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RESEARCH ARTICLE

Protective Effect of *Ocimum sanctum* on Disposition Kinetics of Sulphadimidine in Lead-Treated Cockerel

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ABSTRACT

This study was carried out to evaluate the protective effect *of Ocimum sanctum* (tulsi) on lead-induced alteration in disposition kinetics of sulphadimidine (SD) in cockerels. Twenty-one, four-week male white leghorn (WLH) chicks were randomly divided into groups I, II and III (7 in each) and were fed at dietary with 0 ppm lead and no tulsi (group I, Control), 100 ppm lead (group II), or 100 ppm lead + 100 ppm tulsi (group III). After four weeks, disposition kinetics of intravenous single dose administration of SD (50 mg /kg) was determined in each bird. The mean values of elimination half-life, distribution–half, AUC, and volume of distribution were significantly (p<0.01) higher in lead-treated group than those in lead plus tulsi group (III) The clearance (Cl, mL/kg/h) of SD was slower in lead-treated groups than lead plus tulsi and control groups The priming dose of SD (mg/kg) in chicks following single i.v. administration is proposed as 68.91 and 122.57 for group I (control), 51.42 and 79.21 for group II (lead) and 61.38 and 106.59 for group III (lead plus tulsi), at 8 h and 12 h interval, respectively. The maintenance doses ($D_{m,}$ mg/kg) were minimum in group II (39.31 and 57.54) followed by group III (41.03 and 86.25) and control (47.13 and 100.80) at 8 and 12h interval, respectively. It is concluded that feeding of lead at 100 ppm dietary level altered the kinetics and dosage regimen of SD which were significantly ameliorated following simultaneous feeding with powdered tulsi (100ppm) in cockerels.

Keywords: *Disposition kinetics, Sulphadimidine, Lead, Cockerels, Oscimum Sanctum*

Sulphadimidine, a commonly-used sulfonamide antibacterial drug in veterinary practice, competes with paraminobenzoic acid (PABA) for the catalytic site of the enzyme dihydropteric acid synthetase to inhibit the synthesis of folic acid, required for bacterial proliferation [1]. Sulfonamides are metabolized mainly in liver by acetylation followed by conjugation with sulfate or glucuronic acid, deamination and cleavage of heterocyclic ring [2]. *Ocimum sanctum,* commonly known as tulsi, is a plant widely used in Ayurveda. It has been shown to possess anti-inflammatory, antioxidant and cognition-enhancing properties [3]. Aqueous extract feeding also provided significant liver and aortic tissue protection [4]. *Ocimum sanctum* fixed oil produces hypotensive effect due to its peripheral vasodilatory action; it also increases blood-clotting time due to inhibition of platelet aggregation [5].

Lead is one of the most common heavy metals responsible for toxicity in man and animals. It is absorbed rapidly from gastrointestinal tract and lungs. In the bloodstream, lead binds with hemoglobin in erythrocytes and accumulates in bones. It excretes out very slowly in urine and bile and fractionally by exfoliation of epithelial tissue including hair with a half-life of 20-30 years [6-8]. Thus, persistence of lead impairs the hepato-renal function and subsequently the pharmacokinetic pattern of the drugs that are metabolized by the liver and excreted in the urine. Variation in pharmacokinetics pattern of sulphonamides has also been reported in animals both in normal as well as stressful conditions [9-13]. In view of these facts, sulphadimidine was considered to study its disposition kinetics and to evaluate protective efficacy of *Ocimum sanctum* (tulsi) in chronically lead-intoxicated cockerels.

MATERIALS AND METHODS

Chemical

Pure analytical-grade lead acetate, $Pb(C_2H_3O_2)_2.3H_2O$ (CDH) was used in this study. Mature tulsi plants (*Ocimum sanctum*) were collected from Research and Development Farm, Medicinal and

