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Original Article

Evaluation of systemic effect of *Pycnocycla spinosa* extract in mice using Irwin test

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Abstract

Hydroalcoholic extract of *Pycnocycla spinosa* has spasmolytic action *in vitro*. At oral dose of 250 μg/kg it inhibits castor oil induced diarrhea in mice. If *P. spinosa* extract has no serious adverse effect, it would be a good alternative medicine for treatment of diarrhoea and abdominal spasm. In this research, overall systemic effect of *P. spinosa* extract on mice behavior and reaction was investigated. Single acute dose of the extract (500 μg/kg and 1 mg/kg, i.p.) had negligible effect on animal behavior. However, some changes were seen with dose of 10 mg/kg of the extract. In a group of mice that were treated daily with the extract (10 mg/kg, i.p.) over 3 weeks, a reduction on alertness and marked decrease on defecation was observed on first and second weeks. No significant changes were seen in behavior of the animals that were received the extract in their drinking water over three weeks. Lethal dose causing 50% mortality indicates a large margin of safety.

Key words: Antidiarrhoeal, Behavioral activity, Hydroalcoholic extract, Irwin test, *Pycnocycla spinosa*

INTRODUCTION

Pycnocycla spinosa Decne. exBoiss. var. *spinosa* (Umbelliferae) is a wild plant growing in Iran (1). Hydroalcoholic extract of *P. spinosa* is a potent relaxant of isolated ileum (2). The hydroalcoholic extract is stable and composed of alkaloid, flavonoid and saponin components (3). The antispasmodic action of *P. spinosa* extract is partly due to alkaloid and flavonoid rich fractions (3). In addition, *P. spinosa* extract was shown to have antidiarrhoeal action *in vivo* (2). Physical stability and potent pharmacological activity are characteristics, which make *P. spinosa* extract a suitable candidate to be used as an antidiarrhoeal agent. However, before recommendation for clinical use, other possible pharmacological activity of the extract, including its general systemic and/or toxic effects needs to be determined. Although the extract would be given orally, some of the extract components might be absorbed and may affect other organs. First step in studies of drug effect on systemic and central activity is a general screening methods as described by Turner (4). Therefore, an objective of this research was to investigate central and systemic effect of *P. spinosa* extract in mice, using general method, which was introduced by Irwin (5). Other objective was to determine the lethal doses of *P. spinosa* extract which cause 50% mortality (LD_{50}).

MATERIALS AND METHODS

Plant extract

Aerial parts of *P. spinosa* were collected

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in June from Isfahan University campus and identified by the botanist, Mr Mehregan, in the Biology Department at Isfahan University. A voucher specimen (A24) was authenticated and then deposited in the herbarium of the Faculty of Pharmacy and Pharmaceutical Sciences (Isfahan, Iran). The aerial part of the plant was dried in shade. The total hydroalcoholic extract was obtained by percolation (6). After evaporation of sovent, the amount of dry crude extract was determined (w/w).

Screening for CNS activity

Male albino mice (Pasture, Tehran) weighing 27-32g housed at room temperature with free access to food and water were used in this study. The procedure involved an initial period of observation of relatively undisturbed mouse and subsequently a phase during which the animal is subjected to a series of stimuli, the least innocuous first, followed by tests of increasing stimulus intensity. The observation consists of animal behavior in the cage (undisturbed), animal placed on bench (disturbed), animal held by tail (tail suspension) and when animal was restrained (supine). In undisturbed situation, parameters like body position, locomotor activity, respiration, tremors, bizarre behavior, exophthalmos, and convulsi-ons were assessed. Animal then was placed on the bench and parameters like transfer arousal, spatial locomotion, gait incapacity, palpebral closure, piloerection, startle response, finger approach and touch escape responses were assessed. Then the animal was held by its tail and positional passivity, visual placing, grip strength, pinna, corneal and toe pinch reflexes were assessed. After that, the animal was restrained by grabbing skin on its back and holding it in supine position, then skin color, limb tone, abdominal tone, salivation, provoked biting, tail pinch, diarrhoea, lacrimation and at the end righting reflex were assessed. Throughout the trial any handling irritability, vocalization, urination, defecation and death that occurred were recorded. These observations generally assess behavior, neurological and autonomic activities. Assessment and quantification of above parameters were performed adopting the methods described by Turner (4). For most parameters an increase in score from a norm of 4 to 8 was used to denote stimulation, depression being indicated by an increase in score from 4 to 0. If data couldn't be accurately judged (for example muscle tone), a reduced scale 0, 2, 4, 6, 8 were used.

