



Efficacy and safety of standardized Ashwagandha (*Withania somnifera*) root extract on reducing stress and anxiety in domestic dogs: A randomized controlled trial

Jasleen Kaur^{a,*}, Sujit Seshadri^b, Krishna Hasitha Golla^c, Prasanthi Sampara^d

^a Department of Veterinary Medicine, Allvet Pet Clinic, 8-2-269/A/3, Behind Lumbini Jewel Mall, Hyderabad, Telangana, India

^b Department of Veterinary Medicine, Allvet Pet Clinic, 8-2-269/A/3, Behind Lumbini Jewel Mall, Hyderabad, Telangana, India

^c Department of Veterinary Medicine, Allvet Pet Clinic, 8-2-269/A/3, Behind Lumbini Jewel Mall, Hyderabad, Telangana, India

^d SMART Research Department, Sunshine Hospitals, Hyderabad, Telangana, India

ARTICLE INFO

Article history:

Received 23 July 2021

Revised 7 March 2022

Accepted 25 March 2022

Available online 31 March 2022

Keywords:

Ashwagandha

Canine

CBARQ

Cortisol

Stress

ABSTRACT

Ashwagandha (*Withania somnifera*) is the most revered botanical in Ayurvedic medicine. It has been traditionally used to reduce stress and enhance overall well being in humans. This study aimed to investigate the adaptogenic effects of an Ashwagandha root extract in domestic dogs (*Canis familiaris*). In this 4-week, randomized, double-blind, placebo-controlled study, 24 dogs experiencing stress and anxiety received either placebo or 15 mg/kg bodyweight of Ashwagandha root extract once daily. Outcome measures included urine cortisol to creatine ratio (UCCr), Canine Behavioral Assessment and Research Questionnaire (CBARQ), Canine Brief Pain Inventory scale (CBPI), and Body condition score (BCS). Based on the data collected from all the dogs, Ashwagandha root extract supplementation was associated with significant improvements in stress-related signs. In comparison with the placebo, Ashwagandha intake was associated with a statistically significant reduction in urine cortisol to creatine ratio ($P=0.0005$). Results from CBARQ revealed that Ashwagandha intake was associated with a statistically significant reduction in the fear and anxiety domain ($P=0.03$), compared to placebo. There was also a statistically significant reduction in the CBPI pain severity ($P < 0.0001$) and pain interference ($P < 0.0001$) domains, compared to the placebo group. Ashwagandha intake was well tolerated in all the dogs with no reported adverse events. In conclusion, the intake of Ashwagandha root extract for 4 weeks was associated with significant reductions in UCCr, and signs of fear and anxiety and pain interference.

© 2022 Elsevier Inc. All rights reserved.

Introduction

Animals and human beings are persistently subjected to stressful environments in their everyday activities. Stress is a biological response of the body caused by the extraordinary circumstances defined as a stressor threatening its natural homeostasis (Laugero and Moberg, 2000; Karatsoreos and McEwen, 2011). Stress can negatively affect several physiological systems and increases the risk of disease depending on its characteristics and the individual's reaction to it. Dogs suffer from stress and anxiety as much

as people do, though it is difficult to recognize the signs. In dogs, some commonly reported stressors include stimuli to transportation (Bergeron et al., 2002), social and spatial restriction (Hennessy et al., 1997), and loud noise (Grigg et al., 2021).

Separation anxiety and noise aversions affect the welfare of the dog and the bond between humans and animals. Anxiety is a response to a prospective or imagined threat or uncertainty (Sherman and Mills, 2008). Physiologic indications (e.g., increased respiratory and heart rates, vasomotor changes, shaking or paralysis, increased salivation or sweating, gastrointestinal abnormalities) and behavioral signs (e.g., increased salivation or perspiration, gastrointestinal disorders) are all signs of anxiety in dogs. Changes in activity (e.g., immobility, pacing, circling, restlessness); changes in nearest neighbor distances (e.g., remaining close to a person or conspecific); or changes in appetite, including anorexia, are

* Address for reprint requests and correspondence: Jasleen Kaur, Allvet Pet Clinic, 8-2-269/A/3, Behind Lumbini Jewel Mall, Banjara hills Rd. no. 2, Hyderabad, 500034; Tel: +91 9700827800

E-mail address: kaurj.dr@gmail.com (J. Kaur).