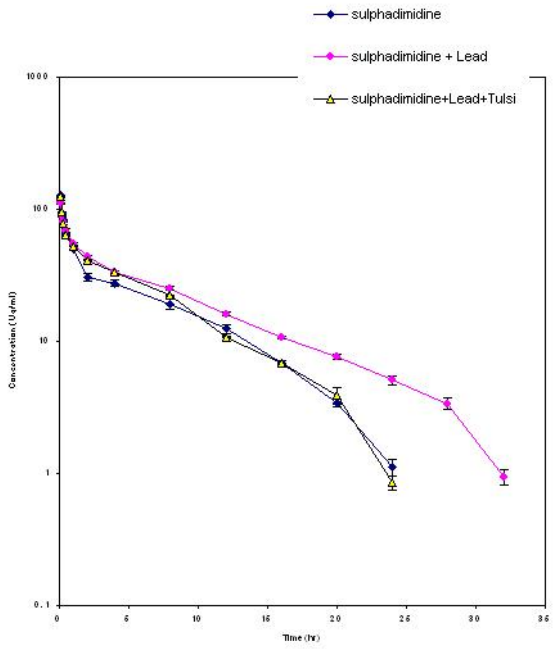

Fig 1. Mean + S.E. Plasma concentration vs time plot of sulphadimidine, sulphadimidine + Lead, sulphadimidine + Lead + Tulsi following single dose (50 mg/kg) i.v. administration in cockerels (n=7)

Aromatic Plants, Department of Horticulture, N.D.U.A.T., and was identified taxonomically.

Experimental animals

Four-week male white leghorn chicks were procured from government poultry farm chak ganjaria, Lucknow and were reared for four weeks for acclimatization before the start of the study. Four weeks chicks were used in this study and kept in deep litter system of housing and maintained on grower ration procured from U.P. state Agro Industries Corporation limited, Lucknow. Food and water were provided *ad libitum* throughout the study.

Medicated ration was prepared for about a week period and kept in close container. Dried tulsi leaves were pulverized to prepare 100 ppm tulsi-medicated feed by mixing 600 mg of powder of tulsi in 6 kg food. Similarly, Lead (100 ppm)-treated ration was prepared by taking 145.21 mg of lead acetate containing lead equivalent to 100 ppm and mixing thoroughly. All the birds were fasted over night prior to the start of the experiment.

The 'Animal Ethical Committee' of Narendra Dev University of Agriculture and Technology, Kumarganj, Faizabad, Utter Pradesh, India approved all the procedures adopted in the study.

Animal treatment

Twenty-one, four weeks old male white leghorn chicks, were randomly divided into three groups of I (control), II (lead 100 ppm), and III (lead 100 ppm + tulsi 100 ppm) (seven birds each). The pharmacokinetics pattern of sulphadimidine was estimated after injecting a single dose (50 mg/kg, I.V.) of that in these groups after 12 weeks of feeding trial [11-13]. Blood samples were collected from wing vein in heparinised sterilized tubes before and after 0.08, 0.17, 0.25, 0.50, 1, 2, 4, 8, 12, 16, 20, 24, 28 and 32 h of drug administration.

Analytical method

The concentration of free and total sulphonamide in plasma (μg/ml) was estimated according to the method of Bratton and Marshal, 1939 and Richterich, 1969. [14, 15] based on what measured by AUTOCHEM 2011 at 580 nm. Dosage regimen was calculated using information of single dose trial [16, 17]. The Pharmacokinetic analysis of plasma concentration time profile of sulphadimidine for each bird was performed with the aid of PHARMAKIT, (M/s Clyde Soft, Glasgow, UK).

Statistical analysis

Comparison of pharmacokinetic parameters of treated and control groups were done using one way analysis of variance in SPSS software, Version 11. The p values ≤ 0.05 were considered significant.

Table 1. Mean plasma concentration (Mean ± SEM))(μg/ml) of sulphadimidine in blood following intravenous administration of single iv dose of sulphadimidine at 50 mg/kg in different groups of cockerel

