A preliminary study on some potential toxic effects of *Rosa damascena* Mill

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Summary

Rosa damascena has been traditionally used as an herbal medicine for different therapeutic purposes. In order to preliminarily study the probable toxic effects of the plant, its infusion was orally administered to 5 groups of 5 dogs at doses 0.5-8 times that of human use in traditional medicine (90-1440 mg/kg/d) for 10 successive days. The dogs in the control group (n=4) received placebo. Serum levels of urea, creatinine, alkaline phosphatase (ALP), alanine aminotransferase (ALT), bilirubin, albumin and protein were measured in all experimental groups at days 0, 1, 3, 7 and 10. Except for a transient increase in bilirubin levels (day 3) and a rise in serum ALT at day 10, both with the highest dose of the treatment, there were no statistical differences between different experimental groups compared to the control. The results suggest minimal nephrotoxic or hepatotoxic effects for the infusion of *R. damascena*, however, the medication may be hepatotoxic at extraordinary high doses.

Key words: Damask rose, *Rosa damascena*, Herbal medicine, Serum enzymes

Introduction

Rosa damascena Mill, commonly known as Damask rose, is mainly cultivated worldwide for the production of rose essential oil. It is also grown as an ornamental plant and for production of rose water and attar of roses (Widrlechner, 1981). The petals of the plant contain carboxylic acid, terpene, myrcene, vitamin C, geraniol, citronellol and nerol (Zargari, 1992; Libster, 2002; Verma *et al*., 2011). The flowers of the plant are also rich sources of flavonoids (Velioglu and Mazza, 1991).

In addition to perfuming effect, the plant has been traditionally used for medical purposes from a long time ago. In ancient medical books, *R. damascena* has been suggested for treatment of menstrual bleeding, treatment of abdominal pain and digestive problems (AveSina, 1990), as well as chest pain and as an anti-inflammatory remedy (Green, 1999).

Recent studies suggest a wide variety of therapeutic effects for *R. damascena*. Several studies suggest strong antibiotic effects against a wide range of bacteria for the plant (Basim and Basim, 2003; Ozkan *et al*., 2004; Ulusoy *et al*., 2009; Shokouhinejad *et al*., 2010). Anti-HIV effect has also been reported for water and methanol extracts of the plant (Mahmood *et al*., 1996). Besides, *R. damascena* has been shown to possess analgesic (Rakhshandeh *et al*., 2008), anticonvulsant (Kheirabadi *et al*., 2008; Ramezani *et al*., 2008), antitussive (Shafei *et al*., 2003), relaxant (Boskabady *et al*., 2006; Hongratanaworakit, 2009) and antidiabetic (Gholamhoseinian *et al*., 2009) effects. Recent studies from this lab suggest laxative effects for *R. damascena* infusion in rats (Arezoomandan *et al*., 2011) and dogs (Abbaszadeh *et al*., 2010). Despite its extensive pharmacologic effects and

numerous therapeutic applications, little is known regarding potential toxic effects of this herbal medicine. The present study was aimed to preliminarily evaluate the potential toxic effects of the plant.

Materials and Methods

Dried flower petals of *R. damascena* were purchased from the market. The genus and the species of the plant were confirmed by the Research Center for Plant Sciences, Ferdowsi University of Mashhad (Herbarium No: 10972, FUMH). The petals were infused in distilled water (120 g/L) for 10 min. The filtrate was collected and stored at 4°C for a maximum period of 3 days before being fed to the animals. Each gram of the original dried flower petals was calculated to yield 0.52 g dry matter in the extract. This was achieved by weighing the dried pellet of the extract. In this study, however, all calculations were based on the original dried petal weights. The basal dose (N) of the extract was extrapolated according to human dose (180 mg/kg Bwt) in traditional medicine (Zargari, 1992). The initial extract was prepared 8 times thicker, contained 72 mg extract dry matter per ml, and was fed to the group receiving the highest dose (8 N; 20 ml/kg Bwt). It was serially diluted with distilled water to yield other doses of 4 N, 2 N, N and 1/2 N, respectively.

The animals were treated in compliance with the guidelines of Animal Welfare Committee of the Research Deputy of Ferdowsi University of Mashhad. The experiment was performed in the Animal House of the Veterinary School, Ferdowsi University of Mashhad. Twenty nine healthy dogs (20.1 ± 1.5 kg) of both sexes (1–4-year-old) were acclimated in experimental conditions for 1 week and were then randomly assigned to 6 experimental groups. Five groups (n=5) received exponentially increasing doses of the herb extract (1/2 N, N, 2 N, 4 N and 8 N: 90-1440 mg/kg) daily, using a 50 ml syringe. The 6th group (n=4) received a similar volume (12 ml/kg) of the placebo (distilled water) and was considered as the control.

The animals in different experimental groups received either the extract or the placebo for 10 successive days. All animals were sampled for blood serum at days 0, 1, 3, 7 and 10 of the experiment. Serum samples were analysed for urea, creatinine, alkaline phosphatase (ALP), alanine aminotransferase (ALT), bilirubin, albumin and protein using commercial kits by an autoanalyzer (Tagra 3000, Italy).

