Research Article

PHARMACOKINETICS AND TESTING OF DOSAGE REGIMEN OF AMIKACIN AFTER REPETITIVE INTRAVENOUS ADMINISTRATION IN HEALTHY BUFFALO CALVES

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ABSTRACT

Testing of dosage regimen of amikacin was carried out in five healthy female buffalo calves after its intravenous (i.v.) administration. The drug was injected at the dose rate of 7.5 mg/kg i.v. and concentrations of amikacin in plasma were estimated at various time intervals by microbiological assay method using *Bacillus subtilis* (ATCC 6633) as the test organism. From log plasma concentrations of amikacin, some important pharmacokinetic parameters like distribution rate constant (α), elimination rate constant (β), volume of distribution (Vd_{area}) and total body clearance (Cl_B) of 1.947 ± 0.222 h^{-1}, 0.140 ± 0.007 h^{-1}, 1.19 ± 0.08 L/Kg and 2.96 ± 0.12 ml/Kg/min, respectively were derived. From these kinetic parameters, the loading (D*) and maintenance (D_0) doses of 8.56 ± 2.08 and 6.39 ± 2.02 mg/kg, respectively were calculated for maintaining the therapeutic concentration (C_P^{∞} min = MIC) of 2.0 µg/ml at the dosage interval (γ) of 8 h. The calculated D* was given initially by i.v. route and after every 8 h dosage interval, the calculated D_0 was administered repetitively (three successive D_0) and plasma concentrations of amikacin were estimated. The minimum therapeutic concentration of 2.0 µg/ml was maintained every 8 h. Thus, the study demonstrates that the calculated dosage regimen from a single injection can be practically used for multiple administrations in clinical condition, where the drug has to be used repetitively for a few days in order to cure the infectious conditions.

Key words: Pharmacokinetics, Testing of dosage regimen, Amikacin, Intravenous administration, buffalo calves.

INTRODUCTION

Aminoglycosides have assumed an important role in the armamentarium of drugs used for the treatment of serious gram negative infections (Chambers and Sande, 1996). Amikacin, an aminoglycoside antimicrobial, is a semisynthetic derivative of kanamycin. It is resistant to almost all the R factor mediated aminoglycoside modifying enzymes (Meyer, 1977; Perlin and Lerner, 1979; Seligman 1978) and thus, is used to treat gram negative bacterial infections for which other aminoglycosides are ineffective.

Pharmacokinetics of amikacin has been studied in dogs (Baggot *et al.*, 1985), cats (Jernigan *et al.,* 1988), goats (Uppal *et al.,* 1997; Agrawal *et al.,* 2001; Agrawal *et al.,* 2002), sheep (Carli *et al.*, 1990; Haritova and Lashev, 2004), horse (Orcini *et al.,* 1985), cow calves (Carli *et al.*; 1990, Saini and Srivastava, 1997), and cross-bred bovine calves (Saini and Srivastava, 1998). Calculation of dosage regimen in most of the pharmacokinetic studies is based on the pharmacokinetic parameters derived from single intravenous or intramuscular administrations. Study regarding testing of dosage regimen of antimicrobials derived from single administrations when administered in multiple dosing is not available. Hence, the present study was undertaken with amikacin to investigate whether the dosage regimen calculated from single intravenous administration actually maintains the minimum inhibitory concentration (MIC) at the end of every dosage interval during repetitive administration.

MATERIALS AND METHODS

Experimental animals and drug administration:

Five healthy female buffalo calves weighing between 120 – 180 kg body weights were used. The animals were maintained under standard conditions and feeding schedule with water *ad libitum* and were adapted to laboratory conditions for 2 weeks prior to the commencement of experiments. To each animal, the drug amikacin sulphate was injected i.v. at the dose of 7.5 mg/kg body weight.

Collection and storage of blood samples:

Blood samples (10 ml) were collected from the contralateral jugular vein into heparinised glass centrifuge tubes before and at 5, 10, 15, 20, 30, 45 min. and 1, 1.5, 2, 3, 4, 5, 6, 8, 10, 12, 24 and 30 h after administration of the drug. The plasma was separated after centrifugation at 3000 rpm for 15 min. at room temperature and stored at – 20° C till analysis.

Assay procedure:

The concentrations of amikacin in plasma were estimated by microbiological bioassay technique (Arret *et al.,* 1971) using *Bacillus subtilis* (ATCC 6633) as the test organism. The minimum detection was 0.1 µg/ml.