Experimental Procedure

All experiments were performed in the morning and at least six mice were used in each group. For acute study single dose of extract (500 μg/kg, 1 mg/kg and 10 mg/kg) or vehicle were administered blindly by intraperitoneal (i.p.) injection. On each experiment day only 2 or 3 mice were used. The observation schedule was made on first mouse and scores were recorded on a record sheet. Upon completion of tests on first animal, injection allocated to that animal was given and the time of injection was recorded. Then the test were continued on the second mouse and injected upon completion of testes. In a similar way, the third mouse was tested and injected. Observational tests were performed again separately on each animal at 30 and 90 minutes interval after injection.

For chronic studies, one group of animal (n=6) was given *P. spinosa* extract solution (10 mg/kg, i.p.) every day for 3 weeks and the observation schedule were made before extract administration, and then after 2, 7, 14 and 21 days. In another group (n=20), the extract was supplied in drinking water (50 μg/ml) for oral administra-tion and similar chronic study procedure was used. Each experiment group had its' own control, which received equivalent volume of the vehicle (ethanol). Diazepam (5 mg/kg, i.p.) was used as positive control for the acute study (n=6).

Lethal dose study

Lethal dose causing 50% mortality (LD_{50}) in mice was determined after i.p.

administration of several successive doses of *P. spinosa* extract (ranging from 15 mg/kg to 240 mg/kg), using two folds increment in doses at 15 minutes intervals. A dose of the extract that stopped both the heart beats and respiration considered as lethal dose.

Drugs and solutions

Diazepam was obtained from Daro-pakhsh, Iran. Dried extract of *P. spinosa* was dissolved as 10 mg/ml solution in 50% ethanol. The extract solution was then further diluted in saline for parental administration to prepare 1 mg/ml stock solution. The intraperitoneal volume of injection depending on weight of the animal, was usually between 0.27-0.32 ml.

Analysis of results

Upon completion of each group of experiment, the data were assemble into summery table comprising all the results. As the score scale, was not isomorphic to the arithmetic scale, the median value (plus interquartile range) was used. Non-parametric statistical tests were used to analyze differences between groups of mice. Wilcoxon matched-pair signed rank test and Mann Whitney U-test were used for related and unrelated data respectively.

Mortality ratios at different dose of the extract were calculated and the LD_{50} was determined after fitting a regression line through the points (7). Origin graphic computer program was used to fit regression line.

RESULTS

Screening for CNS activity

A significant reduction in awareness, grooming, motor activity, motor incoordination, muscle tone and posture was seen in animals treated with diazepam (5 mg/kg). There was less urination and also a decrease in palpebral opening as well as corneal and pinnal reflexes. Equivalent volume of diazepam vehicle (ethanol) also had affected some of the parameters, slightly reducing alertness, reactivity spontaneous activity, grooming and posture. In another group of mice 0.5 ml of saline solution was used as a neutral control in order to determine changes in behavior following injection and adaptation due to repeating of behavioral experimental procedure. Following saline injection slight but no significant changes in alertness, locomotor's activity, urination and defecation were observed.

P. spinosa extract (500 µg/ml, i.p.) slightly reduced alertness, but 90 min after injection alertness returned to its pretreatment control level. There was also a reduction in reactivity, spontaneous activity, and pain response. Urination and defecation were also reduced. There was no change in the other parameters. With 1 mg/kg *P. spinosa* extract there was greater reduction in alertness. A reduction in grooming, reactivity, spontaneous activity, limb activity and locomotor activity was also observed. Palpebral opening was slightly reduced and there was a marked reduction on defecation (Table 1). Acute injection of *P. spinosa* extract (10 mg/kg, i.p.) reduced alertness, grooming, reactivity, limb activity and motor activity. Peak effects being observed 30 min after injection. There was also a marked reduction in both urination and defecation (Table 2). There was no significant change in other parameters in acute studies. It is interesting to note that the equivalent volume of vehicle (ethanol + saline) also caused some reduction in alertness, reactivity and spontaneous activity. Urination and defecation were also slightly reduced in the control groups.

