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Antidiarrheal Effects of *Juniperus phoenicia* L. Leaves Extract in Rats

[1]E.Y. Qnais, [2]F.A. Abdulla and [1]Y.Y. Abu Ghalyun
[1]Department of Biology, Faculty of Art and Science,
[2]Department of Physical Therapy, Allied Health Sciences, Hashemite University, Zarka, Jordan

Abstract: The antidiarrheal effects of the aqueous extract of *Juniperus phoenicia* leaves were studied using several experimental models of diarrhea. Results obtained revealed that the aqueous extract caused a dose dependent protection of rats against castor oil induced diarrhea and reduced castor oil induced enteropooling. The extract also caused a dose dependent decrease in intestinal transient and showed a significant dose dependent relaxant effect (EC_{50}65.1±8.4 mg mL^{-1}) on rat ileal smooth muscle. The intraperitoneal LD_{50} value was found to be 1587±143 mg kg^{-1} in mice. Phytochemical screening of the aqueous extract revealed the presence of flavonoids, alkaloids and tannins. These findings suggest that the aqueous extract of *Juniperus phoenicia* leaves may elicit an antidiarrheal effect at least by reducing intestinal fluid accumulation and inhibiting intestinal motility, thereby justifying its use in ethnomedical practice as an antidiarrheal agent.

Key words: *Juniperus phoenicia*, antidiarrheal effect, intestinal motility, ileum, relaxation

INTRODUCTION

Juniperus phoenicia L. (Cupressaceae) an evergreen plant usually growing as a bush or it can have the shape of a tree. As a tree it can reach the height of 10 m. *Juniperus phoenicia* is distributed in south west of Jordan, where it is popularly known as Arar. The aqueous extract of *Juniperus phoenicia* leaves is used in Jordanian traditional medicine for treatment of variety of diseases such as diarrhea, gout and poor appetite. It also eliminates gastrointestinal bacteria and parasites. Even through some studies have been done on members of the genous[1-5] little information is available about the medical use of *Juniperus phoenicia.* It is used to eliminate both fungi and bacteria[6,7]. Few chemical compounds have been isolated from *Juniperus phoenicia* such as diterpenic acids and α-pinene[6-8]. α-pinene have antifungal activity[6].

Despite the relatively large use of this plant in popular medicine in Jordan for its antidiarrheal properties, surprisingly no effort has been done to examine its anti-diarrhoeal activity. The present study was done to evaluate the antidiarrheal effect of aqueous extract of *Juniperus phoenicia* leaves using various validated models and to find out if the folk medicinal use has a scientifically justified basis.

MATERIALS AND METHODS

Plant material: Fresh leaves of *Juniperus phoenicia* were collected from Dana area of Altafila (Jordan) in 2004. The plant material was identified and authenticated taxonomically at the Hashemite university herbarium.

Preparation of aqueous extract: Aqueous extract was obtained by boiling 150 g of the ground air dried leaves of *Juniperus phoenicia* in 3 L of distilled water for 15 min with continuous stirring. The resultant solution was filtered. The filtrate was completely evaporated under reduced pressure at 60°C. Solutions were prepared by dissolving the resultant powder in Physiological Salt Solution (PSS). PSS was prepared daily and had the following composition (mM): 118 NaCl, 4.7 KCl, 25 $NaHCO_3$, 1 NaH_2PO_4 H_2O, 0.5 Na_2HPO_4, 11.1 glucose, 2.5 $MgCl_2$. $6H_2O$ and 2.5 $CaCl_2$ $2H_2O$. The pH of stock solution was adjusted to 7.4.

The aqueous extract was tested for the presence of tannins, alkaloids and flavonoids using standard procedures[9] followed by thin layer chromatography technique.

Animals: Adult albino rats of either sex, weighting between 150 and 200 g were used. Animals were provided with the standard animal feed and tap water. Food was withdrawn 18 h before the experiments but water was allowed.