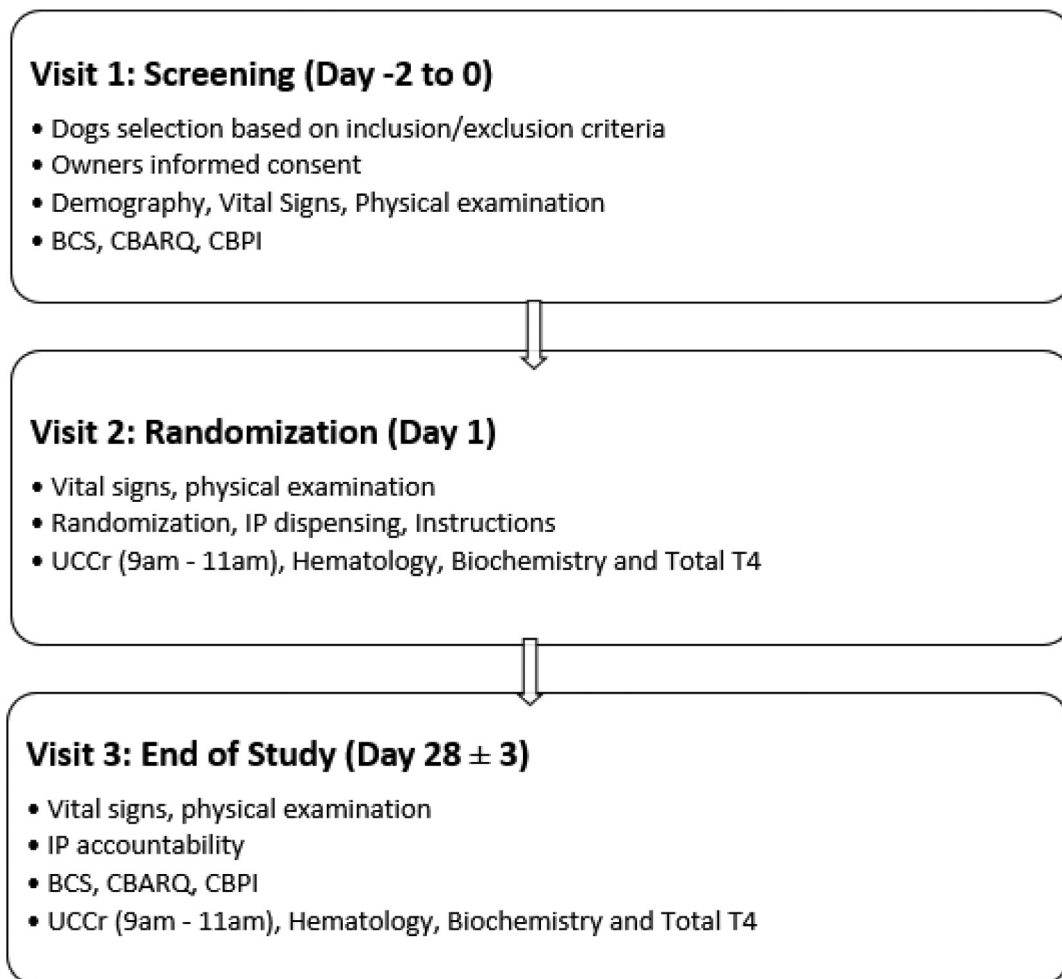


Figure 1. Overview of study design (BCS, Body condition score; CBARQ, Canine Behavioral Assessment & Research Questionnaire; CBPI, Canine Brief Pain Inventory; UCCr, urine cortisol creatinine ratio; Total T4 – total thyroxine).

examples of behavioral indicators of anxiety (Sherman and Mills, 2008). Regular exposure to anxiety-inducing stimuli over a period can negatively affect the physical, mental, or social health of the dog and thus reduce the quality of life (Dreschel, 2010). According to a study conducted in the United States, 10.3% of cases had a specific fear, anxiety, or phobia, 14% experienced separation anxiety, and 22% exhibited fear-related aggression toward individuals (Bamberger and Houpt, 2006).

Distress responses to sounds take a variety of forms, ranging from more mild reactions, such as panting, hiding, hyperactivity, or escape attempts (Shull-Selcer and Stagg, 1991), to more extreme reactions, such as destructiveness and self-trauma. Between 40% and 50% of dog owners report that their dog is “scared” of some sort of noise (Beaver, 1999; Blackwell et al., 2013). As a result, many owners may seek help and advice from veterinarians, behaviorists, and dog trainers (Rugbjerg et al., 2003) or elect relinquishment, abandonment, or euthanasia (Wells and Hepper, 2000; Segurson et al., 2005). Chronic stress and anxiety in dogs can cause an increase in cortisol levels. Urine cortisol to creatinine ratio (UCCr) can be used to assess stress in dogs (Part et al., 2014; Citron et al., 2020) and it also is sensitive enough to measure stress associated with trivial daily activities, such as a car ride.

Treatment is often delayed until abnormal responses reach extreme levels such as panic attacks or reactions to multiple stimuli. Animals with these issues may appear normal at other times

and have a temperament that is not fearful. Therapeutic options for the management of anxiety include tricyclic antidepressants (e.g., clomipramine), H1 antagonists (e.g., fexofenadine), and selective serotonin reuptake inhibitors such as fluoxetine. These medications may have undesired effects such as intermittent vomiting, diarrhea, lethargy, depression, loss of appetite, and skin rash in dogs (King et al., 2000; Seksel and Lindeman, 2001; Simpson et al., 2007; Alja et al., 2009), although these are not commonly reported. Natural and botanical supplements that can manage stress responses may have fewer undesired effects, but they are seldom measured.

Ashwagandha, scientifically known as *Withania somnifera* (L.) Dunal, is a member of the Solanaceae family. The roots are extensively used for their adaptogenic properties and numerous health benefits. Ashwagandha is a highly versatile herb that acts on various human body systems, including the nervous system, the immune system, the endocrinal system, and the reproductive system. Ashwagandha is widely known to reduce stress responses in both humans and animals (Weiner and Weiner, 1994). In humans, various studies have demonstrated the stress-reducing effects of Ashwagandha (Chandrasekhar et al., 2012; Choudhary et al., 2017; Salve et al., 2019). In a study conducted in geriatric canines, Ashwagandha root extract supplementation demonstrated hepatoprotective, anti-peroxidative, and antioxidant effects (Nabi et al., 2014). There is no published clinical study assessing stress mitigating

effects of the standardized ashwagandha root extract in domestic dogs. Hence, we used a standardized herbal formulation to test any potential effects. By using a standardized formulation, findings can be replicated and compared with pharmaceutical formulations (Sayed et al., 2019) in the future.

The study was aimed to evaluate the effect of standardized Ashwagandha root extract as an adaptogen and an anxiolytic herb in domestic dogs (*Canis familiaris*). We hypothesized ashwagandha root extract supplement will result in a greater reduction in UCCr concentration compared to a placebo over 4 weeks.

Materials and Methods

Study design

The study was a 4-week, single-center, randomized, double-blind, placebo-controlled trial. It was conducted from February 2019 to May 2019 at an Allvet private pet clinic, in Hyderabad, India. The study protocol was approved by the Institutional Animal Ethics Committee (IAEC) and Committee for Control and Supervision of Experiments on Animals (CPCSEA), New Delhi, India (Ref no: II/2018-2-IAEC/CVSc, Hyderabad). The study avoided any discomfort for the animals using proper handling and clinical management by the experienced veterinarian.

Study Animals

All the 24 dogs with signs of stress and anxiety enrolled in the study were client-owned, lived in Hyderabad, India, and were recruited at the private clinic during routine visits. The dog's owner signed informed consent at the screening visit before the study procedures.

