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Research Article

Disposition Kinetics of *Withania somnifera* (Ashwagandha) In Healthy Buffalo Calves

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ABSTRACT

The disposition kinetic 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 *Escherichia coli* (ATCC 25922) as test organism. Following a single oral dose of *Withania somnifera* in healthy buffalo calves, mean peak plasma concentration at 0.75 hr 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. The mean therapeutic concentration (≥ 0.1 mg/ml) of *Withania somnifera* was maintained from 10 min to 3 h in plasma of healthy buffalo calves. The mean elimination half life ($t_{1/2}\beta$) 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 mg/ml.h and 246.26 ± 17.66 mg/ml.h^2. The total body clearance (Cl_B) ranged from 2.26 to 3.09 L/kg/h with a mean value of 2.78 ± 0.12 L/kg/h. Considering the AUC/MIC and C_{max} /MIC ratios obtained in the present study, it can be stated that *Withania somnifera* administered orally in the dosing schedule applied is efficacious against bacteria with MIC values under 0.1 mg/ml in buffalo calves. The high values of AUC/MIC (1814.4) and C_{max} /MIC (2481.7) obtained in the present study, provides support for excellent clinical and bacteriological efficacy of *Withania somnifera* in buffalo calves.

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

INTRODUCTION

WHO has predicated that the microorganisms are becoming resistant to most of the antibiotics and by 2020 antibiotics (so called wonder drug of the 20th Century) will loss their effectiveness and be no more in use to cure diseases in man and animals. Most of the 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 as phagocytic system through macrophages. WHO has also advised to its all member countries to explore and use the traditional wisdom for the health management. Due to these facts (harmful chemical residue and antibiotic resistance) there is a race in the healthcare personnel/scientists throughout the world to find suitable and sustainable methods of treating the ailments. Now the attention of the international scientific forum has been diverted towards the alternative therapies.

Withania somnifera (*W. somnifera*) commonly known as Ashwagandha or Indian Ginseng is an important herb in indigenous medicinal systems (Ayurveda) for over 3000 years. Among all parts of this plant, *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 antistress. The roots of *W. somnifera* contain several alkaloids, withanolides, a few flavanoides and reducing sugars. *W. somnifera* contains number of phytoconstituents, withanolides as the major constituent. It is one of the most commonly used drugs as natural antimicrobial agent [1]. *W. somnifera* is commonly used Indian medicinal plant for antimicrobial activity since the ancient time. The antibacterial activity of *W. somnifera* is now approaching for evaluation of their therapeutic efficacy and valuable use as antibacterial agents in the present study. Their therapeutic use should be based on the correlation between antibacterial activity and its concentration achieved *in vivo*. Among various factors, that determines 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 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 drug of plant origin could reach clinical use and the National formulary could not adopt even a dozen of plant medicines. For this reason a special effort is needed for development of herbal drug having therapeutic utility.

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Table 1: Plasma concentration (mg/ml) of *W.somnifera* following single oral dose of 500 mg/kg in healthy buffalo calves

Time (h)	Experimental Calf						Mean ± S.E.M.
	C1	C2	C3	C4	C5	C6	
0.167	6.13	6.19	6.31	6.88	6.59	6.29	6.39 ± 0.11
0.25	14.04	15.20	14.80	13.3	14.31	13.20	14.14 ± 0.32
0.333	58.12	51.27	60.1	60.03	60.71	55.21	57.57 ± 1.50
0.50	103.8	103.88	106.8	104.91	105.23	103.9	104.75± 0.47
0.75	252.9	259.5	200.6	290.21	285.1	200.7	248.16±16.12
1	110.5	107.91	55.1	66.1	110.23	105.25	92.51± 10.22
1.5	61.20	28.81	112.30	119.46	65.87	61.31	74.82± 14.09
2	19.7	11.7	34.2	31.89	29.06	80.6	34.54 ± 9.83
2.5	10.32	16.20	18.20	19.20	17.14	15.71	16.12 ± 1.27
3	6.81	6.21	6.33	6.76	6.88	6.31	6.55 ± 0.12
4	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	-

N.D. = Non-detectable

Table 2: Kinetic parameters of *W. somnifera* calculated by non-compartmental analysis following single oral administration of 500 mg/kg in healthy buffalo calves