Calculation of kinetic parameters and computation of dosage regimen:

The log plasma concentration versus time profile showed a biphasic curve, hence kinetic parameters were

obtained from formulae derived for a two-compartment open model (Gibaldi and Perrier, 2007). An average of ≥2 µg/ml of amikacin has been reported to be the minimum therapeutic concentration (Leroy *et al.*, 1978). Hence, in the present investigation, the dosage regimen of amikacin was calculated at the therapeutic level of 2 µg/ml as minimum inhibitory concentration (MIC). The loading or priming dose (D*) and maintenance dose (D_o) were calculated as per the formulae of Saini and Srivastava (1997) as follow:

$$D^* = C_P^{\infty}\,(\min).\ Vd_{area}.\ (e^{\beta\gamma}),$$

$$D_o = C_P^{\infty}\,(\min).\ Vd_{area}.\ (e^{\beta\gamma} - 1)$$

where e is the base of natural logarithm, β is the elimination rate constant, γ is the dosage interval and C_P^{∞} (min) is the MIC of the drug to be maintained.

Statistical analysis

The pharmacokinetic parameters were calculated for each animal and the data are presented as means + SEM.

RESULTS

The mean concentrations of amikacin in the plasma, plotted on a semilogarithmic scale as a function of time, are presented in Figure 1. The concentration was 25.72 ± 2.19 µg/ml at 5 min and then decreased rapidly to 8.42 ± 0.67 µg/ml at 1 h. Thereafter, the concentration of amikacin fell gradually and only traces (0.21 ± 0.03 µg/ml) were detected by 24 h after dosing. The pharmacokinetic parameters which describe the distribution and elimination of amikacin in bovine calves are given in Table 1. Based on the pharmacokinetic parameters, suggestive dosage regimen have been calculated which has been presented in Table 2.

The calculated doses i.e. loading and maintenance doses based on single i.v. dose of 7.5 mg/kg have been administered repetitively for three consecutive dose intervals and plasma concentrations of amikacin were monitored. The study clearly demonstrates that the minimum therapeutic concentration (MIC) of ≥2 µg/ml was maintained during the period of study (Figure 2).

Fig.1:
A semilogarithmic plot of the plasma concentration-time profile of amikacin in cross-bred calves following a single intravenous dose of 7.5 mg/kg. Distribution (α) and elimination (β) phases are represented by least-squares regression lines.

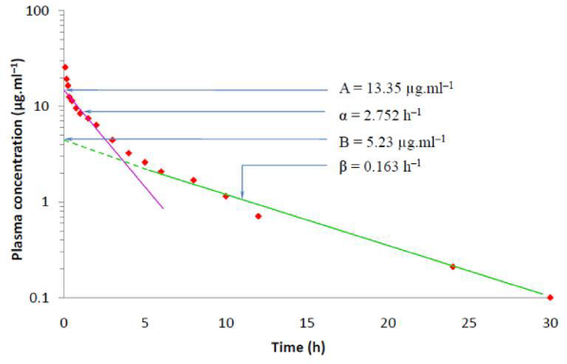
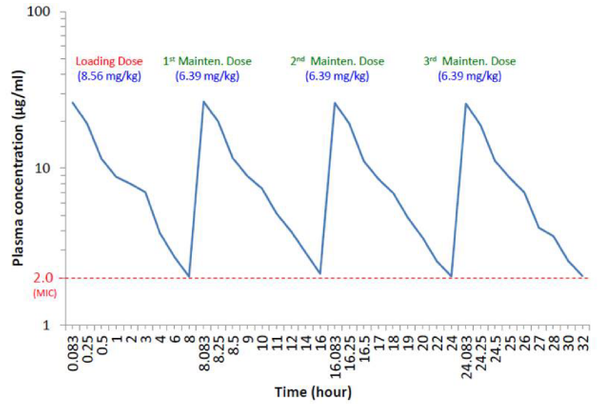


Fig. 2:
Plasma concentrations (µg/ml) of Amikacin in buffalo calves (n=5) during repetitive administration to verify the maintenance of MIC of 2.0 µg/ml at the dosage interval of 8 h.