Eligibility Criteria

Male and female dogs with mild to moderate anxiety aged 3–8 years of any breed, intact or neutered, and with no significant medical histories were eligible to participate. All the dogs had to have at least 2 behavioral signs related to stress and anxiety: avoiding eye contact, avoidance of the feared object, crouching or cringing with tail lowered or tucked between the legs, whimpering, and whining, freezing, and shaking and trembling. In addition, they were diagnosed with mild to moderate anxiety using modified C-BARQ questionnaire fear and anxiety domain where the average score should be between 1 and 3, suggesting the presence of mild to moderate anxiety, and dogs also had to have an average body condition score between 3 and 6. Dogs were excluded from participating in the study if they were concurrently on anti-psychotic medication, steroids, anti-inflammatories, nutritional energy supplements, herbal products, or had contraindications or hypersensitivity to Ashwagandha. Other reasons for exclusion were the presence of any medical condition or disease and pregnancy and lactation.

Study Visits

A veterinarian examined the dog and interviewed the owner at the baseline, randomization, and the end of the study. At the screening visit (day -2 to 0), a physical examination, eligibility evaluation based on the inclusion and exclusion criteria, and psychometric scales took place. At the randomization visit (day 1), physical examination, blood sampling, and dispensing of trial intervention were performed. At the end of the study visit (day 28), an evaluation of physical examination, blood sampling, psychometric scales, and adverse events took place. [Figure 1](#) provides an

overview of the study design and details of procedures at each visit.

Sample Size and Randomization

As this was an exploratory study, the sample size was a convenience sample of 24 dogs. The enrolled dogs were randomly assigned to 1 of the 2 groups (Ashwagandha and Placebo) in a 1:1 randomization ratio. Thus, 12 dogs were in the ashwagandha group, and another 12 dogs were in the placebo group. Randomization was performed using an automated method of random number generation which was pre-determined for the site. The investigational product and the placebo group products were manufactured and packed into identical containers and labeled equivalently to ensure blinding. All dogs were assessed at baseline, and week 4.

Trial Interventions

The investigational product contained an Ashwagandha root extract (KSM-66 Ashwagandha root extract), received from the manufacturer, Ixoreal Biomed Inc., CA, USA. It contains more than 5% of total withanolides and is manufactured as per Current Good Manufacturing Practices (cGMP) defined by US-FDA. All extractions are done using following a green chemistry-based process that is devoid of any alcohol-based extraction. The placebo (starch) was identical to the investigational product in appearance, color, odor, taste and packaging. Both the investigational product and the placebo were packaged in identical containers. Dogs received either Ashwagandha root extract or placebo powder at a dose of 15mg/kg body weight once daily. All the dog owners were instructed to mix the powder based on their dogs' weight in a regular meal at night.

Efficacy Assessments

Urine cortisol creatinine ratio

The urine cortisol creatinine ratio is a screening test for measuring the stress and the amount of cortisol and creatinine (Citron et al., 2020). UCCr is sensitive to daily activities such as a car ride or a visit to the veterinarian. These activities may cause an increase in this ratio. A UCCr test involves the collection of a urine sample taken early in the morning. For our study, the veterinarian assistant took urine samples at the same time (9–11 am) between both visits before the veterinary physician assessment. The collected urine was stored at -20° Celsius until the analysis. Analysis was performed using a direct method without extraction. Cortisol concentration was measured in duplicates by using a DetectX® Cortisol Enzyme Immunoassay Kit (Arbor Assays Inc.). The urinary creatinine concentration was determined by the Jaffe' kinetic method (initial rate reaction). Thereafter, the UCCr was calculated.

Behavioral Assessments

C-BARQ – Canine Behavioral Assessment and Research Questionnaire. Canine Behavioural Assessment and Research Questionnaire (C-BARQ) is a standardized questionnaire predominantly focused on assessing owner-rated frequency and severity of behavioral signs in dogs (Bray et al., 2019). The questionnaire was customized to use relevant questions simply to provide raw scores associated with 3 domains: excitability, aggression, and fear and anxiety. Dog owners were interviewed by the experienced veterinarian to help them score their dog's behavior on a 0–4 rating scale: 0 – no signs of behavior; 1–3 – mild to moderate signs of behavior, 4 – severe signs of behavior at baseline and end of the study visit.

CBPI – Canine Brief Pain inventory. The Canine Brief Pain Inventory (Canine BPI) is a psychometric evaluation that allows owners to rate the severity of their dog's pain and the effect of pain on their daily functions (Brown et al., 2007). The evaluation is a validated questionnaire that asks dog owners 4 questions about the severity of pain evident in a dog (the pain severity score) and 6 questions about how pain interferes with a dog's activity level (the pain interference score).

Body Condition Score

Body condition score is a quantitative and the most common indirect method to evaluate the body fat accumulation of pets based on visual observation and palpation (Laflamme, 1997). The evaluation is done at the ribs, waist, and hips. A 9-point body condition score is commonly used for dogs. The ideal weight corresponds to 4 and 5 scores on the 9-point scale in dogs.

Safety Assessments. Clinical safety was assessed based on the frequency of adverse events reported by the dog owner. During the study, all adverse events were reported to the investigator who noted the event characteristics including severity and occurrence.

Blood samples to assess the concentrations of hemoglobin, platelet count, serum glutamic pyruvic transaminase (SGPT), alkaline phosphatase, serum creatinine, and total thyroxine (T4) were collected from the jugular vein into tubes with and without ethylene diamine tetraacetic acid (EDTA). Plasma and serum samples were separated from the collected blood samples by centrifugation at 4000 rpm for 15 minutes. The analysis was carried out using commercially manufactured kits.