In chronic study in the group of mice that were treated daily with *P. spinosa* extract (10 mg/kg, i.p.) over 3 weeks, a reduction on alertness was observed on the first and second weeks. However, there was an increase in spontaneous activity on week 2 and 3 of treatments. Limb activity and motor activity were also slightly increased on second week. Other significant

change that was observed during period of chronic treatment with *P. spinosa* extract was a mark reduction on defecation. No significant change was observed in other parameters during the course of studies. In the parallel time matched control group which were treated daily with equivalent volume of vehicle (ethanol) there was a significant reduction on alertness at weeks 2 and 3 and decrease on reactivity on third week ($P<0.05$). There was also a slight reduction on defecation after 1 week but thereafter it remained constant. In the group that received the extract in their drinking water, they had an average daily intake of 6 ml of the extract over three weeks. This is equivalent of 8 mg/kg of the extract intake per day. Control group also had drunk a similar amount of fluid. No significant changes were seen in the test or control groups in this study (Table 3). No mortality occurred in any group of the mice that were treated with *P. spinosa* extract in acute or chronic studies.

Lethal dose (LD_{50})

Intraperitoneal injection of *P. spinosa* extract at doses above 30 mg/kg had a pronounced effect on the mice, the animals became sedated and sleepy and when provoked to move, they had a severe ataxia. Muscle tonicity was severely reduced, eyes were almost closed and there was a clear reduction in respiratory rate.Mortality was mainly observed with doses above 100 mg/kg, which caused severe respiratory depression. The lethal dose causing 50% mortality calculated to be about 140 mg/kg (n=12). Respiratory depression seems to be the main cause of death.

DISCUSSION

Hydroalcoholic extract of *P. spinosa* at oral dose of 250 μg/kg has antidiarrhoeal action (2). At 1 mg/kg oral dose it almost abolishes castor oil induced diarrhoea in mice (2). Loperamide and Diphenoxylate are reported to inhibit castor oil induced diarrhoea in mice at dose of 3 mg/kg and 2.5 mg/kg respectively (8, 9). Despite *P. spinosa* extract is mixtures of active and inactive substance, in comparative doses, it has an antidiarrhoeal action as good as that of loperamide or diphenoxylate. Nevertheless, such compounds may have other pharmacological or adverse effect, which need to be investigated. If *P. spinosa* extract has no serious adverse effect, then it would be a good alternative herbal medicine for treatment of diarrhoea and abdominal spasm.

The experimental procedure used for detecting central and systemic effect of *P. spinosa* was essentially designed to screen drug actions as rapidly, comprehensively and inexpensively as possible. The experiment consists of systematic observations on mice, where it is possible to detect gross changes in behavior. Observation, although subjective, allows observer to escape limitations of mechanical instruments, notably the ability in many experiments to monitor only one or two parameters concurrently. The information collected in observation screening tests was assessed by quantal (all or none) or quantitative (graded) ways. Both these methods have their advantages and disadvantages. The first is faster, cheaper and an initial drug profile can be prepared rapidly. The second is slower but usually more information can be obtained. It is often difficult, however, to design quantitative test procedure to measure certain parameters, for example, muscle weakness produced by a relevant drug. On the other hand, the quantal test also hasdisadvantages, for examples the observer may find it difficult to establish a cut off or end point. Despite limitation of the procedure, this test clearly shows the central effect of diazepam and confirms its reliability. In this way, we have examined central and systemic action of *P. spinosa* extract using single dose and three weeks administration of the extract. Hydroalcoholic extract of *P. spinosa* at antidiarrhoeal does (500 μg/kg and 1 mg/kg) have no

Table 1. Assessment of animal behavioral response before and after injection of antidiarrhoeal dose of *P.spinosa* extract (1mg/kg, i.p.). Each number is a median (plus interquartile range) of behavioral scores in 6 separate mice.