Antidiarrheal test: Rats were housed in 5 cages containing 6 rats each. Rats in Group A, B and C received 150, 300 and 450 mg kg^{-1}, respectively. The doses were selected on a trail basis, those in Group D received 5 mg kg^{-1} of diphenoxylate as positive control. Group E

Corresponding Author: Dr. Younes Y. Abu Ghalyun, Department of Biology, Faculty of Art and Science, Hashemite University, Zarka, Jordan Tel: 00962 3826600 4820 Fax: 00962 53826368

which served as a control received PSS only. All administrations were intraperitoneally. The rats were then housed singly in cages lined with white blotting paper. One hour after the above treatments, the rats were given 1 mL of castor oil orally. The rats were observed at time intervals, up to 5 h after the castor oil administration, for the presence of diarrhea. Diarrhea for the purpose of this study was taken to mean watery (wet), unformed stool. The number of wet droppings was counted every hour for a period of 5 h.

Anti-enteropooling test: Intraluminal fluid accumulation was determined by the method of Robert *et al.*[10]. Fasted rats were divided into four groups of six animals each. Group A received PSS intraperitonially and served as control. Group B, C and D received 150, 300 and 450 mg kg^{-1} intraperitoneally of the plant extract, respectively. The above treatments were given 1 h before administration of 1 mL of castor oil orally. Two hours later the rats were sacrificed, the small intestine was ligated both at pyloric sphincter and at the ileocaecal junctions and dissected out. The small intestine was weighted. The intestinal contents were collected by milking into a graduated tube and their volume was measured. The intestine was rewighted and the difference between full and empty intestines was calculated.

Gastrointestinal motility: 0.5 mL of charcoal food (5 g of activated charcoal suspended in 50 mL PSS) was given to five groups of six animals. In Group A, B and C, the charcoal food was administrated to animals intragastrically 60 min after the intraperitoneal injection of aqueous extract of *Juniperus phoenicia* (150, 300 and 450 mg kg^{-1}, respectively). Group D was treated with 1 mg kg^{-1} of atropine sulphate instead of the aqueous extract. Controls (Group E) were treated with PSS before receiving the charcoal food.

The animals were killed after 60 min of charcoal administration and the small intestine, from the pylorus to the caecum, was rapidly removed and laid out on white filter paper for inspection and measurement of the distances traversed by the front of the charcoal food. This distance was calculated as a percentage of the whole intestine length.

Ileal preparation: Rats were lightly anaesthetized with ether and were sacrificed by a sharp blow to the head and the abdomen was opened. Segments of the ileum about 1-2 cm long were removed and dissected free of adhering mesentery. The lumen was flushed with PSS. The tissue was mounted in 10 mL organ bath containing PSS at 37±1°C and aerated with air (95 and 5% of O_2 and CO_2, respectively). A tension of 1 g was applied. The responses were recorded isometrically on a minigraph (Lafayette instrument company) after a 60 min equilibrium period during which the PSS was changed every 15 min as a precaution against tissue metabolites[11]. After equilibrium period, aqueous extract of *Juniperus phoenicia* leaves was added directly to the organ bath. The responses of the ileum to aqueous extract of *Juniperus phoenicia* leaves were expressed as a percentage of the maximum relaxation to papaverine (10^{-3} M) which was added at the end of the experiment.

Acute toxicity test: The LD_{50} of the aqueous extract was determined in mice using the method of Lorke[12].

Statistical analysis: The values were expressed as the mean±SE. A student's t-test was used for the evaluation of data and $p<0.05$ accepted as significant. The effective concentration producing 50% of the maximum response (EC_{50}) was obtained by the best visual fit from the plot of the individual experiments.

RESULTS

Sixty minutes after administration of castor oil the diarrhea was apparent in all the animals of control group, for the next 4 h. This was largely eliminated by the intraperitoneal injection of diphenoxylate. The effect of the aqueous extract was not as potent as the standard drug (diphenoxylate) used, but it was dose-related (Table 1). The preliminary acute toxicity test in mice showed the intraperitoneal injection LD_{50} to be 1587±143 mg kg^{-1}.

Castor oil caused accumulation of water and electrolytes in intestinal loop. Treatment with the *Juniperus phoenicia* extract (150, 300 and 450 mg kg^{-1}) produced a significant and dose dependent reduction in intestinal weight and volume (Table 2).