Statistical analysis

All 24 dogs completed the study and de-identified data were analyzed using MedCalc Version 20.009. A descriptive statistical analysis was performed. Data were presented as Mean \pm SD and Median \pm IQR. The frequency and percentages were reported where applicable. The categorical demographic data were analyzed using the Chi-square test (Fisher's exact test). The primary end-point of the study, UCCr, was used to evaluate differences between the 2 experimental groups (ashwagandha and placebo) of dogs at baseline and after week 4. All the efficacy and safety outcomes were analyzed using a student t-test. In all situations, conformity with assumptions of normality and homogeneity of variance were assessed with a Shapiro-Wilk test (significance level set *a priori* at $\alpha = 0.05$). The change in mean values between baseline and week 4 was also reported using percentage. The multiple comparisons were adjusted using Bonferroni correction, where applicable and significance is reported with a 2-tailed *P*-value less than 5%.

Results

A total of 24 client-owned dogs were enrolled in the study and randomly allocated to receive either an Ashwagandha root extract ($n = 12$) or a placebo ($n = 12$). The demographic data and baseline characteristics were well balanced concerning age, weight, and body condition scores between the 2 treatment groups (Table 1). More than half of the dogs were male (16.67% females; 83.33% males- all intact), representing 6 breeds: mixed ($n = 18$), American Akita ($n = 1$), beagle ($n = 1$), German shepherd ($n = 1$), Labrador retriever ($n = 2$), and Rottweiler ($n = 1$). All the dogs completed the study and remained healthy throughout the study.

Table 1
Demographic characteristics.

Variable	Ashwagandha (n = 12)	Placebo (n = 12)
Age (years)		
Mean (SD)	4.08 (0.99)	4.16 (0.83)
Median (IQR)	4.5 (3 - 5)	4 (4 - 4.75)
Sex [#]		
Female, n (%)	3 (25.0)	1 (8.33)
Male, n (%)	9 (75.0)	11 (91.67)
Breed		
Mixed, n (%)	10 (83.33)	8 (66.67)
Any other, n (%)	2 (16.67)	4 (33.33)
Weight (kg)		
Mean (SD)	24.75 (10.15)	28 (9.94)
Median (IQR)	23 (18 - 26.75)	25 (21 - 35)
Body Condition Score		
Mean (SD)	3.66 (0.65)	3.33 (0.49)
Median (IQR)	4 (3 - 4)	3 (3 - 4)

By independent sample t-test;

[#] Fisher exact test.

Efficacy Assessments

Urine Cortisol Creatinine Ratio

Changes in mean UCCr across the Ashwagandha and placebo group over time and significance levels are detailed in Table 2 and Figure 2. The mean UCCr decreased in both the groups over the 4 weeks of the study, with the decrease being significantly greater among the Ashwagandha group when compared to the placebo group.

Relative to the baseline, the UCCr was significantly lower Ashwagandha group ($P = 0.002$). A between-group analysis revealed that there was a statistically significant difference in the change in the mean urine cortisol creatinine ratio between both Ashwagandha and placebo groups ($P = 0.0005$). In the Ashwagandha group, there was a 26.9% reduction in UCCr from baseline to week 4. In the placebo group, there was a 3.9% reduction in the UCCr from baseline to week 4. The reduction was approximately 6 times higher in the Ashwagandha group when compared to the placebo group.

Canine Behavioral Assessment and Research Questionnaire

Changes in the CBARQ subscale scores across the Ashwagandha and placebo groups over time are detailed in Table 3. Relative to the baseline, the mean CBARQ scores for the excitability subscale ($P = 0.002$), was significantly lower in the Ashwagandha group. A between the group analysis revealed a non-significant difference in the change in the CBARQ excitability subscale scores ($P = 0.06$) between Ashwagandha and placebo groups.

In the Ashwagandha group, there was a statistically significant reduction in the aggression ($P = 0.01$) and fear and anxiety ($P = 0.01$) scores over time. In addition, there were no statistically significant reductions in the aggression ($P = 0.47$) and fear and anxiety ($P = 0.09$) score over time in the placebo group. An examination of between-group changes revealed there was a statistically significant change in the fear and anxiety score between the Ashwagandha and placebo groups ($P = 0.03$). There was no statistically significant change in the aggression scores ($P = 0.13$) between the 2 groups.

Canine Brief Pain Inventory

Changes in the CBPI subscale scores across the Ashwagandha and placebo groups over time are detailed in Table 4. Relative to the baseline, the mean CBPI scores for pain severity ($P = 0.002$), was significantly lower in the Ashwagandha group. A between the group analysis revealed there was a statistically significant decrease in pain severity scores ($P < 0.0001$) between Ashwagandha and placebo groups.