DISCUSSION

An average concentration of ≥2 µg/ml of amikacin has been reported to be the minimum therapeutic concentration (Leroy *et al.*, 1978). The minimum therapeutic plasma concentration was maintained up to 6 h after administration. The semilogarithmic plot of the observed plasma concentrations of amikacin against time showed two distinct phases. Hence, we considered that the data were best fitted to a two-compartment open model, adequately described by the equation

$$C_P = Ae^{-\alpha t} + Be^{-\beta t}$$

where C_P is the concentration at time t, A is the zero time intercept of distribution phase, B is the zero time intercept of elimination phase, α is the distribution rate constant, and β is the elimination rate constant.

The high values of Vd_{area} (1.19 ± 0.08 L/Kg) and T≈P ratio (1.97 ± 0.34) suggest that amikacin is rapidly and extensively distributed in the various body fluids and tissues of buffalo calves. In contrast, lower volume of distribution of 0.4 ± 0.02 L/kg was reported in goats (Agrawal *et al.,* 2002), 0.40 ± 0.03 L/kg in cross-bred calves (Saini & Srivastava, 1998), 0.23 ± 0.04 in dogs (Baggot *et al.,* 1985) and 0.27+0.04 L/kg in humans (Bauer and Blouin, 1983). The elimination half-life of amikacin in calves found in the present investigation (4.96 ± 0.24 h) was much higher than that in horses (1.57 h; Orsini *et al.*, 1985) and cats (1.31 h; Jernigan *et al.*, 1988). Saini and Srivastava (1998) reported similar values for the area under curve (111.2 ± 3.6 mg/L.h) and the total body clearance (0.09 L/kg/h) of amikacin in calves following single intravenous

Table 1:
Disposition kinetics of amikacin in cross-bred calves (n = 5) after a single intravenous administration of 7.5 mg/kg.

Parameter	Unit	Mean ± SEM
Zero time concentration:	$\mu g.ml^{-1}$	
A (Distribution)		13.82 ± 1.07
B (Elimination)		4.91 ± 0.54
$C_P^{\infty} = A+B$		18.74 ± 1.21
Rate constant:	h^{-1}	
α (Distribution)		1.947 ± 0.222
β (Elimination)		0.140 ± 0.007
Half life:	h	
$t_{1/2}$ α (Distribution)		0.36 ± 0.26
$t_{1/2}$ β (Elimination)		4.96 ± 0.24
Micro-rate constants for drug transfer:	h^{-1}	
K_{12} (Central to peripheral compartment)		1.029 ± 0.126
K_{21} (Peripheral to central compartment)		0.614 ± 0.064
K_{el} (Elimination from central compartment)		0.445 ± 0.039
Area under curve ($AUC_{0-\infty}$)	mg/L.h	45.07 ± 3.25
Area under first moment curve (AUMC)	$mg/L.h^2$	260.56 ± 13.42
Mean residential time (MRT)	h	5.61 ± 0.15
Fraction of drug available for elimination from central compartment, Fc		0.39 ± 0.05
Tissue to plasma concentration ratio, T≈P		1.97 ± 0.34
Volume of distribution, Vd_{area}	L/kg	1.19 ± 0.08
Total body clearance, Cl_B	ml/kg/min	2.96 ± 0.12

Table 2:
Calculated dosage regimen of amikacin in buffalo calves (n=5) used for repetitive administration for maintaining $C_P^{"}$ min (MIC) of 2 µg/ml at the selected dosage interval of 8 h.

	Mean ± SEM
Loading or Priming (D*) dose in mg/kg	8.56 ± 2.08
Maintenance dose (D_o) in mg/kg	6.39 ± 2.02

administration.

The ultimate objective of the disposition kinetic study is to determine an appropriate dosage regimen. Using a convenient dosage interval, the calculation of the maintenance dose (D_o) is based on the minimum effective concentration (MEC) and may be calculated from the formula

$$D_o = C_P\infty \text{ (min)}. Vd_{area}. (e^{\beta\gamma} - 1)$$

where β is the elimination rate constant and γ is the dosing interval. The priming dose is calculated by omitting the –1 from this equation. Taking 8 h as a suitable dosage interval, with a minimum therapeutic plasma level of 2 µg/ml (Leroy *et al.*, 1978) and using the values of β and Vd(area) in Table 1, an appropriate dosage regimen for amikacin in bovine calves would be 8.56 mg/kg followed by 6.39 mg/kg at 8 h intervals.