Statistical analysis and drawing of the figures were performed using GraphPad Prism version 5.0 Software (GraphPad Software, USA). Statistical comparisons were performed using two-way analysis of variance (ANOVA) followed by Bonferroni posttests. Unless otherwise mentioned, all data are represented as mean and SEM.

Results

Serum ALT activity averaged 22.3 IU at the beginning of the experiment in the control group and did not change significantly during the 10 days of the experiment (Table 1). ALT activity increased significantly at the end of the experiment in dogs receiving the highest dose of the extract compared to the control. However, there were no other significant differences between the control group and those that received the extract throughout the experiment.

At the beginning of the experiment, the average serum ALP activity was 123.7 IU/L in the control group and did not change significantly throughout the experiment (Table 1). Consistently, there were no statistical differences in the enzyme activity among different experimental groups due to the time course or treatment with the extract.

No statistical differences were observed in serum creatinine concentrations, serum albumin and urea levels and the amount of serum total protein either within or between different experimental groups (Table 1).

Comparison of the results from serum bilirubin levels did not show significant changes throughout the experiment (Table 1). Consistently, apart from 8 N group at day 3 (P<0.05), there were no significant differences between the test groups and the control.

It is noteworthy that the test animals,

Table 1: Serum parameters in dogs that received placebo (control) or different doses of *R. damascena* infusion (N=180 mg/kg Bwt) for 10 consecutive days

Parameters	Day	Control	1/2 N	N	2 N	4 N	8 N
ALT (IU/L)	0	22.3±2.8	26.2±4.7	37.9±6.8	30.5±7.5	27.2±6.1	33.7±5.0
	1	22.8±3.2	27.6±5.1	32.4±4.4	25.0±4.9	22.3±1.2	28.2±1.9
	3	23.0±3.0	27.1±5.9	29.0±3.0	23.5±4.2	24.8±3.8	37.5±6.8
	7	22.7±3.1	27.6±5.2	31.1±3.0	41.8±18.5	22.8±1.9	36.2±5.7
	10	20.9±4.0	26.0±4.6	31.7±4.0	38.2±15.4	25.8±3.5	49.4±10.6[*]
ALP (IU/L)	0	123.7±11.3	97.2±11.9	140.4±43.8	114.8±20.2	129.8±33.5	98.3±3.2
	1	114.9±9.4	96.2±11.5	154.2±36.2	207.7±101.9	89.9±10.0	100.6±8.9
	3	124.5±13.1	90.8±8.0	159.4±45.8	199.7±95.0	125.6±33.7	120.0±7.7
	7	128.4±16.2	80.9±22.0	167.7±50.5	182.0±81.1	119.2±25.7	112.3±8.6
	10	135.6±23.7	85.9±9.2	177.8±56.9	166.8±54.6	96.3±15.6	121.6±12.7
Urea (mg/dL)	0	26.8±2.8	26.3±2.2	25.6±3.5	30.1±4.3	27.1±2.1	27.5±2.5
	1	27±2.5	24.9±2.4	34.6±7.9	31.8±12.4	32.0±7.8	33.3±10.6
	3	28.1±3.2	20.3±1.4	19.7±2.6	20.5±2.6	21.5±2.5	23.9±2.6
	7	27.8±2.5	20.9±1.3	19.7±0.8	24.2±3.3	20.0±3.2	23.7±1.5
	10	27.0±2.5	22.7±3.2	20.5±1.3	24.1 ±4.1	20.0±2.3	24.0±2.7
Creatinine (mg/dL)	0	0.912±0.186	0.991±0.126	0.993±0.244	1.019±0.207	0.889±0.149	0.954±0.142
	1	0.920±0.205	0.970±0.122	0.849±0.145	0.814±0.155	0.834±0.153	0.948±0.130
	3	0.908±0.189	0.882±0.101	1.035±0.195	0.923±0.137	1.005±0.111	0.937±0.150
	7	0.902±0.190	0.961±0.187	0.833±0.285	0.907±0.123	0.874±0.110	0.921±0.138
	10	0.872±0.182	0.944±0.118	0.876±0.158	0.984±0.100	0.871±0.082	1.007±0.159
Bilirubin (mg/dL)	0	0.292±0.022	0.329±0.016	0.135±0.032	0.288±0.027	0.260±0.042	0.255±0.028
	1	0.292±0.020	0.251±0.033	0.132±0.037	0.247±0.065	0.224±0.025	0.231±0.033
	3	0.290±0.019	0.249±0.036	0.201±0.058	0.289±0.052	0.226±0.033	0.518±0.105[*]
	7	0.290±0.026	0.250±0.034	0.218±0.037	0.221±0.053	0.267±0.046	0.309±0.053
	10	0.267±0.037	0.230±0.048	0.146±0.030	0.299±0.042	0.236±0.040	0.254±0.032
Albumin (g/dL)	0	3.12±0.35	3.07±0.23	3.12±0.14	2.98±0.19	3.68±0.19	3.13±0.25
	1	3.31±0.28	3.05±0.25	3.06±0.12	2.82±0.28	3.41±0.19	2.86±0.26
	3	3.10±0.22	3.03±0.20	3.01±0.15	2.75±0.20	3.29±0.16	3.04±0.29
	7	3.12±0.22	3.14±0.15	3.17±0.13	2.84±0.18	3.17±0.21	2.75±0.12
	10	3.07±0.37	3.18±0.20	3.13±0.06	2.72±0.26	3.32±0.22	2.94±0.13
Protein (g/dL)	0	6.24±0.30	7.10±0.25	7.03±0.28	5.69±0.32	7.35±0.19	6.80±0.15
	1	6.29±0.38	7.05±0.33	6.72±0.30	5.69±0.23	7.20±0.29	6.71±0.24
	3	6.62±0.38	7.10±0.16	6.73±0.46	6.00±0.21	6.72±0.27	6.83±0.36
	7	6.43±0.36	7.01±0.13	7.33±0.37	6.25±0.28	6.82±0.26	6.55±0.32
	10	6.42±0.40	7.13±0.22	7.15±0.48	6.18±0.35	6.92±0.05	6.67±0.41