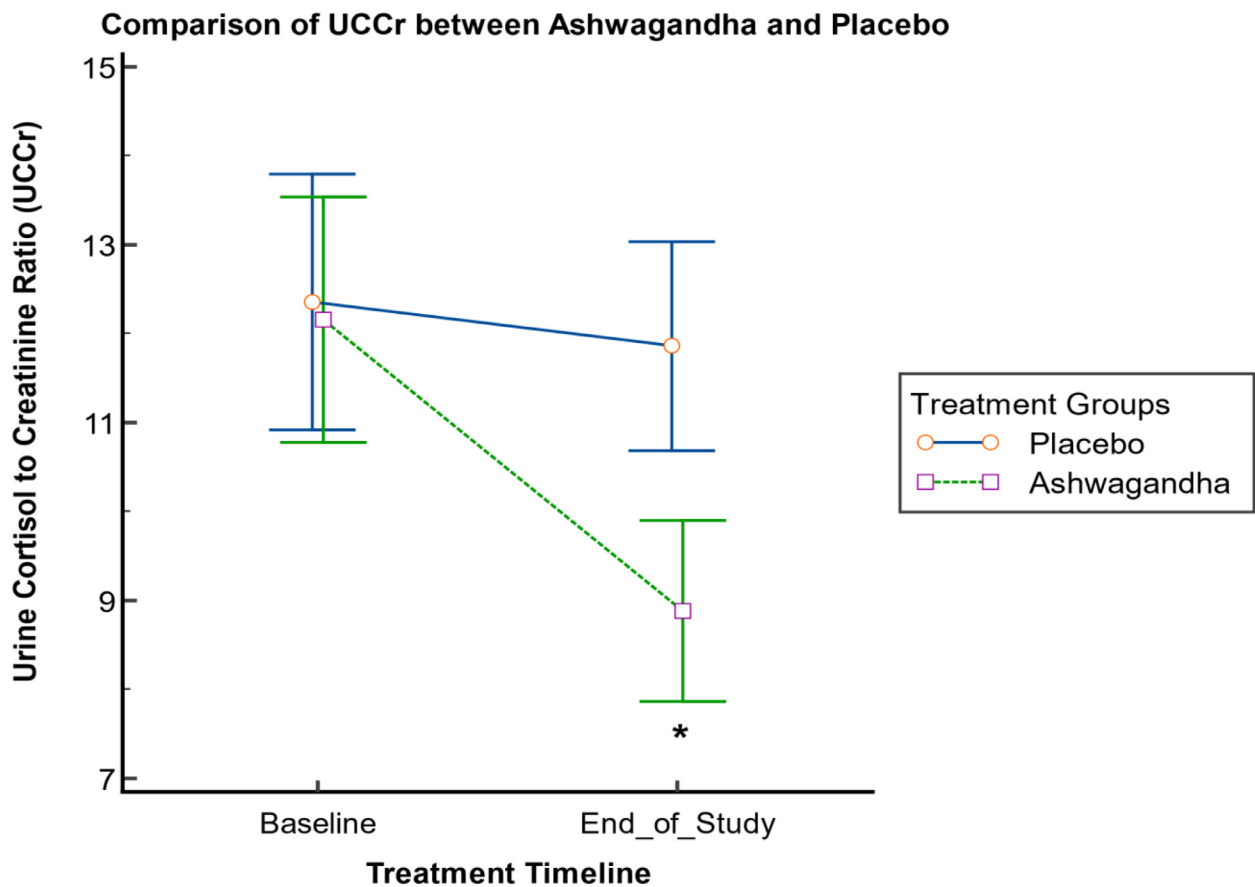


Figure 2. Mean Change in urine cortisol creatine ratio between ashwagandha and placebo at baseline (day 1) and end of study (day 28).

Table 2
Urine cortisol creatine ratio.

Variable		KSM-66 – Ashwagandha (n = 12)		Placebo (n = 12)	
		Baseline	End of Study	Baseline	End of Study
UCCr Ratio	Mean ± SD	12.16 ± 2.17	8.88 ± 1.59* [#]	12.35 ± 2.26	11.86 ± 1.85
	Median (IQR)	11.88 (10.79 – 13.23)	8.58 (7.79 – 10.33)	11.79 (10.30 – 14.15)	(10.29 – 12.54)

* P < 0.001 – between-group analysis by independent sample t-test
P < 0.001 – within the group analysis by dependent sample t-test.

Table 3
Canine behavioral assessment and research questionnaire scores.

Variable		KSM-66 – Ashwagandha (n = 12)		Placebo (n = 12)	
		Baseline	End of Study	Baseline	End of Study
Excitability	Mean ± SD	2.44 ± 0.73	1.56 ± 0.60 [#]	2.33 ± 0.50	2.06 ± 0.46
	Median (IQR)	2.45 (1.72 – 3.14)	1.56 (1.06 – 2.06)	2.33 (2.24 – 2.41)	2.06 (1.97 – 2.14)
Aggression	Mean ± SD	2.72 ± 0.50	2.16 ± 0.56 [#]	2.66 ± 0.35	2.53 ± 0.41
	Median (IQR)	2.71 (2.62 – 2.79)	2.16 (1.97 – 2.35)	2.66 (2.31 – 3.01)	2.53 (2.05 – 3.00)
Fear & Anxiety	Mean ± SD	3.17 ± 0.38	2.54 ± 0.50* [#]	3.06 ± 0.43	2.87 ± 0.23
	Median (IQR)	3.17 (3.14 – 3.20)	2.54 (2.23 – 2.84)	3.05 (3.00 – 3.11)	2.87 (2.68 – 3.06)

* P < 0.05 – between-group analysis by independent sample t-test
P < 0.001 – within the group analysis by dependent sample t-test.

In the Ashwagandha group, there was a statistically significant reduction in the pain interference score ($P=0.002$) over time. An examination of between-group changes revealed there was a statistically significant reduction in the pain interference score between the Ashwagandha and placebo group ($P < 0.0001$). In the Ashwagandha group, there was a 44% reduction in the pain severity and a 42% reduction in the pain interference scores from baseline to week 4. In the placebo group, there was a 9% reduction in the

pain severity and a 13% reduction in pain interference scores from baseline to week 4.

Body Condition Score

Changes in the Body Condition Score across Ashwagandha and placebo groups over time are detailed in Table 5. Relative to the baseline, the mean body condition score was significantly higher in the Ashwagandha group. A between the group analysis revealed

Table 4
Canine brief pain inventory.

Variable		KSM-66 – Ashwagandha (n = 12)		Placebo (n = 12)	
		Baseline	End of Study	Baseline	End of Study
Pain severity	Mean ± SD	3.58 ± 0.51	1.99 ± 0.37 ^{#,*}	3.59 ± 0.20	3.26 ± 0.19
	Median (IQR)	4 (3 - 4)	2.25 (1.62 - 2.25)	3.62 (3.6 - 3.64)	3.25 (3.05 - 3.5)
Pain Interference	Mean ± SD	2.79 ± 0.26	1.61 ± 0.28 ^{#,*}	2.89 ± 0.37	2.5 ± 0.04
	Median (IQR)	3 (2.5 - 3)	1.5 (1.5 - 1.5)	3 (2.8 - 3)	2.5 (2.5 - 2.5)

* P < 0.0001 – between-group analysis by independent sample t-test

P < 0.001 – within the group analysis by dependent sample t-test.

Table 5
Body condition score.

Variable		KSM-66 – Ashwagandha (n = 12)		Placebo (n = 12)	
		Baseline	End of Study	Baseline	End of Study
Body Condition Score	Mean ± SD	3.67 ± 0.65	4.08 ± 0.66*	3.33 ± 0.49	3.16 ± 0.38
	Median (IQR)	4 (3 - 4)	4 (4 - 4.75)	3 (3 - 4)	3 (3 - 3)

* P = 0.001 – between-group analysis by independent sample t-test

Table 6
Vital signs and biochemical parameters.