Time (h)	Mean Plasma Concentration (Mean ± SEM)		
	Control (0 ppm)	Lead (100 ppm)	Lead (100 ppm) +Tulsi (100 ppm)
0.08	126.57 ± 1.30	111.28 ± 1.64	125.00 ± 1.70
0.17	94.14 ± 1.01	90.28 ± 2.14	95.42 ± 0.92
0.25	83.00 ± 0.48	79.28 ± 2.20	78.71 ± 1.55
0.5	63.42 ± 1.21	69.00 ± 2.14	64.00 ± 1.13
1	50.14 ± 1.59	54.57 ± 0.92	52.85 ± 0.70
2	38.71 ± 2.14	43.28 ± 1.44	41.00 ± 0.53
4	27.57 ± 1.65	33.28 ± 0.80	33.28 ± 0.99
8	19.14 ± 1.62	25.28 ± 0.96	22.14 ± 0.46
12	12.44 ± 0.88	16.14 ± 0.50	10.77 ± 0.17
16	6.85 ± 0.38	10.74 ± 0.28	6.91 ± 0.24
20	3.4 ± 0.22	7.64 ± 0.31	3.92 ± 0.54
24	1.12 ± 0.16	5.07 ± 0.42	0.85 ± 0.11
28	n. d.	3.37 ± .34	nd
32	n. d.	0.94 ± 0.13	nd

nd = not detected

RESULTS

Mean plasma concentration (μg mL^{-1}) of sulphadimidine following single dose (50 mg kg^{-1}) i.v. administration in different groups of chicks (control, 100 ppm lead and 100 ppm lead plus 100 ppm tulsi) is given in Table 1 and disposition curve has been depicted in Fig 1. The minimum therapeutic concentration (25 μg mL^{-1}) of sulphadimidine was maintained for 4 hours in control and lead plus tulsi groups whereas 8 h in lead-intoxicated group. Sulphadimidine was detected up to 24 hours in control and lead plus tulsi groups whereas lead-intoxicated chicks were found to have sulphadimidine up to 32 hours of post administration. The plasma concentration-time profiles of sulphadimidine in all groups indicated that disposition of the drug followed two-compartmental open models. The values of various pharmacokinetic parameters were computed from plasma levels of free sulphadimidine in control and treated groups following single i.v. administration at the dose of 50 mg kg^{-1} (Table 2).

The mean value of extrapolated drug concentration during distribution phase (A, ug/mL) was also less in lead-treated groups as compared to other groups whereas the value of extrapolated drug concentration during elimination phase (B, ug/mL) did not reveal any variation among control and treated groups. The mean values of distribution rate constant (α, h^{-1}) and elimination rate constant (β, h^{-1}) were significantly ($p < 0.01$) less in lead-intoxicated groups as compared to control and lead plus tulsi group. The values of distribution rate constant (α, h^{-1}) were 5.227 ± 0.464, 3.856 ± 0.356 and 6.062 ± 0.292, respectively, in control, lead and lead plus tulsi groups. The value of elimination rate constants (β, h^{-1}) were in the order as 0.144 ± 0.010, 0.105 ± 0.002 and 0.137 ± 0.001, respectively, in control, lead and lead plus tulsi groups.

The mean value of elimination half-life ($t_{1/2}$β, h) was significantly ($p < 0.01$) higher in lead treated group as it was 6.582 ± 0.119 in lead treated group as compared to 4.918 ± 0.310 are in control and 5.018 ± 0.068 in lead plus tulsi group. The mean value of distribution half-life ($t_{1/2}$α, hours) was significantly ($p<0.01$) higher in lead-treated group as it was 0.189 ± 0.019 in lead-treated group, 0.138 ± 0.012 in control and 0.114 ± 0.004 in lead plus tulsi group. There was a significantly ($p<0.01$) lower rate of transfer of the drug from peripheral to central ($k_{12,}$ h^{-1}) compartment in lead-treated chicks in comparison to other groups whereas the rate of transfer from central to peripheral compartment (k_{21}) did not reveal any variation among treated and control groups. The volume of distribution (Vd_{area}, mL kg^{-1}) did not reveal any significant variation among all the groups as the values of Vd_{area} were 0.870 ± 0.040, 0.866 ± 0.014 and 0.814 ± 0.008 in controls, lead and lead plus tulsi group, respectively. The clearance (Cl, mL h^{-1}) of sulphadimidine was slower in lead-treated birds than control and lead plus tulsi as the value of Cl was 0.090 ± 0.002 in lead, 0.124 ± 0.008 in control and 0.111 ± 0.001 in lead plus tulsi group. The retention of sulphadimidine in the body was significantly ($p<0.01$) higher in lead-treated chicks than that in other groups as the values of mean residential time (MRT, h) was 9.156 ± 0.174 in lead-treated group, 6.734 ± 0.429 in control and 6.956 ± 0.095 in lead plus tulsi treated group.