Treatments	Dose (Extract) 1 mg/kg	Control (before inj.)	30 min (after inj.)	90 min (after inj.)
Awareness	Awareness	4.5(4-5)	2(0.5-4)*	2.25(1-3)*
	Visual Placing	4(4-4)	4(4-4)	4(4-4)
	Passivity	0(0-0)	0(0-0)	0(0-0)
	Stereotypy	0(0-0)	0(0-0)	0(0-0)
Mood	Grooming	4(4-4)	3.5(3-4)	3.5(2-4)
	Vocalization	0(0-0)	0.5(0-1)	0(0-1)
	Restlessness	0(0-0)	0(0-0)	0(0-0)
	Irritability (aggression)	0(0-0)	0(0-0)	0(0-0)
	Fearfulness	0(0-0)	0(0-0)	0(0-0)
Motor Activity	Reactivity (envir.)	4(4-4)	3(2-4)	2.5(2-4)
	Spontaneous Activity	4(4-5)	4(3-4)♣	3(2-4)
	Touch Response	4(4-4)	4(3.5-4)	4(3-4)
	Pain Response	4(4-4)	4(4-4)	4(4-4)
CNS Excitation	Startle Response	0(0-0)	0(0-0)	0(0-0)
	Straub Tail	0(0-0.5)	0(0-0)	0(0-0)
	Tremors	0(0-0)	0(0-0)	0(0-0)
	Twitches	0(0-0)	0(0-0)	0(0-0)
	Convulsions	0(0-0)	0(0-0)	0(0-0)
Posture	Body Posture	4(4-4)	4(4-4)	3(3-4)
	Limb Position	4(4-4)	4(4-4)	3(3-4)
Motor Incoord	Staggering Gait	0(0-0)	0(0-0)	0(0-0)
	Gait Incapacity	0(0-0)	0(0-0)	0(0-0)
	Righting Reflex	0(0-0)	0(0-0)	0(0-0)
Muscle Tone	Limb Tone	4(4-4)	4(4-4)	4(4-4)
	Grip Strength	4(4-4)	4(3.5-4)	4(4-4)
	Body Sag	0(0-0)	0(0-0)	0(0-0)
	Abdominal Tone	4(4-4)	4(4-4)	4(4-4)
Reflex	Pinna	1(1-1)	1(1-1)	1(1-1)
	Corneal	1(1-1)	1(1-1)	1(1-1)
	IFR (Toe Pich)	4(4-4)	4(4-4)	4(4-4)
Autonomic	Salivation	0(0-0)	0(0-0)	0(0-0)
	Writhing	0(0-0)	0(0-0)	0(0-0)
	Palpebral opening	4(4-4)	4(3-4)	4(3-4)
	Exopthalmos	0(0-0)	0(0-0)	0(0-0)
	Urination	1(0-2)	1(0-1)	1(0-1)
	Piloerection	0(0-0)	0(0-0)	0(0-0)
	Skin colour	1(1-1)	1(4-4)	1(1-1)
	Respir. Rate	6(6-6)	5(5-6)	5(5-5)
Miscellaneous	Lacrimation	0(0-0)	0(0-0)	0(0-0)
	Defecation	4(4-5)♣	0(0-1)*	0.5(0-2)*
	Diarrhea	0(0-0)	0(0-0)	0(0-0)
Dead	No. Acute	0	0	0
	No. delayed	0	0	0

* =P<0.05 compared with pretreatment control, (Wilcoxan-test).♣ = P<0.05 compared with vehicle treated control, (Mann-Whitney U test).

Table 2. Assessment of animal behavioral response before and after injection of *P. spinosa* extract (10 mg/kg, i.p.).