This calculated dosage regimen was tested after dosing first with the priming dose and then with three consecutive maintenance doses at 8 h interval. The plasma concentration measured confirmed that MIC of ≥2 µg/ml was maintained during the period of study. This proved that dosage regimen calculated from single intravenous administration can very well be used in multiple administrations.

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Research Article

HYPOLIPIDEMIC ACTIVITY OF COW URINE ARK AND *ALOE VERA* IN POULTRY

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ABSTRACT

This study was conducted to explore the hypolipidemic activity of Cow urine ark and Aloe vera extract in broiler birds. Broiler birds were administered cow urine ark, aloe vera extract and their combination by oral route. Upon treatment, significant reduction was observed in serum cholesterol, triglycerides, total lipids, LDL and VLDL with significant increase HDL value in treated groups. Finding of the present study indicates hypolipidemic potential of cow urine ark, aloe vera and their combination.

Key words: Serum cholesterol, triglycerides, LDL, VLDL, HDL.

INTRODUCTION

High levels of cholesterol in the blood can trigger the emergence of cardiovascular disease (Goldstein *et al.,* 1973). One way of lowering cholesterol is to incorporate cholesterol reducing substance into the diets of meat producing animals. Natural substances with antilipidemic potential, decreases the accumulation of intracellular fat and lowers adiposities in meat without affecting body weight. Cow urine has been described in 'Sushruta Samhita' and 'Ashtanga Sangraha' as most effective secretion of animal origin with innumerable therapeutic values (Kekuda *et al.,* 2010). Aloe vera has a long history of use as an herbal medicine with innumerable therapeutic properties. Despite its widespread use as folk remedy over long period of time, the biochemical details of its action on living systems particularly broiler birds have not systematically investigated. Hence, this study was conducted to study the hypolipidemic activity of Cow urine ark and Aloe vera extract in broiler birds.

MATERIALS AND METHODS

Preparation of Cow urine ark and Aloe vera extract

The cow urine was collected from Indigenous cows raised under standard feeding and management condition from Dayodaya Dairy Farm, Jabalpur. The ark of cow urine was prepared according to the method of Khanuja *et al.* 2002. The indigenous plant Aloe vera was collected from department of Aromatic and Medicinal plants, J.N.K.V.V. Jabalpur (M.P.). The leaves of the plant were shed dried at room temperature and powdered leaves were used for preparation of alcoholic extract as per the method described by Pandey and Shrivastava (1989).

Forty, day old healthy broilers chicks were procured from Phoenix poultry farm, Jabalpur and were used in this study. Birds were divided into four groups A, B, C and D containing 10 birds in each group. Birds were kept separately and maintained under hygienic conditions; standard ration and water was given *ad libitum*. Birds of group A served as control. In treatment group B and C, 1 ml Cow urine ark and 1 ml Aloe vera was given respectively by oral route. In group D 0.5 ml Cow urine ark with 0.5 ml of Aloe vera extract were given daily, by oral route. Treatments were given continuously for 42 days. Two ml blood was collected from jugular vein using 24 gauge needle in clean and dry test tube without anticoagulant. Blood collection was done on 14th, 28th and 42nd day of experiment. The serum samples were separated and centrifuged at 3000 rpm for 10 minutes at room temperature and stored at -20ºC till further analysis.

RESULTS

In the present study, significant effect of cow urine ark, aloe vera and their combination was observed on the serum lipid profile of birds. Among different treatment groups, least value of cholesterol was observed in Aloe vera treated group on 42nd day of the study. On 42nd day of the study the value of triglyceride was observed to be 269.21±0.04 and 229.53±0.07 respectively for cow urine

Table 1.
Effect of cow urine ark and *Aloe vera* on serum cholesterol (mg/dl) level

Group	Day 14th	Day 28th	Day 42nd
A	165.79$^{a;4}$ ±0.85	166.01$^{b;4}$ ±0.63	166.78$^{c;4}$±0.54
B	164.51$^{c;3}$±0.30	163.34$^{b;3}$ ±0.13	162.75$^{a;3}$±0.16
C	163.15$^{a;2}$ ±0.12	153.05$^{b;1}$ ±0.03	150.64$^{a;1}$ ±0.11
D	159.33$^{c;1}$ ±0.04	158.32$^{b;2}$±0.10	157.34$^{a;2}$±0.06

Mean with different superscript differ in rows (alphabet) and columns (numerical) differ significantly (P< 0.05)