The aqueous extract of *Juniperus phoenicia* decreased the propulsion of the charcoal meal through the

Table 1: Effect of *Juniperus phoenicia* extract on 1 mL castor oil induced diarrhea in rats

Treatments	Dose	Mean of wet/loose faeces in 5 h	(%) of inhibition
PSS	3 mL kg^{-1}	15.0±3.2	----
Diphenoxylate	5 mg kg^{-1}	3.0±0.6*	80
J. phoenicia	150 mg kg^{-1}	13.2±2.3	12
J. phoenicia	300 mg kg^{-1}	10.3±1.8*	31
J. phoenicia	450 mg kg^{-1}	7.2±0.9*	52

Values are expressed as means±SE from six rats

* Significantly different from control: ($p<0.05$) (Student's t-test)

Table 2: Effect of *Juniperus phoenicia* extract on 1 mL castor oil induced enteropooling in rats

Treatments	Dose	Weight of intestinal content (g)	(%) of inhibition (weight)	Volume of intestinal content (mL)	(%) of inhibition (volume)
PSS	3 mL kg^{-1}	2.10±0.3	---	1.50±0.26	---
J. phoenicia	150 mg kg^{-1}	1.60±0.11	24	1.20±0.23	20
J. phoenicia	300 mg kg^{-1}	1.23±0.35*	41	0.91±0.39*	39
J. phoenicia	450 mg kg^{-1}	0.90±0.42*	57	0.70±0.21*	53

Values are expressed as means±SE from six rats
* Significantly different from control: ($p<0.05$) (Student's t-test)

Table 3: Effect of *Juniperus phoenicia* extract on intestinal motility expressed as distance traveled by the charcoal food as % of the total intestinal length

Treatments	Dose	Movement of charcoal meal (%)	(%) of inhibition
PSS	3 mL kg^{-1}	70.2±4.3	----
Atropine	1 mg kg^{-1}	31.3±5.5*	55
J. phoenicia	150 mg kg^{-1}	66.1±2.6	6
J. phoenicia	300 mg kg^{-1}	50.6±1.7*	28
J. phoenicia	450 mg kg^{-1}	41.8±2.2*	40

Values are expressed as means±SE from six rats
* Significantly different from control: ($p<0.05$) (Student's t-test)

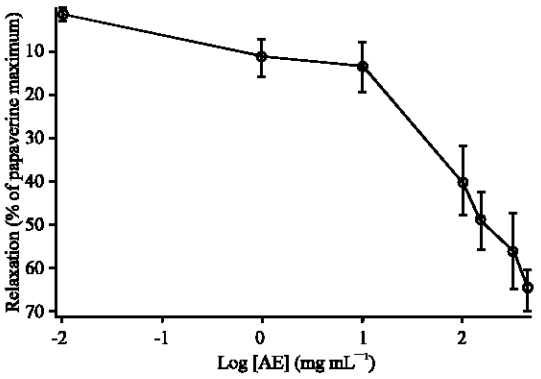

Fig. 1: Concentration effect curve of the Aqueous Extract (AE) of *Juniperus phoenicia* on rat isolated ileum. Values are mean±SE of six experiments

gastrointestinal tract significantly with respect to the control group. The highest inhibition of intestinal transit was obtained with atropine sulphate (Table 3).

In concentrations ranging from 1 to 450 mg mL^{-1} aqueous extract caused a concentration-dependent decrease in the amplitude of the contractions and relaxed the tone of the longitudinal segments of the ileum (Fig. 1). The EC_{50} of aqueous extract for relaxation of ileal segments was 65.1±8.4 mg mL^{-1} (n= 6). The relaxant effect of aqueous extract was fully reversible following washout of the preparations.

DISCUSSION

The results of this study suggest that the aqueous leaves extract of *Juniperus phoenicia* in graded doses (150, 300 and 450 mg kg^{-1} body wt.) reduced diarrhea by inhibiting intestinal motility and intestinal fluid accumulation. The inhibitory effect of the aqueous leaves extract of *Juniperus phoenicia* justifies the use of the plant in folk medicine and its use as a nonspecific anti-diarrhoeal agent. The extract meets some of the criteria for acceptance of a drug as an antidiarrhoeal[13,14]. These criteria include inhibition of the production of wet or unformed faeces in animals and inhibition of gastrointestinal propulsive action.

