ABSTRACT

The pharmacokinetic study of *Withania somnifera* (Ashwagandha) was investigated after single dose administration of 500 mg/kg, orally in six non-descript healthy buffalo calves. Estimation of concentration of *Withania somnifera* in plasma was carried out by microbiological assay technique (Agar gel diffusion technique) by using *E. coli* (ATCC 25922) as the test organism. Following a single oral dose of *Withania somnifera* in healthy buffalo calves, the mean peak plasma concentration at 0.75 h was 248.16±16.12 μg/ml and was detected up to 3 h with a mean plasma concentration of 6.55±0.12 μg/ml.

The mean therapeutic concentration (≥ 0.1 mg/ml) of *Withania somnifera* was maintained from 10 minutes to 3 h in plasma of healthy buffalo calves. The mean elimination half life (t1/2 β) of *Withania somnifera* was observed to be 0.92 ± 0.032 h. The mean value of area under curve in plasma (AUC) and area under first moment curve (AUMC) were found to be 181.44 ± 8.84 μg/ml.h and 246.26 ± 17.66 μg/ml.h². The total body clearance (Clb) ranged from 2.26 to 3.09 L/kg/h with a mean value of 2.78 ± 0.12 L/kg/h.

Keywords: pharmacokinetics, *Withania somnifera*, Ashwagandha, buffalo calves

INTRODUCTION

WHO has predicated that microorganisms are becoming resistant to most antibiotics and by 2020 antibiotics (the so-called wonder drugs of the 20th Century) will lose their effectiveness and no longer be used to cure diseases in man and animals. Most antibiotics are bacteriostatic in nature and as such they do not kill the bacteria; rather, they suppress their growth and the bacteria have to be killed by the body’s defense mechanisms named the phagocytic system through macrophages. WHO has also advised its all member countries to explore and use traditional wisdom for the health management. Due to these facts (harmful chemical residues and antibiotic resistance) there is a race among healthcare personnel/scientists throughout the world to find suitable and sustainable methods of treating ailments. Now the attention of the international scientific forum has been diverted towards alternative therapies.

*Withania somnifera* (*W. somnifera*), commonly known as Ashwagandha or Indian ginseng, has been an important herb in indigenous medicinal systems (Ayurveda) for over 3000 years. Among all parts of this plant, the *W. somnifera* root is considered to be the most active for therapeutic purposes like strengthening the body and for helping to prevent disease. *W. somnifera* is used in several indigenous drug preparations for maintaining health.
as well as treatment of several disease conditions. Its main use is as an immunomodulator and as an antistressor. The roots of *W. somnifera* contain several alkaloids, withanolides, a few flavonoids and reducing sugars. *W. somnifera* contains number of phytoconstituents, withanolides as the major constituent. It is one of the most commonly used drugs as a natural antimicrobial agent (Jaffer and Jawad, 1998). *W. somnifera* commonly used Indian medicinal plant for antimicrobial activity since the ancient time. The antibacterial activity of *W. somnifera* is now approaching for evaluation of its therapeutic efficacy and valuable use as an antibacterial agent in the present study. Its therapeutic use should be based on the correlation between antibacterial activity and its concentration achieved in vivo. Among various factors that determine the variation in intensity and duration of pharmacological effects, dosage, route of administration and disease status of animal are of much importance.

The majority of the population, particularly those living in villages, depends largely on herbal medicine. Scientific data on a good number of medicinal plants investigated is well documented. However, only very few drugs of plant origin have reached clinical use and the National Formulary could not adopt even a dozen plant medicines. For this reason, a special effort is needed for development of herbal drugs having therapeutic utility.

There is no data available for the kinetic study of *W. somnifera* in any species of animal so far. Therefore, the study was conducted in expectation to enhance to a remarkable extent the use of *W. somnifera* judiciously in animal practices, and also to consider species variations due to differences in pharmacokinetics of antibacterial agents. Hence, the present study was undertaken, to investigate the pharmacokinetics of *W. somnifera* in healthy buffalo calves.