Table 2.
Effect of cow urine ark and *Aloe vera* on serum triglyceride (mg/dl) level

Group	Day 14th	Day 28th	Day 42nd
A	273.48$^{b;4}$±0.17	273.20$^{b;4}$±0.04	272.21$^{a;4}$±0.04
B	267.98$^{a;3}$ ±0.31	269.20$^{b;3}$±0.04	269.21$^{c;3}$±0.04
C	249.27$^{c;1}$±0.22	239.27$^{b;1}$±0.22	229.53$^{a;1}$ ±0.07
D	259.57$^{c;2}$ ±0.09	248.12$^{b;2}$±0.59	239.56$^{a;2}$±0.05

Mean with different superscript differ in rows (alphabet) and columns (numerical) differ significantly (P< 0.05)

Table 3.
Effect of cow urine ark and *Aloe vera* on serum total lipid (mg/dl) level

Group	Day 14th	Day 28th	Day 42nd
A	297.19$^{a;3}$ ±0.05	297.67$^{ab;4}$±0.13	298.21$^{b;4}$±0.23
B	295.79$^{c;3}$±0.44	294.46 $^{b;3}$ ±0.06	293.32$^{a;3}$±0.10
C	285.38$^{c;1}$±0.36	281.44$^{b;1}$±0.06	272.57$^{a;1}$±0.59
D	292.45$^{b;2}$ ±0.03	291.33$^{ab;2}$ ±0.37	290.74$^{a;2}$±0.63

Mean with different superscript differ in rows (alphabet) and columns (numerical) differ significantly (P< 0.05)

Table 4.
Effect of cow urine ark and *Aloe vera* serum very low density lipoprotein (mg/dl) level

Group	Day 14th	Day 28th	Day 42nd
A	54.69 $^{b;4}$±0.03	54.64$^{b;3}$±0.09	54.47$^{a;4}$ ±0.02
B	53.59$^{a;3}$ ±0.06	53.84$^{b;3}$±0.09	53.84$^{b;3}$ ±0.08
C	49.85$^{c;1}$±0.04	47.85$^{b;1}$±0.04	45.90$^{a;1}$ ±0.01
D	51.91$^{c;2}$±0.01	49.62$^{b;1}$ ±0.11	47.91$^{a;2}$ ±0.01

Mean with different superscript differ in rows (alphabet) and columns (numerical) differ significantly (P< 0.05)

Table 5.
Effect of cow urine ark and *Aloe vera* on serum low density lipoprotein (mg/dl) level

Group	Day 14th	Day 28th	Day 42nd
A	79.44 $^{b;3}$ ±0.11	80.59 $^{c;4}$ ±0.13	78.51 $^{a;3}$ ±0.08
B	78.44 $^{b;3}$ ±0.11	76.59 $^{b;2}$ ±0.13	74.51 $^{a;3}$±0.08
C	65.52 $^{c;1}$ ±0.03	62.96 $^{b;1}$ ±0.22	56.04 $^{a;1}$ ±0.18
D	70.58 $^{c;2}$ ±0.10	68.23 $^{b;1}$ ±0.08	65.90 $^{a;2}$ ±0.54

Mean with different superscript differ in rows (alphabet) and columns (numerical) differ significantly (P< 0.05)

Table 6.
Effect of cow urine ark and *Aloe vera* on serum high density lipoprotein (mg/dl) level

[Group	Day 14th	Day 28th	Day 42nd
A	44.24^{1}± 0.07	43.75 ^{1}±0.32	44.27^{1} ±0.40
B	45.34 $^{a;2}$±0.03	46.39$^{b;2}$ ±0.08	46.50$^{c;2}$ ±0.07
C	46.50$^{a;3}$±0.07	49.36$^{b;4}$ ±0.12	52.51$^{c;3}$±0.10
D	46.56$^{a;3}$ ±0.10	47.46 $^{b;3}$±0.08	48.56$^{c;3}$ ±0.14

Mean with different superscript differ in rows (alphabet) and columns (numerical) differ significantly (P< 0.05)

treated group B and Aloe Vera treated group C.

For serum total lipid, the values observed was 281.44 ± 0.06 and 272.57 ±0.59 respectively on 28th and 42nd day of the study in group C; however in group D, the value at same duration was observed to be 291.33 ±0.37 and 290.74±0.63. The value of very low density lipoprotein on 42nd day was observed to be 45.90± 0.01 and 47.91±0.01 respectively in group C and D, which was significantly less in comparison to control group. Significant improvement in the high density lipoprotein values was observed in the treatment group B, C and D particularly on 42nd day of the studies.