Variable		KSM-66 – Ashwagandha (n = 12)		Placebo (n = 12)	
		Baseline	End of Study	Baseline	End of Study
Respiratory Rate (breaths/min)	Mean ± SD	19.41 ± 4.52	16.33 ± 3.20 [#]	18.16 ± 2.48	19.33 ± 3.44
	Median (IQR)	20 (16.5 - 23.5)	16 (13.2 - 20)	18 (16 - 20)	19 (16.5 - 21.5)
Hemoglobin (14.2 -19.2 g/dL)	Mean ± SD	16.10 ± 2.44	17.22 ± 2.18 [#]	15.21 ± 2.40	15.47 ± 2.59
	Median (IQR)	16.44 (14.7 - 17.9)	17.9 (16.1 - 18.8)	15.45 (13.2 - 16.5)	14.5 (13.1 - 18.5)
T4 (1-4 µg/dL)	Mean ± SD	1.66 ± 0.74	2.02 ± 0.58 [#]	1.46 ± 0.37	1.6 ± 0.33
	Median (IQR)	1.5 (1.1 - 1.9)	2.05 (1.5 - 2.42)	1.35 (1.2 - 1.75)	1.7 (1.3 - 1.8)

P < 0.05 – within the group analysis by dependent sample t-test.

there was a statistically significant increase in the body condition scores (P=0.001) between Ashwagandha and placebo groups. At the end of the study, the Ashwagandha root extract supplemented dogs attained an ideal body condition score of 4.

Adverse Events

No major adverse events were reported in the study. One dog, from the placebo group, experienced fever, which was resolved without any medication. All the hematological and biochemical parameters assessed were in the normal reference range both at baseline and end of the study.

Changes in respiratory rate, hemoglobin concentration, and Thyroxine (T4) levels across the Ashwagandha and placebo groups over time are detailed in Table 6. Relative to the baseline the mean respiratory rate was significantly lower in the Ashwagandha group (P=0.03). A between-group analysis revealed that there was a statistically significant difference in the change in the mean respiratory rate between both Ashwagandha and placebo groups (P = 0.01).

Relative to the baseline, Ashwagandha intake resulted in a statistically significant increase in hemoglobin concentration (P = 0.01) over time. A between the group analysis revealed there was a non-significant difference in change in the hemoglobin concentration between the Ashwagandha and placebo group (P=0.18). In the Ashwagandha root extract supplemented group there was a statistically significant increase in the T4 concentration over time (P=0.01). An examination of the between-group changes revealed there was no statistical significance between the group differences in the T4 concentrations (P=0.22).

Discussion

In this 4-week, randomized, double-blind, placebo-controlled study, the administration of an Ashwagandha root extract (KSM-66 Ashwagandha®) at a dose of 15mg/kg body weight was associated with significant changes in the UCCr in dogs experiencing stress responses and anxiety when compared to the placebo. Ashwagandha root extract was also associated with statistically significant improvements in rating scales pertaining to fear and anxiety and aggression, and pain interference over time. Ashwagandha supplementation was well tolerated with no reported adverse events.

The stress-relieving and calming effect of Ashwagandha root extract has been confirmed in several human studies (Chandrasekhar et al., 2012; Choudhary et al., 2017; Salve et al., 2019). These results provide initial evidence of the potential value of using Ashwagandha root extract in dogs with stress and anxiety.

Ashwagandha has been suggested to have anti-stress, anti-inflammatory, antioxidant, immunomodulatory, adaptogenic, neuroprotective, and hepatoprotective activities (Singh et al., 2010; Dar et al., 2015). UCCr is likely the most extensively used physiological index of canine welfare in published studies, and it is regarded as a credible marker of mild stress in dogs (van Vonderen et al., 1998; Citron et al., 2020). In our study, a significant reduction of 26% UCCr was observed in the dogs administered with Ashwagandha root extract after 4 weeks demonstrating a significant stress response reduction. Extensive studies have been conducted on the biological model of animals for evaluating the adaptogenic/anti-stress properties of Ashwagandha (Gajarmal et al., 2014). In one study, researchers demonstrated that male Wistar rats administered with *Withania somnifera*, an hour before a foot

shock experienced a significantly reduced level of stress responses (Bhattacharya and Muruganandam, 2003). In horses, Ashwagandha root extract has been found to have statistically significant adaptogenic, antioxidant, hemopoietic, and immune-stimulant properties (Priyanka et al., 2020).

In our study, Ashwagandha root extract supplementation reported a 7% increase in the hemoglobin concentration over time. The increase in hemoglobin concentration can be correlated to the hemopoietic effect observed in the equine study (Priyanka et al., 2020). While Ashwagandha root extract supplementation produced a statistically significant change in the T4 levels over time, there was no difference between the groups.

Consistent individual-specific patterns in behavior and stress physiology have been recorded in various animal species, although the relationship between the 2 categories of characteristics remains ambiguous (Carere et al., 2010; Rayment et al., 2015). Exposure to stressors and the resultant response can cause an observable change in the behavior of the dogs, such as fear and anxiety, aggression, and excitability. Among the specific C-BARQ findings from our study dogs, Ashwagandha supplementation resulted in significant reductions in excitability, fear, and anxiety ratings of 36 percent and 19.8 percent, respectively. The placebo group experienced a 12% and 6% reduction in excitability and fear and anxiety domain, respectively. Pain and stress are 2 distinguishable but overlapping processes physiologically. Both phenomena challenge the body's homeostasis and necessitate decision-making to help animals adapt to their environment (Abdallah and Geha, 2017). The brain plays a central role in stress and pain processes (McEwen, 2007; Baliki and Apkarian, 2015). CBPI was originally developed as an owner-completed questionnaire designed to quantify the severity and effect of chronic pain in companion dogs with osteoarthritis (Brown et al., 2007). The primary goal of the treatment of OA in dogs is to relieve body pain and thus improve quality of life. As our study result showed, the ashwagandha root extract reduces the pain severity and pain interference by 44% and 42%, respectively, which is 3 times more than the placebo's 9% and 13% reduction in the pain severity and pain interference, respectively.