Castor oil is reported to produce changes in intestinal mucosal membrane permeability to electrolytes and water and thus produces diarrhea[15,16]. The antidiarrheal activity of *Juniperus phoenicia* extract could be due to several mechanisms. These mechanisms include: (1) The extract may increase the reabsorption of water and NaCl by decreasing intestinal motility as observed by the reduction in intestinal motility by charcoal meal. (2) the presence of tannates in the aqueous leaves extract of *Juniperus phoenicia* may make the intestinal mucosa more resistant and reduces secretion[17-19]. Tannic acid and tannins are water soluble polyphenols that are present in many plants[20]. (3) the extract may reduce prostaglandins secretion from intestinal mucosa. Liberation of ricinoleic acid by castor oil results in irritation and inflammation of intestinal mucosa, which lead to the release of prostaglandins. Prostaglandins then result in stimulation of secretion[21,22]. Flavonoids and alkaloids are known for inhibiting release of autocoids and prostaglandins, thereby inhibit secretion induced by castor oil[23,24]. Phytochemical analysis of the aqueous extract of *Juniperus phoenicia* revealed the presence of flavonoids and alkaloids. These constituents may mediate the antidiarrheal property of *Juniperus phoenicia* extract.

Juniperus phoenicia aqueous extract caused a concentration dependent relaxation of rat ileal smooth muscle. The contractions of smooth muscles are known to depend on the concentration of intracellular Ca^{+2} [25,26]. Since plant extract caused an inhibition of the contractions of the ileum, this implies that this extract decreased the cytosolic calcium, either by inhibiting Ca^{+2} influx or by inhibiting Ca^{+2} release from intracellular stores, or both. The relaxant effect could also have resulted from other mechanisms such as a decrease in the sensitivity of contractile apparatus to existing concentrations of Ca^{+2} and/or inhibition of the binding of Ca^{+2} to the contractile proteins[27]. Further studies are required to ascertain the precise mechanism of action of aqueous extract on ileal smooth muscles.

It is conclude that the present study supports claims by traditional medicine practitioners about the use of the leaves aqueous extract of *Juniperus phoenicia* in the treatment of diarrhea. Moreover, the active constituents responsible for the antidiarrheal activity remain to be identified and further studies are needed to understand

the mechanism of antidiarrheal action and relaxant effect of *Juniperus phoenicia* extract.