The dosage regimens for minimum plasma therapeutic concentration of 25 μg mL^{-1} at 8 and 12 h interval for control and treated groups have been given in Table 3. At 8 h interval, the priming dose (D, mg kg^{-1}) for the cockerels fed on lead (100 ppm) after 12 weeks feeding trial was less than control and lead plus tulsi fed cockerels as it was computed to be 51.42 for lead-intoxicated group in comparison to 61.38 for lead plus tulsi and 68.91 for control group. Similarly, at 12 h interval, the D was only 79.21 for the cockerels of lead-treated groups, 106.59 for lead plus tulsi and 122.57 for the control group. Correspondingly, the maintenance doses (D_m, mg kg^{-1}) at 8 and 12 h interval were less for lead-treated chicks (39.3 and 57.54) as compared to lead plus tulsi (41.03 and 86.25) and control group (47.13

Table 2. Pharmacokinetic parameters after single dose 50mg kg^{-1} iv sulphadimidine in control, lead-intoxicated and lead plus tulsi-medicated cockerels (Mean ± S.E.M.) n = 7

Parameter	Control	Lead (100 ppm)	Tulsi + Lead (each 100ppm)
A (μg mL^{-1})	106.750 ± 4.235	72.580 ± 1.794	107.434 ± 4.053
B (μg mL^{-1})	55.210 ± 2.474	55.918 ±0.998	59.136 ± 0.718
α (h^{-1})	5.227 ± 0.464	3.856 ± 0.356	6.062 ± 0.292
β (h^{-1})	0.144 ± 0.010	0.105 ± 0.002	0.137 ± 0.001
AUC (μg mL^{-1}h)	410.444 ± 25.023	550.811 ± 13.324	445.979 ± 6.978
$t_{1/2}$ α (h)	0.138 ± 0.012	0.189 ± 0.019	0.114 ± 0.004
$t_{1/2}$ β (h)	4.918 ± 0.310	6.582 ± 0.119	5.018 ± 0.068
k_{10} (h^{-1})	0.401 ± 0.030	0.233 ± 0.007	0.373 ± 0.013
k_{12} (h^{-1})	3.084 ± 0.030	2.00 ± 0.216	3.585 ± 0.216
k_{21} (h^{-1})	1.882 ± 0.172	1.734 ± 0.140	2.239 ± 0.083
MRT (h)	6.734 ± 0.429	9.165 ± 0.174	6.956 ± 0.095
Cl (mL h-1kg-1)	0.124 ± 0.008	0.090 ± 0.002	0.111 ± 0.001
Vd_{area} (mL kg^{-1})	0.870 ± 0.040	0.866 ± 0.014	0.814 ± 0.008

A and B = extrapolated zero time plasma drug concentration during distribution and elimination phases, respectively = theoretical zero time concentration; α and β = distribution and elimination rate constants, respectively; AUC = area under plasma concentration time curve; $t_{1/2}$ α and $t_{1/2}$ β = distribution and elimination half lives, respectively; K_{10} = rate constant of drug elimination from central compartment; K_{12} and K_{21} = micro-rate constant of drug transfer from central to peripheral, peripheral to central compartment, respectively; MRT = mean residential time; Cl = total body clearance; Vd_{area} = volume of distribution from AUC

Table 3 Intravenous Dosage regimens of sulphadimidine to maintain desired therapeutic concentration of 25 μg mL^{-1} at 8 and 12 h intervals in control, lead-intoxicated and lead plus tulsi-medicated cockerels**.**

Groups	Interval (h)	D (mg kg^{-1})	C_{ss} (min) following D (μg mL^{-1})	C_{ss} (max) following D (μg mL^{-1})	Dm (mg kg^{-1})	C_{ss} (min) following Dm(μg mL^{-1})	C_{ss} (max) following Dm (μg mL^{-1})
Control	8	51.42	43.21	102.52	39.31	25	39.31
	12	122.57	30.40	171.13	100.80	25	140.73
Lead (100ppm)	8	51.42	43.21	102.52	39.31	25	39.31
	12	79.21	34.42	125.78	57.54	25	91.37
Lead (100 ppm) + tulsi (100 ppm)	8	61.38	37.39	112.80	41.03	25	75.41
	12	106.59	30.89	161.85	86.25	25	130.96

D= Priming dose
Dm= Maintenance dose
C_{ss} (min) = Minimum steady state concentration
C_{ss} (max) = Maximum steady state concentration

and 100.80) respectively, as shown in Table 3.

