shown that An effect of Phoenix dactylifera fruit at dose 1000 mg /kg possess antidiarrheal activity of the aqueous extract that has a component of the phytochemical analysis of the date and leaves extracts of *P. dactylifera*.

In conclusion, date methanolic extract and leave oily and methanolic extracts that are used for treating diarrhea induced by castor oil in rats as antidiarrheal activity and transit because they have inhibiting effects on GIT disorder. These bases use date and leaves of *P. dactylifera* for the treatment of diarrhea in Iraq

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