**MATERIALS AND METHODS**

Six clinically healthy male buffalo calves of non-descript breed between 6 to 8 months of age and 100-150 kg body weight were used. The experiment was approved by the institute ethical committee and the synopsis committee of Madhya Pradesh Pashu Chikitsa Vigyan Vishwavidyalaya, Jabalpur, Madhya Pradesh, India as a part of post graduate degree programme of the first author. These buffalo calves were housed in an animal shed and maintained on dry fodder and greens as well as routine grazing for at least 4-5 h a day. Clean drinking water was available ad libitum.

The roots of *W. somnifera* were obtained from the Department of Aromatic and Medicinal Plants, Agriculture College, J.N.K.V.V., Jabalpur. The roots of *W. somnifera* were shed dried and crushed in a mixer and grinder to prepare a fine powder. 100 g of *W. somnifera* powder was dissolved in 1 L of sterile triple distilled water for 24 h to make a cold aqueous extract of *W. somnifera*. The cold aqueous extract of *W. somnifera* was administered at a dose rate of 500 mg /kg body wt. orally by drenching tube in each of the six healthy buffalo calves. Before collection of blood, the sites around the jugular vein on either site of the neck of the animals were aseptically prepared. The sites were sterilized prior to each collection with rectified spirit. Blood samples (approx. 1 ml) were withdrawn from jugular vein into heparinized glass centrifuge tubes at 0, 10, 15, 20, 30, 45 minutes and 1, 1.5, 2, 2.5, 3, 4 and 6 h after administration of the drug. Plasma was separated by centrifugation at 3,000 r.p.m. for 15 minutes at
room temperature and kept at -4°C until analysis. For preparation of standards, normal plasma prior to drug administration was also collected.

Aqueous extract of *W. somnifera* was prepared as 100 g of *W. somnifera* powder with 1 L of sterile triple distilled water; after 24 h, this solution was used for preparation of stock solution of 100 mg/ml of *W. somnifera*. One millilitre of stock solution (100 mg/ml) was dissolved in 1 ml of triple distilled water under constant stirring to obtain 50 mg/ml and also 50 mg/ml was diluted in triple distilled water to make different strengths viz., 25, 12.50, 6.25, 3.13, 1.56 and 0.78 mg/ml in water. From each standard solution of *W. somnifera* in water, 50 μl was added to a centrifuge tube containing 450 μl of plasma collected prior to drug administration. This yielded *W. somnifera* standards of 10, 5, 2.5, 1.25, 0.625, 0.313, 0.156 and 0.078 mg/ml in plasma. Blank plasma containing no *W. somnifera* was also prepared.

The test organism used for the microbiological assay technique (agar gel diffusion technique) of *W. somnifera* was *E. coli* (ATCC 25922). The organism was grown on the slant of culture tube containing nutrient agar slants at 37°C for overnight. Then it was stored under refrigeration. The organism was transferred weekly to fresh media to maintain its normal activity (Arora *et al.*, 2004).

Pharmacokinetic analysis of *W. somnifera* after single oral administration was calculated from a semi-logarithmic scale as a plot of plasma drug concentration versus time curve. The log plasma drug concentration versus time profile showed a non-linear curve and hence, non-compartmental analysis was done through statistical moment approach as described by Singh (1999). The mean therapeutic concentration (≥ 0.1 mg/ml) of *W. somnifera* was maintained from 10 minutes to 3 h in plasma of healthy buffalo calves as stated by Arora *et al.* (2004) who reported the minimum inhibitory concentration (MIC) of *W. somnifera*, which came out to be 0.1 mg/ml for *S. typhimurium* and *E. coli*.

**RESULTS AND DISCUSSION**

Plasma concentrations of *W. somnifera* at various time intervals following a single oral dose of 500 mg/kg in healthy buffalo calves are shown in Figure 1.

The mean plasma concentration of the drug at 0.16 h was found to be 6.39 ± 0.11 mg/ml and the value ranged from 6.13 to 6.88 mg/ml. The drug was detectable in all six animals up to 3 h with the mean plasma concentration was 6.55 ± 0.12 mg/ml. The drug was not detectable in any of six animals after 4 h. The peak concentration of *W. somnifera* was found at 0.75 h with mean concentration of 248.16 ± 16.12 mg/ml as shown in the Figure. 1.