Treatments	Dose (Extract) 1 mg/kg	Control (before inj.)	30 min (after inj.)	90 min (after inj.)
Awareness	Alertness	4(4-4)	1(0-4)	3.5(0.5-4)
	Visual Placing	4(4-4)	4(4-4)	4(4-4)
	Passivity	0(0-0)	0(0-0)	0(0-0)
	Stereotypy	0(0-0)	0(0-0)	0(0-0)
Mood	Grooming	4(4-4)	1(0-4)	4(0-4)
	Vocalization	0(0-0)	0(0-0)	0(0-0)
	Restlessness	0(0-0)	0(0-0)	0(0-0)
	Irritability (aggression)	0(0-0)	0(0-0)	0(0-0)
	Fearfulness	0(0-0)	0(0-0)	0(0-0)
Motor Activity	Reactivity (envir.)	4(4-4)	3(1-4)	3(2-4)
	Spontaneous Activity	4(4-4)	4(3.5-4)	♣ 4(4-4)
	Touch Response	4(3-4)	3.5(1-4)	3.5(3-4)
	Pain Response	4(4-4)	4(3-4)	4(4-4)
CNS Excitation	Startle Response	0(0-0)	1(0-2)	0(0-1)
	Straub Tail	0(0-0)	0(0-0)	0(0-0)
	Tremors	0(0-0)	0(0-0)	0(0-0)
	Twitches	0(0-0)	0(0-0)	0(0-0)
	Convulsions	0(0-0)	0(0-0)	0(0-0)
Posture	Body Posture	4(4-4)	2(2-4)	3.5(3-4)
	Limb Position	4(4-4)	2(2-4)	3.5(3-4)
Motor Incoord	Staggering Gait	0(0-0)	0(0-0)	0(0-0)
	Gait Incapacity	0(0-0)	0.5(0-1)	0(0-0)
	Righting Reflex	0(0-0)	0(0-0)	0(0-0)
Muscle Tone	Limb Tone	4(4-4)	4(4-4)	4(4-4)
	Grip Strength	4(4-4)	4(4-4)	4(4-4)
	Body Sag	0(0-0)	0(0-0)	0(0-0)
	Abdominal Tone	4(4-4)	4(4-4)	4(4-4)
Reflex	Pinna	1(1-1)	1(1-1)	1(1-1)
	Corneal	1(1-1)	1(1-1)	1(1-1)
	IFR (Toe Pich)	4(4-4)	4(4-4)	4(4-4)
Autonomic	Salivation	0(0-0)	0(0-0)	0(0-0)
	Writhing	0(0-0)	0(0-0)	0(0-0)
	Palpebral opening	4(4-4)	4(3-4)	4(3-4)
	Exopthalmos	0(0-0)	0(0-0)	0(0-0)
	Urination	0.5(0-1)	0(0-0)	0(0-1)
	Piloerection	0(0-0)	0(0-0)	0(0-0)
	Skin colour	1(1-1)	1(1-1)	1(1-1)
	Respir. Rate	6(6-6)	6(5-6)	6(6-6)
Miscellaneous	Lacrimation	0(0-0)	0(0-0)	0(0-0)
	Defecation	1.5(1-2)	0.5(0-1)	*0(0-0)
	Diarrhea	0(0-0)	0(0-0)	0(0-0)
Dead	No. Acute	0	0	0
	No. delayed	0	0	0

Each number is a median (plus interquartile range) of behavioral scores in 6 separate mice. There was no significant change compared with pretreatment control (Wilcoxan- test).♣= $P<0.05$ compared with vehicle treated control, (Mann-Whitney U test).

Table 3. Assessment of animal behavioral response before and after regular oral consumption of *P. spinosa* extract (8 mg/kg per day) over 2 days, 1 week, 2 weeks and 3 weeks.