Exposure to stressors can affect grooming and may alter overall body conditions in dogs. The body condition score is a common method to evaluate animal body fat mass. In this study, Ashwagandha intake was able to improve the body condition score in dogs to an ideal score of 4, whereas the placebo group didn't have any significant changes in the body condition score. All of the dogs were below the ideal body condition score at the baseline, which may be due to the owner's feeding regimen being low quality. The present study also demonstrated a significant decrease in the respiratory rate, upon the supplementation of Ashwagandha root extract. An increased respiratory rate in dogs can be identified as an important physiological marker of stress and stress-related anxiety (Stellato et al., 2019).

Except for one ADR (fever) in the placebo group, no adverse events were reported. Thus, ashwagandha can be considered safe and acceptable. All the hematological and biochemical parameters were within normal laboratory limits.

One important limitation of this study is the small sample size. A large-scale study with a longer duration should be conducted with various breeds, different environmental conditions, and specific anxiety disorders to confirm the observations. We used CBARQ in mildly stressed dogs and observed a modest improvement. It would be necessary to conduct a study in severely stressed dogs to see if the changes are commensurate to determine treatment effect. A similar approach might be used to measure the body condition scores. Although the CBARQ and the BCS findings were statistically significant, therapeutic applicability should be established by multiple clinical trials.

Conclusion

The findings of this exploratory study suggest that Ashwagandha root extract may aid in attenuating signs of stress and anxiety in dogs.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Ethics Statement

All the authors declare that this study has been conducted following the animal ethical and welfare guidelines provided by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA). The study was approved by the Institutional Animal Ethics Committee (IAEC) and Committee for Control and Supervision of Experiments on Animals (CPCSEA), New Delhi, India (Ref no: II/2018-2-IAEC/ CVSc, Hyderabad).

Acknowledgments

The authors thank Ixoreal BioMed Inc., Los Angeles, California, USA, for supplying the KSM-66 high concentration root extract used in this study.

Conflict of Interest

The authors declare that they have no conflicts of interest with the publication of this study.

References

- Abdallah, C.G., Geha, P., 2017. Chronic pain and chronic stress: two sides of the same coin? *Chronic Stress (Thousand Oaks)* 1, 1–10.
- Alja, P., Silvestra, K., Aleksandra, D.P., Tina, K., 2009. The efficacy of antihistamine fexofenadine versus methylprednisolone in the treatment of atopic dermatitis in dogs. *Slov. Vet. Res.* 46, 5–12.
- Baliki, M.N., Apkarian, A.V., 2015. Nociception, pain, negative moods, and behavior selection. *Neuron* 87, 474–491.
- Bamberger, M., Houpt, K.A., 2006. Signalment factors, comorbidity, and trends in behavior diagnoses in dogs: 1,644 cases (1991–2001). *J. Am. Vet. Med. Assoc.* 229, 1591–1601.
- Beaver, B.V.G. 1999. *Canine Behavior: A Guide for Veterinarians*, W.B. Saunders Company, Philadelphia, 125.
- Bergeron, R., Scott, S.L., Émond, J.P., Mercier, F., Cook, N.J., Schaefer, A.L., 2002. Physiology and behavior of dogs during air transport. *Can. J. Vet. Res.* 66, 211.
- Bhattacharya, S.K., Muruganandam, A.V., 2003. Adaptogenic activity of *Withania somnifera*: an experimental study using a rat model of chronic stress. *Pharmacol. Biochem. Behav.* 75, 547–555.
- Blackwell, E.J., Bradshaw, J.W.S., Casey, R.A., 2013. Fear responses to noises in domestic dogs: prevalence, risk factors and co-occurrence with other fear related behaviour. *Appl. Anim. Behav. Sci.* 145, 15–25.
- Bray, E.E., Levy, K.M., Kennedy, B.S., Duffy, D.L., Serpell, J.A., MacLean, E.L., 2019. Predictive models of assistance dog training outcomes using the canine behavioral assessment and research questionnaire and a standardized temperament evaluation. *Front. Vet. Sci.* 6, 49.
- Brown, D.C., Boston, R.C., Coyne, J.C., Farrar, J.T., 2007. Development and psychometric testing of an instrument designed to measure chronic pain in dogs with osteoarthritis. *Am. J. Vet. Res.* 68, 631–637.
- Carere, C., Caramaschi, D., Fawcett, T.W., 2010. Covariation between personalities and individual differences in coping with stress: Converging evidence and hypotheses. *Curr. Zool.* 56, 728–740.
- Chandrasekar, K., Kapoor, J., Anishetty, S., 2012. A prospective, randomized double-blind, placebo-controlled study of safety and efficacy of a high-concentration full-spectrum extract of Ashwagandha root in reducing stress and anxiety in adults. *Indian J. Psychol. Med.* 34, 255–262.
- Choudhary, D., Bhattacharyya, S., Joshi, K., 2017. Body weight management in adults under chronic stress through treatment with ashwagandha root extract: a double-blind, randomized, placebo-controlled trial. *J. Evidence-Based Complement. Altern. Med.* 22, 96–106.
- Citron, L.E., Weinstein, N.M., Littman, M.P., Foster, J.D., 2020. Urine cortisol-creatinine and protein-creatinine ratios in urine samples from healthy dogs collected at home and in hospital. *J. Vet. Intern. Med.* 34, 777–782.