Parameter (Unit)	Experimental Calf						Mean ± S.E.M.
	C1	C2	C3	C4	C5	C6	
β (h^{-1})	0.75	0.82	0.69	0.67	0.81	0.71	0.74 ± 0.025
$t_{1/2}$ β (h)	0.92	0.83	0.99	1.02	0.84	0.96	0.92 ± 0.032
AUC (mg/ml.h)	189.75	161.64	177.84	220.31	163.21	175.91	181.44 ± 8.84
AUMC (mg/ml.h^2)	252.89	197.17	254.69	327.19	199.82	245.80	246.26 ±17.66
MRT (h)	1.33	1.21	1.43	1.48	1.22	1.39	1.34 ± 0.045
Vdss (L/kg)	3.50	3.76	4.07	3.37	3.77	4.01	3.68 ± 0.12
CL_B (L/Kg/h)	2.63	3.09	2.81	2.26	3.06	2.84	2.78 ± 0.12
C_{max} (mg/ml)	252.9	259.5	200.6	290.21	285.1	200.7	248.16±16.12
C_{max}/MIC (Ratio)	2529	2595	2006	2902	2851	2007	2481.7±161.2
AUC/MIC (Ratio)	1897.5	1616.4	1778.4	2203.1	1632.1	1759.1	1814.4 ± 88.5

There is no data available for the disposition 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 under taken, to investigate the disposition kinetic study 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 institutional 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 animal shed and maintained on dry fodder and greens as well as routine grazing for at least 4-5 hours a day. Clean drinking water was supplied *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 mixer and grinder to prepare fine powder. 100 g of *W. somnifera* powder was dissolved in 1 L of sterile triple distilled water for 24 h to make cold aqueous extract of *W. somnifera.* The cold aqueous extract of *W. somnifera* was administered at dose rate of 500 mg /kg body wt., orally by drenching tube in each of 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. 2 ml) were withdrawn from jugular vein into heparinized glass centrifuge tubes at 0, 10, 15, 20, 30, 45 min and 1, 1.5, 2, 2.5, 3 and 4 h after administration of the drug. Plasma was separated by centrifugation at 3,000 r.p.m. for 15 min at room temperature and kept at -4ºC 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 hr, this solution was used for preparation of stock solution of 100 mg/ml of *W. somnifera*. One ml 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. Various standard samples (0.078 to 10 mg/ml) in plasma were prepared and used simultaneously with test samples in assay plates for obtaining the zone of inhibition (measured as diameter in mm). The diameter of zone of inhibition was measeared ≥ 9 mm is only considered. The relationship between standards and zone of inhibition was linear from 1.25 to 10 mg/ml. The test samples with higher concentrations were diluted and remeasured. The sensitivity of the assay method was as low as 1.25 mg/ml.

The test organism used for the microbiological assay technique (Agar gel diffusion technique) of *W. somnifera* was *Escherichia coli* (ATCC 25922) [2]. 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.

Pharmacokinetic analysis of *W. somnifera* after single oral administration was calculated from 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 [3]. The mean therapeutic concentration (≥ 0.1 mg/ml) of *W. somnifera* was maintained from 10 min to 3 h in plasma of healthy buffalo calves as stated by Arora *et al.* [2] who reported the minimum inhibitory concentration (MIC) of *W. somnifera*, which came out to be 0.1 mg/ml for *Salmonella typhimurium* and *Escherichia coli*.

Clinical and microbiological outcomes of *W. somnifera* therapy can be predicted by the site of infection and in terms of pharmacokinetic-pharmacodynamic (PK/PD) surrogate relationships based on $C_{max:}$ MIC and the AUC: MIC ratio [4]. The pharmacodynamic efficacy of *W. somnifera* was determined by calculating C_{max} / MIC and AUC/MIC ratios following oral administration of *W. somnifera*. In order to calculate the PK/PD efficacy predictors hypothetical MIC values were used. The minimum therapeutic concentration (MIC) value of *W. somnifera* was noted to be 0.1 mg/ml for *S. typhimurium* and *Escherichia coli* [2]. Keeping in mind the synergistic effect of the body immune system and other in vivo factors as well as to cover most of the susceptible organisms, the MIC of 0.1 mg/ml of *W. somnifera* is taken into consideration.

RESULTS AND DISCUSSION

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

The mean plasma concentration of the drug at 0.167 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 Table 1.

The pharmacokinetic parameters of *W. somnifera* following single oral administration of 500 mg/kg in healthy buffalo calves are shown in Table 2.

The plasma *W. somnifera* 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 ($t_{1/2}$ β) values of the drug were observed to be 0.92 ± 0.032 h.