Treatments	Dose (Extract) 8 mg/kg/day	Control	2 days after oral dosing	7 days after oral dosing	14 days after oral dosing	21 days after oral dosing
Awareness	Awareness	4(4-4)	4(4-4)	4(4-4)	4(4-4)	4(4-4)
	Visual Placing	4(4-4)	4(4-4)	4(4-4)	4(4-4)	4(4-4)
	Passivity	0(0-0)	0(4-4)	0(0-0)	0(0-0)	0(0-0)
	Stereotypy	0(0-0)	0(0-0)	0(0-0)	0(0-0)	0(0-0)
Mood	Grooming	4(4-4)	4(4-4)	4(4-4)	4(4-4)	4(4-4)
	Vocalization	0(0-0)	0(0-0)	0(0-0)	0(0-0)	0(0-0)
	Restlessness	0(0-0)	0(0-0)	0(0-0)	0(0-0)	0(0-0)
	Irritability (aggression)	0(0-0)	0(0-0)	0(0-0)	0(0-0)	0(0-0)
	Fearfulness	0(0-0)	0(0-0)	0(0-0)	0(0-0)	0(0-0)
Motor Activity	Reactivity (envir.)	4(4-4)	4(4-4)	4(4-4)	4(4-4)	4(4-4)
	Spontaneous Activity	4(4-4)	4(4-4)	3.75(3-4)	2.5(2-3.5)	3(3-3)
	Touch Response	4(4-4)	4(4-4)	4(4-4)	4(4-4)	4(4-4)
	Pain Response	4(4-4)	4(4-4)	4(4-4)	4(4-4)	4(4-4)
CNS Excitation	Startle Response	0.5(0-2)	2(2-2)	3(2-3)	2.75(2-3)	*3(3-4)
	Straub Tail	0(0-0)	0(0-0)	0(0-0)	0(0-0)	0(0-0)
	Tremors	0(0-0)	0(0-0)	0(0-0)	0(0-0)	0(0-0)
	Twitches	0(0-0)	0(0-0)	0(0-0)	0(0-0)	0(0-0)
	Convulsions	0(0-0)	0(0-0)	0(0-0)	0(0-0)	0(0-0)
Posture	Body Posture	4(4-4)	4.25(4-5)	4(4-4.5)	4(4-4)	4(4-4)
	Limb Position	4(4-4)	(4-4)	4(4-4)	4(4-4)	4(4-4)
Motor Incoord	Staggering Gait	0(0-0)	0(0-0)	0(0-0)	0(0-0)	0(0-0)
	Gait Incapacity	0(0-0)	0(0-0)	0(0-0)	0(0-0)	0(0-0)
	Righting Reflex	0(0-0)	0(0-0)	0(0-0)	0(0-0)	0(0-0)
Muscle Tone	Limb Tone	4(4-4)	4(4-4)	4(4-4)	4(4-4)	4(4-4)
	Grip Strength	4(4-4)	4(4-4)	4(4-4)	4(4-4)	3.75(3.5-4)
	Body Sag	0(0-0)	0(0-0)	0(0-0)	0(0-0)	0(0-0)
	Abdominal Tone	4(4-4)	4(4-4)	4(4-4)	4(4-4)	4(0-0)
Reflex	Pinna	1(1-1)	1(1-1)	1(1-1)	1(1-1)	1(1-1)
	Corneal	1(1-1)	1(1-1)	1(1-1)	1(1-1)	1(1-1)
	IFR (Toe Pich)	4(4-4)	4(4-4)	4(4-4)	4(4-4)	4(4-4)
Autonomic	Salivation	0(0-0)	0(0-0)	0(0-0)	0(0-0)	0(0-0)
	Writhing	0(0-0)	0(0-0)	0(0-0)	0(0-0)	0(0-0)
	Palpebral opening	4(4-4)	4(4-4)	4(4-4)	4(4-4)	4(4-4)
	Exopthalmos	0(0-0)	0(0-0)	0(0-0)	0(0-0)	0(0-0)
	Urination	0(0-1)	0(0-0)	0(0-0)	0(0-0)	0(0-0)
	Piloerection	0(0-0)	0(0-0)	0(0-0)	0(0-0)	0(0-1)
	Skin colour	1(1-1)	1(1-1)	1(1-1)	1(1-1)	1(1-1)
	Respir. Rate	6(6-6)	6(6-6)	6(6-6)	6(6-6)	6(6-6)
Miscellaneous	Lacrimation	0(0-0)	0(0-0)	0(0-0)	0(0-0)	0(0-0)
	Defecation	1(1-2)	♣1(1-1)	2(2-2)	2(1-2)	2(2-3)
	Diarrhea	0(0-0)	0(0-0)	0(0-0)	0(0-0)	0(0-0)
Dead	No. Acute	0	0	0	0	0
	No. delayed	0	0	0	0	0