- Dar, N.J., Hamid, A., Ahmad, M., 2015. Pharmacologic overview of *Withania somnifera*, the Indian Ginseng. *Cell. Mol. Life Sci.* 72, 4445–4460.
- Dreschel, N.A., 2010. The effects of fear and anxiety on health and lifespan in pet dogs. *Appl. Anim. Behav. Sci.* 125, 157–162.
- Gajarmal, A.A., Shende, M.B., Chothe, D.S., 2014. Antistress activity of Ashwagandha (*Withania somnifera* Dunal) – a review. *Int. Ayurvedic Med. J.* 2, 386–393.
- Grigg, E.K., Chou, J., Parker, E., Gatesy-Davis, A., Clarkson, S.T., Hart, L.A., 2021. Stress-Related behaviors in companion dogs exposed to common household noises, and owners' interpretations of their dogs' behaviors. *Front. Vet. Sci.* 8, 1–10.
- Hennessy, M.B., Davis, H.N., Williams, M.T., Mellott, C., Douglas, C.W., 1997. Plasma cortisol levels of dogs at a county animal shelter. *Physiol. Behav.* 62, 485–490.
- Karatsoreos, I.N., McEwen, B.S., 2011. Psychobiological allostasis: resistance, resilience and vulnerability. *Trends Cogn. Sci.* 15, 576–584.
- King, J.N., Simpson, B.S., Overall, K.L., Appleby, D., Pageat, P., Ross, C., Chaurand, J.P., Heath, S., Beata, C., Weiss, A.B., Muller, G., Paris, T., Bataille, B.G., Parker, J., Petit, S., Wren, J., 2000. Treatment of separation anxiety in dogs with clomipramine: results from a prospective, randomized, double-blind, placebo-controlled, parallel-group, multicenter clinical trial. *Appl. Anim. Behav. Sci.* 67 (4), 255–275.
- Laflamme, D., 1997. Development and validation of a body condition score system for dogs. *Canine practice (Santa Barbara)*, 22(4):10–15.
- Laugero, K.D., Moberg, G.P., 2000. Energetic response to repeated restraint stress in rapidly growing mice. *Am. J. Physiol. Endocrinol. Metab.* 279, E-33–E-43.
- McEwen, B.S., 2007. Physiology and neurobiology of stress and adaptation: central role of the brain. *Physiol. Rev.* 87, 873–904.
- Nabi, S.U., Wani, A.R., Dey, S., 2014. Therapeutic effect of ashwagandha (*Withania somnifera* L.) in liver dysfunction of old dogs. *Appl. Biol. Res.* 16, 232.
- Part, C.E., Kiddie, J.L., Hayes, W.A., Mills, D.S., Neville, R.F., Morton, D.B., Collins, L.M., 2014. Physiological, physical and behavioural changes in dogs (*Canis familiaris*) when kennelled: Testing the validity of stress parameters. *Physiol. Behav.* 133, 260–271.
- Priyanka, G., Anil Kumar, B., Lakshman, M., Manvitha, V., Kala Kumar, B., 2020. Adaptogenic and immunomodulatory activity of Ashwagandha root extract: an experimental study in an equine model. *Front. Vet. Sci.* 0, 700.
- Rayment, D.J., De Groef, B., Peters, R.A., Marston, L.C., 2015. Applied personality assessment in domestic dogs: Limitations and caveats. *Appl. Anim. Behav. Sci.* 163, 1–18.
- Rugbjerg, H., Proschowsky, H.F., Ersbøll, A.K., Lund, J.D., 2003. Risk factors associated with interdog aggression and shooting phobias among purebred dogs in Denmark. *Prev. Vet. Med.* 58, 85–100.
- Salve, J., Pate, S., Debnath, K., Langade, D., 2019. Adaptogenic and anxiolytic effects of ashwagandha root extract in healthy adults: a double-blind, randomized, placebo-controlled clinical study. *Cureus* 11.
- Sayed, N., Khurana, A., Godugu, C., 2019. Pharmaceutical perspective on the translational hurdles of phytoconstituents and strategies to overcome. *J. Drug Deliv. Sci. Technol.* 53, 101201.
- Segurson, S.A., Serpell, J.A., Hart, B.L., 2005. Evaluation of a behavioral assessment questionnaire for use in the characterization of behavioral problems of dogs relinquished to animal shelters. *J. Am. Vet. Med. Assoc.* 227, 1755–1761.
- Seksel, K., Lindeman, M.J., 2001. Use of clomipramine in treatment of obsessive-compulsive disorder, separation anxiety and noise phobia in dogs: A preliminary, clinical study. *Aust. Vet. J.* 79, 252–256.
- Sherman, B.L., Mills, D.S., 2008. Canine anxieties and phobias: an update on separation anxiety and noise aversions. *Vet. Clin. North Am. Small Anim. Pract.* 38, 1081–1106.
- Shull-Selcer, E.A., Stagg, W., 1991. Advances in the understanding and treatment of noise phobias. *Vet. Clin. North Am. Small Anim. Pract.* 21, 353–367.
- Simpson, B.S., Landsberg, G.M., Reisner, I.R., Ciribassi, J.J., Horwitz, D., Houpt, K.A., Kroll, T.L., Luescher, A., Moffat, K.S., Douglass, G., Robertson-Plouch, C., Veenhuizen, M.F., Zimmerman, A., Clark, T.P., 2007. Effects of reconcile (fluoxetine) chewable tablets plus behavior management for canine separation anxiety. *Vet. Ther.* 8, 18–31.
- Singh, G., Sharma, P.K., Dudhe, R., Singh, S., 2010. Biological activities of *Withania somnifera*. *Ann. Biol. Res.* 1, 56–63.
- Stellato, A.C., Hoffman, H., Gowland, S., Dewey, C.E., Widowski, T.M., Niel, L., 2019. Effect of high levels of background noise on dog responses to a routine physical examination in a veterinary setting. *Appl. Anim. Behav. Sci.* 214, 64–71.
- van Vonderen, I.K., Kooistra, H.S., Rijnberk, A., 1998. Influence of veterinary care on the urinary corticoid: creatinine ratio in dogs. *J. Vet. Intern. Med.* 12, 431–435.
- Weiner, M.A., Weiner, J.A., 1994. Ashwagandha (Indian Ginseng). *Herbs that heal. Mill Vallery, CA., Quantum Books* p. 70–2.
- Wells, D.L., Hepper, P.G., 2000. Prevalence of behaviour problems reported by owners of dogs purchased from an animal rescue shelter. *Appl. Anim. Behav. Sci.* 69, 55–65.