Plasma drug concentration versus time profile has shown non-linear curve. Hence, kinetic parameters were derived from the formula of non-compartmental analysis through statistical moment approach. The values of different kinetic parameters calculated by the above noted non-compartmental analysis. The elimination rate constant (β) ranged from 0.67 to 0.82 h⁻¹ with a mean value of 0.74 ± 0.025 h⁻¹. The mean elimination half life (t₁/₂ β) values of the drug were observed to be 0.92 ± 0.032 h as shown in Table 1.

There is no data available for the kinetic study of *W. somnifera* in any species of animal so far; therefore, the kinetic parameters calculated in the present study are discussed as follows: Plasma concentration of *W. somnifera* versus time disposition curves after oral administration
were best fit to non compartmental analysis in all six buffalo calves, which is in accordance with results reported for pharmacokinetics of oral administration of sulphur mustard decontaminant CC-2 in rats (Lal et al., 2003).

Following a single oral dose of *W. somnifera* in healthy buffalo calves, mean peak plasma concentration at 0.75 h was 248.16 ± 16.12 mg/ml and was detected up to 3 h with a mean plasma concentration of 6.55 ± 0.12 mg/ml.

Most of the kinetic parameters of *W. somnifera* (500 mg/kg, orally) as elimination rate constant (β) was calculated 0.74 ± 0.025 h⁻¹ which suggested that slightly faster rate of elimination of *W. somnifera* when administered orally. This is best supported by elimination half-life (t₁/₂ β) which were noted 0.92 ± 0.032 h in healthy buffalo calves.

The high values of AUC∞, AUMC∞ and MRT reflect that most of the body area is covered with the drug concentrations. The AUC∞ value was calculated 181.44 ± 8.84 mg/ml.h in healthy buffalo calves. Similar to this AUMC∞ value was 246.26 ± 17.66 mg/ml.h² and MRT values 1.34 ± 0.045 h were found. That clearly indicated that the maximum area covered by drug *W. somnifera* after

![Figure 1. Plasma concentrations of *W. somnifera* following a single oral dose of 500 mg/kg in healthy buffalo calves.](image)

<table>
<thead>
<tr>
<th>Parameter (Unit)</th>
<th>Mean ± S.E.M.</th>
</tr>
</thead>
<tbody>
<tr>
<td>β (h⁻¹)</td>
<td>0.74 ± 0.025</td>
</tr>
<tr>
<td>t₁/₂ β (h)</td>
<td>0.92 ± 0.032</td>
</tr>
<tr>
<td>AUC∞ (mg/ml.h)</td>
<td>181.44 ± 8.84</td>
</tr>
<tr>
<td>AUMC∞(mg/ml.h²)</td>
<td>246.26±17.66</td>
</tr>
<tr>
<td>MRT (h)</td>
<td>1.34 ± 0.045</td>
</tr>
<tr>
<td>Vdss (L/kg)</td>
<td>3.68 ± 0.12</td>
</tr>
<tr>
<td>Clᵣ (L/kg/h)</td>
<td>2.78 ± 0.12</td>
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</table>
oral administration in the body of buffalo calves.

The relatively high value of Vd\textsubscript{ss} (3.68 ± 0.12 L/kg) was observed in healthy buffalo calves. A large volume of distribution (>1 L/kg) indicates wide distribution throughout the body or extensive tissue binding or rapid excretion of a drug or combination of all the above. A high value of Vd\textsubscript{ss} obtained in the present study showed the wide distribution of *W. somnifera* in the body of buffalo calves.

The total body clearance (Cl\textsubscript{B}) value of *W. somnifera* in healthy buffalo calves was 2.78 ± 0.12 L/kg/h which showed slightly increased clearance from the body of buffalo calves, which is in accordance with results of Cl\textsubscript{B} = 2.45 ± 0.21 L/kg/h after oral administration of sulphur mustard decontaminant CC-2 in rats (Lal et al., 2003).

Herbs are the backbone of therapeutic strategies. India, having huge wealth of plant biodiversity, holds excellent potential for herbal treatment. After evaluating the efficacy and pharmacokinetics of medicinal plants, extracts will be recommended for clinical trials in animals under controlled conditions. The ethano medicinal data on indigenous plant *W. somnifera* will serve as useful tool to pharmacologists and clinicians for development of herbal preparations of indigenous plants. Pharmacokinetics of *W. somnifera* will provide valuable clues to the clinician for its large scale use in future.

REFERENCES