Each number is a median (plus interquartile range) of behavioral scores in 20 separate mice. There was no significant change compared with pretreatment control (Wilcoxan-test).♣ = $P<0.05$ compared with vehicle treated control, (Mann- Whitney U test).

significant central effect although slight sedation sometimes observed. Furthermore, with administ-ration of 10 mg/kg (i.p.) of *P. spinosa* extract (10 times of antidiarrhoeal dose) only minor behavioral changes were observed even after three weeks daily administration of the extract. As it was seen in the control group, part of the observed central effect was due to the vehicle (ethanol). However, at extreme high doses of the extract (over 30 mg/kg) a clear central and peripheral effect was seen. The lethal dose causing 50% mortality following i.p. injection of the extract (above 100 mg/kg) indicates a large margin of safety. For treatment of diarrhoea, the extract intended to be used orally, therefore, systemic action of the extract expected to be even less, because absorption may not be complete and would be less rapid than i.p. administration. Daily intake of the extract over 3 weeks (about 8 mg/kg/day) produced no central effect or behavioral changes in mice. This is either because the extract is not fully absorbed or more likely is due to gradual absorption and distribution and possibly as a result of tolerance. However, it is important to point out that hydroalcoholic extract of *P. spinosa* is a potent relaxant of the ileum and at high concentration, can totally inhibit ileum smooth muscle contraction (2) and that is the main cause of reduction in defecation. The control group in the chronic study shows that some of the observed behavioral changes were either due to effect of the vehicle or as a result of animal adaptation.

Thus, from this study it can be concluded that hydroalcoholic extract of *P. spinosa* at antidiarrhoeal doses had no serious adverse effect and has a more selective action on ileum, nevertheless, because of its potent pharmacological action constipation would be expected. In addition, the lethal dose causing 50% mortality (LD_{50}) in mice after i.p. injection of *P. spinosa* extract indicates a relatively good margin of safety. This study therefore confirms general safety of *P. spinosa* hydroalcoholic extract as a useful medicinal plant for treatment of diarrhoea and gastrointestinal spasm. Therefore, it is suggested detailed toxicological testing to be done to role out any toxic effect at doses that would be used for treatment of diarrhoea.

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REFERENCES

1. Mozaffarian V. A dictionary of Iranian plant names. Tehran: Farhang Moaser; 1995. p. 443-444.
2. Sadraei H, Asghari G, Naddafi A. Relaxant effect of essential oil and hydro-alcoholic extract of *Pycnocycla spinosa Decne. & exBoiss* on ileum contractions. Phytother Res. 2003;17: 645-649.
3. Sadraei H, Asghari G, Hekmatti AA. Antispasmodic effect of three fractions of hydroalcoholic extract of *Pycnocycla spinosa*. J Ethnopharmacol. 2003;86:187-190.
4. Turner RA. Screening methods in pharmacology. New York: Academic Press; 1969. p. 22-41.
5. Irwin S. Comprehensive observation assessment: 1a. A systematic, quantitative procedure for assessing the behavioral and physiological state of the mouse. Psycopharmacol. 1968;13:222-257.
6. Samuelsson G. Drugs of natural origin. 4th ed. Stockholm: Swedish Pharmaceutical Press; 1999. p. 48-49.
7. Klaassen CD. Casarett & Doull's toxicology the basic science of poisons. 5th ed. New York: McGrow-Hill; 1996. p. 13-33.
8. Sairam K, Hemalath S, Kumar A, Srinivasan T, Ganesh J, Sahnker M, et al. Evaluation of antidiarrhoeal activity in seed extract of *Mangifera indica*. J Ethnopharmacol. 2003;84: 11-15.
9. Adzu B, Amos S, Amizan MB, Gamaniel K. Evaluation of the antidiarrhoeal effect of *Zizyphus spina-christi* stem bark in rats. Acta Tropica. 2003;387:245-250.