Data are represented as mean ± SEM. [*]$P<0.05$

especially those receiving higher doses of the extract, showed signs of faeces softness and diarrhea, in a dose dependent manner, as described before (Abbaszadeh *et al.*, 2010).

Discussion

Rosa damascena is traditionally used as an herbal medicine. Several pharmacological effects have also been proposed for the petals of the plant in recent studies. The safety of the plant, however, has not received enough attention in scientific studies. We previously reported laxative effects for *R. damascena* infusion both in rats and dogs (see above). Using different doses of the infusion (90-1440 mg/kg/d) or

placebo for 10 consecutive days, the present study was aimed to preliminarily investigate the potential toxic effects of *R. damascena* infusion in dogs.

Potential adverse effects of herbal drugs have received intense attention in recent years. Although most body organs can be affected by drugs and toxins, liver and kidney are of greatest importance in this regard (Chitturi and Farrell, 2000; Lewis, 2000; Perazella, 2005; Izzedine *et al.*, 2006; Derakhshanfar *et al.*, 2009).

Many drugs may induce glomerulopathies and damage the glomerulus, and hence increase its permeability to serum proteins including albumin (Izzedine *et al.*, 2006). One of the outcomes of the latter

disorder is the loss of proteins in urine which results in hypoproteinemia and hypoalbuminemia. Serum creatinine and urea are among the main markers of damage to kidney tissue (Cowell, 2004; Meyer and Harvey, 2004). In this research, serum levels of creatinine and urea did not change significantly during the 10 days of the treatment.

More than 600 herbal and chemical drugs have been identified with hepatotoxic effects (Lee, 2003; Mosallanejad et al., 2012). Serum levels of ALT and ALP are among the most significant laboratory markers of liver tissue damage (Lewis, 2000). In this study, except for a significant increase in ALT level with the highest dose of the infusion at day 10, serum activities of these enzymes did not change significantly due to the treatment.

Liver malfunction can also affect serum levels of other biochemical markers such as proteins and bilirubin. In this research, except for a transient increase in bilirubin with the highest dose of the infusion, these markers did not change significantly due to the treatment. These findings suggest that R. damascena infusion may be easily tolerated by hepatocytes, especially if the dose is not extremely high. This is while the previous study from this lab (Arezoomandan et al., 2011) demonstrated laxative effects even with the lowest dose of the infusion (90 mg/kg/d). It simply suggests that even 16 times the therapeutic dose for 10 successive days has minor adverse effects on studied parameters. In fact, R. damascena has shown protective effects against CCl_4 toxicity, suggesting hepatoprotective effects for the plant (Achuthan et al., 2003). This effect has been attributed to the acetone fraction of the flower with potent antioxidant activity. In the latter study, oral administration of the fraction significantly reduced serum ALP, glutamine pyruvate transaminase (GTP) and glutamine transaminase (GOT) activity in CCl_4 intoxicated rats.

This research did not focus on underlying mechanisms for the observed toxic effect of the infusion. In fact, most researches on chemical ingredients of the petals have studied the essential oil. Chemical ingredients of rose oil such as geraniol, citronellol, farnesol, nerol and linalool are volatile (Kovats, 1987) and are unlikely to resist 10 min boiling in this research. Further research is needed to identify the potentially toxic ingredients.

In conclusion, the present research studied the potential toxic effects of R. damascena infusion using exponential doses of the petals (90-1440 mg/kg/d) and over a rather extended duration of time (10 days). The results suggest that the infusion is easily tolerated by the kidney and the liver, and it exerts minimal nephrotoxic or hepatotoxic effects, unless it is used at unusually extreme doses.

Acknowledgement

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