DISCUSSION

The disposition kinetics of sulphadimidine revealed that its minimum therapeutic concentration (25 μg mL^{-1}) was maintained for 8 h in lead-treated cockerels which was significantly higher as compared to 4 h in lead plus tulsi and control groups. The drug was detected for 32 h in lead-treated cockerels as compared to other groups. Drug disposition suggests slow elimination and retention of drug for longer duration as well as maintenance of therapeutic concentration in lead-treated groups. Thus, dosage regimen varies among various treated and control groups.

The distribution half life ($t_{1/2}\alpha$, h) explains the distribution of sulphadimidine. The slow distribution of drug in lead-treated cockerels might be attributed to the alteration in transportation of drug as a result of toxic effect of lead on capillaries [18] and other tissues causing disruption in the transport of drug across membrane. The mean value of sum of K_{12} and K_{21} was also higher than K_{10} (elimination rate constant). Thus, the elimination of the drug is slower than its distribution in the body. This might be due to renal insufficiency caused by lead as previously reported [19, 20]. The mean value of elimination rate constant was significantly lower in lead-treated cockerels than other groups suggesting slow rate of elimination of sulphadimidine from body. Lead has been reported to produce nephrotoxic effect in poultry [19, 20]. The value of drug transfer rate constant k_{12} and k_{21} are significantly less in lead-treated cockerels which also suggests slower rate of the movement of the drug across these compartments in lead intoxicated cockerels [21]. The elimination half life ($t_{1/2}\beta$, h) was higher in lead-treated group than lead plus tulsi, which indicates slow elimination of drug from lead-treated cockerels. The slow rate of elimination of sulphadimidine is suggestive of slow hepatic metabolism and slow excretion through in lead treated group. As reported earlier, lead have hepatotoxic and nephrotoxic properties [14, 15] but tulsi nullifies these effects [4].

The volume of distribution (Vd_{area}) was considered as indicator of extravascular distribution of the drug. However, it was comparable in control and other groups indicating equal distribution of the drug in body tissues. Area under curve (AUC) of concentration-time profile explains the relative availability of the drug in the body. The values of AUC of sulphadimidine were the highest in lead-treated cockerels followed by lead plus tulsi and control groups. The effectiveness of drug depends not only on the plasma concentration of drug achieved but also duration for which these concentrations exist in the body [22]**.** That may be correlated with availability of therapeutic concentration for longer duration i.e. 8 h in lead-treated group in comparison to only 4 h in other groups.

The value of MRT was higher in lead-treated cockerels which are suggestive of retention of the drug for longer duration in lead-intoxicated cockerels. The clearance (Cl mL Kg^{-1} h^{-1}) of sulphadimidine was slower in lead-treated cockerels than that in other groups. The pharmacokinetic pattern of drug in lead-treated cockerels is attributed to impaired hepatic metabolism of sulphadimidine and renal excretion of the drug as the sulphonamides are metabolized by acetylation in the liver and excreted by kidneys. Altered pharmacokinetic pattern of sulphonamides with longer half-life in comparison to untreated control was also observed in cockerels treated with pesticide [23] and carbon tetrachloride (CTC) [24]. Similarly, allyl alcohol-induced hepatotoxic sheep also revealed prolonged retention of the sulphonamides [25].

The main objective of pharmacokinetic study of a drug is to calculate its dosage regimen. The cockerels fed on lead (100 ppm) were computed to have least priming and maintenance doses in comparison to lead plus tulsi and control groups at both 8 and 12 h interval to maintain minimum therapeutic concentration of 25 μg mL^{-1}. It is concluded from this study that lead-induced toxic effects altered the disposition kinetic of sulphadimidine in cockerels as reported earlier that lead have hepatotoxic and nephrotoxic effects which might alter the kinetic of drug metabolized by body enzyme system. When lead-treated animals were simultaneously treated with tulsi (100 ppm), the tulsi nullified the toxic effect of lead. These findings may be correlated with other species of animals and other drugs as well.

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