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REFERENCES

1. Janku, I., M. Hava and O. Motl, 1957. Diuretic substance from juniper (*Juniperus communis* L.). Experientia, 13: 255-256.
2. Moreno, L., R. Bello, B. Beltran, S. Calatayud, E. Primo-Yufera and A Esplugues, 1998. Pharmacological screening of different *Juniperus oxycedrus* L. extracts. J. Pharmacol. Toxicol., 82: 108-112.
3. Schneider, I., S. Gibbons and F. Bucar, 2004. Inhibitory activity of *Juniperus communis* on 12(S)-HETE production in human platelets. Planta Med., 70: 471-474.
4. Karaman, I., F. Sahin, M. Gulluce, H. Ogutcu, M. Sengul and A. Adiguzel, 2003. Antimicrobial activity of aqueous and methanol extracts of *Juniperus oxycedrus* L. J. Ethnopharmacol., 85: 231-235.
5. Sanchez de Medina, F., M. Gamez, I. Jimenez, J. Jimenez, J. Osuna and A. Zarzuelo, 1994. Hypoglycemic activity of juniper berries. Planta Med., 60: 197-200.
6. Cosentino, S., A. Barra, B. Pisano, M. Cabizza, F. Pirisi and F. Palmas, 2003. Composition and antimicrobial properties of *Sardinian Juniperus* essential oils against foodborne pathogens and spoilage microorganisms. J. Food Prot., 66: 1288-1291.
7. Angioni, A., A. Barra, MT. Russo, V. Coroneo, S. Dessi and P. Cabras, 2003. Chemical composition of the essential oils of Juniperus from ripe and unripe berries and leaves and their antimicrobial activity. J. Agric. Food Chem., 51: 3073-3078.
8. Barrero, A., J. Quilez del Moral, M. Herrador, M. Akssira, A. Bennamara, S. Akkad and M. Aitigri, 2004. Oxygenated diterpenes and other constituents from Moroccan *Juniperus phoenicea* and *Juniperus thurifera* var. africana. Phytochemistry, 65: 2507-2515.
9. Trease, G. and M. Evans, 1978. A Textbook of Pharmacognosy. 11th Edn., Baillier, London: Tindall and Cassell, pp: 134-138.
10. Robert, A., J. Nezamis, C. Lancaster, A. Hanchar and M. Klepper, 1976. Enteropooling assay: A test for diarrhea produced by prostaglandins. Prostaglandins, 11: 809-828.
11. Abu Ghalyun, Y., A. Masalmeh and S. Al-khalil, 1997. Effects of allocryptopine, an alkaloid isolated from *Glaucium arabicum* on rat isolated ileum and urinary bladder. Gen. Pharmacol., 41: 713-716.
12. Lorke, D., 1983. A new approach to practical acute toxicity testing. Arch. Toxicol., 54: 275-287.
13. Akah, P., 1988. Purgative potentials of *Euphorbia heterophylla*. Fitoterapia, LX: 45-48.
14. Bertram K., 2003. Basic and Clinical Pharmacology, 9th Edn., McGraw-Hill Medical, New York.
15. Bruton, L., 1996. Agent Affecting Gastrointestinal Water Flux and Motility, Digestants and Bile Acids. In: Pharmacological Basis of Therapeutics, 9th Edn., McGraw-Hill, New York, pp: 916.
16. Galves, J., A. Zavzuelo, M. Crespo, M. Lorente, M. Ocete and J. Jimenez., 1993. Antidiarrhoeic activity of *Euphorbia hirta* extract and isolation of an active flavonoid constituent. Planta Med., 59: 333-36.
17. Tripathi, K., 1994. Essential of Medical Pharmacology. New Delhi: Jaypee Brothers Medical Publishers, pp: 187.
18. Offiah, V. and U.A. Chikwendu, 1999. Antidiarrhoeal effects of *Ocimum gratissimum* leaf extract in experimental animals. J. Ethnopharmacol., 68: 327-330.
19. Das, S., R. Prakash and S.N. Devaraj, 2003. Antidiarrhoeal effects of methanolic root extract of Hemidesmus indicus (Indian sarsaparilla) an *in vitro* and *in vivo* study. Ind. J. Exp. Biol., 41: 363-366.
20. Ofuji, K., H. Hara, T. Sukamoto and S. Yamashita, 1998. Effects of an antidiarrhoeica containing an extract from geranium herb on astringent action and short-circuit current across jejunal mucosa. Nippon Yakurigaku Zasshi, 111: 265-275.
21. Pierce, N., C. Carpenter, H. Elliot and W. Greenough, 1971. Effects of prostaglandins, theophylline and cholera exotoxin upon transmucosal water and electrolyte movement in the canine jejunum. Gastroenterology, 60: 22-32.
22. Ramakrishna, B., M. Mathan and V. Mathan, 1994. Alteration of colonic absorption by long-chain unsaturated fatty acids. Influence of hydroxylation and degree of unsaturation. Scand J. Gastroenterol., 29: 54-58.
23. Vimala, R., S. Nagarajan, M. Alam, T. Susan and S. Joy, 1997. Antiinflammatory and antipyretic activity of *Michelia champaca* Linn., (white variety), *Ixora brachiata* Roxb. and *Rhynchosia cana* (Willd.) D.C. flower extract. J. Exp. Biol., 35: 1310-1314.

24. Veiga, V., L. Zunino, J. Calixto, M. Patitucci and A. Pinto, 2001. Phytochemical and antioedematogenic studies of commercial copaiba oils available in Brazil. Phytother. Res., 15: 476-480.
25. Jiang, H. and N.L. Stephens, 1994. Calcium and smooth muscle contraction. Mol. Cell Biochem., 135: 1-9.
26. Ganitkevich, V., V. Hasse and G. Pfitzer, 2002. Ca^{2+}-dependent and Ca^{2+}-independent regulation of smooth muscle contraction. J. Muscle Res. Cell Motil., 23: 47-52.
27. Savineau, J. and R. Marthan, 1997. Modulation of the calcium sensitivity of the smooth muscle contractile apparatus: Molecular mechanisms, pharmacological and pathophysiological implications. Fundam. Clin. Pharmacol., 11: 289-299.