There is no data available for the disposition kinetic study of *W. somnifera* in any species of animal so far. 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 [5].

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), the 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 ($t_{1/2}$ 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.h^2 and MRT values 1.34 ± 0.045 h were found. That clearly indicated that the maximum area covered by drug *W. somnifera* after oral administration in the body of buffalo calves.

The relatively high value of Vd_{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_{SS} obtained in the present study showed the wide distribution of *W. somnifera* in the body of buffalo calves.

The total body clearance (Cl_B) value of *W. somnifera* was in healthy buffalo calves were 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_B = 2.45 ± 0.21 L/kg/h after oral administration of sulphur mustard decontaminant CC-2 in rats [5].

There is general consensus that the clinical and microbiological outcomes of *W. somnifera* therapy are favourable and selection of a mutant subpopulation is

preventable if an AUC/MIC ≥ 1000 and a C_{max}/MIC of 500 may achieved in bacterial infections. For Gram-positive pathogens, the minimum required C_{max}/MIC is also 10, while the optimum AUC/MIC target values are still a topic of debate. An AUC/MIC of 30–50 is claimed to be optimal in numerous studies performed mainly in *in vitro* or animal models [6]. Other studies conducted on different patient populations suggested a minimum AUC/MIC of 87-125 to achieve a favourable outcome and to avoid development of resistance regardless of whether the organism is Gram-positive or Gram-negative [7].

Considering the AUC/MIC and C_{max} /MIC ratios obtained in the present study, it can be stated that *W. somnifera* administered orally in the dosing schedule applied is efficacious against bacteria with MIC values under 0.1 mg/ml in buffalo calves. The high values of AUC/MIC (1814.4) and C_{max} /MIC (2481.7) obtained in the present study, provides support for excellent clinical and bacteriological efficacy of *W. somnifera* in buffalo calves. However, it is necessary to note that the numerical values of AUC/MIC and C_{max}/MIC used as a surrogate marker to predict optimal dosage, have been generated in experimental infections in laboratory animals or in human clinical trials [8].

CONCLUSION

Herbs are the backbone of therapeutic strategies. India having huge wealth of plant biodiversity holds an excellent potential for herbal treatment. After evaluating the efficacy and pharmacokinetics of the medicinal plants, the 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 clinician for development of herbal preparation of indigenous plants. Pharmacokinetics of *W. somnifera* will provide valuable clues to the clinician for their large scale use in future.

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REFERENCES

1. Jaffer HJ, Jawad ALM. Evaluation of antimicrobial activity of *Withania somnifera* extracts. *Fitoterapia* 1998; 6: 497-500.
2. Arora S, Dhillon S, Rani G, Nagpal A. The in vitro antibacterial /synergistic activities of *Withania somnifera* extracts. *Fitoterapia* 2004; 75: 385-388.
3. Singh B. Non compartmental pharmacokinrtic analysis of plasma level data through statistical moment approach: a worksheet distance. *ICAR short course on "Recent Approach in Clinical Pharmacokinetic and Therapeutic Monitoring of Drugs in Farm Animals"* Oct 25-Nov 3. Division of Pharmacology and Toxicology IVRI Izatnagar, India, 1999, 36-40.
4. Peloquin CA, Cumbo TJ, Nix DE. Intravenous ciprofloxacin in patients with nosocomial lower respiratory tract infections: impact of plasma concentrations, organism MIC, and clinical condition on bacterial eradication. *Archieve of Internal Medicine* 1989; 149: 2269–2273.
5. Lal J, Kumar V, Gupta RC. Pharmacokinetics of oral and transdermal administration of sulphur mustard decontaminant CC-2 in rats: A preliminary study. *Indian Journal of Pharmacology* 2003; 35: 297-303.
6. Lister PD. Impact of AUC/MIC ratios on the pharmacodynamics of the des-F (6) quinolone garenoxacin (BMS-284756) is similar to other fluoroquinolones, *Journal of Antimicrobial and Chemothrapy* 2003; 51: 199-202.
7. Forrest A, Nix DE, Ballow CH, Goss TF, Birmingham MC, Schentag JJ. Pharmacodynamics of intravenous ciprofloxacin in seriously ill patients. *Antimicrobial Agents Chemotherapy* 1993; 37: 1073-1081.
8. Touitan PL, Lees P. Integration and modelling of pharmacokinetic and pharmacodynamic data to optimize dosage regimens in veterinary medicine. *Journal of Veterinary Pharmacology and Therapeutics* 2004; 27: 467–477.