Most significant observations were observed in aloe vera treated group C for most of the lipid parameters. No significant improvement observed in combination group over the individual treatment of group B and C.

DISCUSSION

Cow urine ark and Aloe vera has long been used as a therapeutic agent with numerous reports for medicinal properties (Reynolds and Dweck, 1999). The hypolipidemic effect observed in the present study of Aloe vera may be due to the presence of powerful anti-cholestromatic agent β-sitosterol, found in its juice (Rajeswari *et al.,* 2012). Better results are obtained on the 42nd day of the study indicating more effect of treatment on long term administration of the medicament. Significant reduction in lipid parameter observed in cow urine ark treated group may be due to free radical scavenging activity of Cow urine (Murthi *et al.,* 2004). Gururaja *et al.* (2011) found reduction in blood glucose, serum cholesterol and serum triglyceride activity and significant increase in HDL level in diabetic rats treated with cow urine. The present study revealed significant reduction in serum cholesterol, triglycerides, total lipids, VLDL and LDL levels in birds treated with Cow urine ark, Aloe vera and their combination.

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Research Article

HAEMATOLOGICAL STUDY ON EFFICACY OF *WITHANIA SOMNIFERA* IN PESTICIDE INTOXICATED COCKERELS

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ABSTRACT

The present study was undertaken to know the protective efficacy of aswagandha in pesticides intoxicated cockerels. Ninety, 8-week old male white leghorn cockerels were randomly divided into 9 groups of 10 birds each. Group I was considered as control and group II(Endosulphan100ppm), III(Chlorpyriphos 250ppm), IV(Deltamethrin 100ppm), V(Fenvalarate 250ppm), VI(Endosulphan100ppm + Ashwagandha 100ppm), VII(Chlorpyriphos 250ppm + Ashwagandha 100ppm), VIII(Deltamethrin 100ppm + Ashwagandha 100ppm), IX(Fenvalarate 250ppm + Ashwagandha 100ppm) were considered as treated groups. They were fed medicated ration for 24 weeks. Blood samples (4-6 ml) were collected at 12 and 24 week interval from wing vein of cockerels using sterilized disposable syringes in a heparinised test tube for estimation of different haematological parameters. There were significant ($P < 0.01$) decrease in TEC, TLC, PCV, Hb and Lymphocytes in pesticides intoxicated cockerel at 12 and 24 weeks interval and simultaneously feeding of PRWS subsides the levels in comparison to control. Similarly Significantly ($P < 0.01$) increases the hetrophil, monocytes, eosinophil and basophil in pesticides intoxicated cockerel at 12 and 24 weeks interval and simultaneously feeding of PRWS subsides the levels in comparison to control.

Key words: Endosulphan, Deltamethrin, Chlorpyriphos, Fenvelarates, Cockerels, Aswagandha.

INTRODUCTION

Endosulfan is an organochlorine insecticide of cyclodiene group, a well known insecticide used against invertebrates. It has a higher solubility in water and hence lower affinity for lipids. As a result of this biomagnifications of this compound in food chain is comparatively very less and hence lower chances of cumulative toxicity (WHO, 1984). Chronic exposure to endosulfan in rats has shown to inhibit 5 HT uptakes. It might also induce monoamine alterations by acting directly on the enzymes related to their synthesis and degradation (Lakshmana and Raju, 1994). A variety of haematological changes were reported by Barnard *et al.* (1984) after 13 week oral exposure of rats to 10-360 ppm of endosulfan. Total erythrocyte count and PCV was reduced in males at 30-360 ppm. In females MCV was increased after a dose of 60-360 ppm. In a 15 and 30 day investigation using dose of 5, 10 and 15 mg/kg, carried out on male rats by Chaudhery and Joshi (2002) they observed a gradual decrease in TEC, PCV and Hb values. TLC showed a significant increase in the values. Various workers have studied the effect of monocrotophos and reported a decrease in PCV, TLC, TEC, lymphocyte and Hb content after prolong sub lethal dosing (Siddiqui *et al.*1991; Singh, 2001). Kumar, *et al.,* 2010 reported that the lymphocytic activity decreased which leads to low HA titre and globulin level in CPF treated group and not alter significantly in HA titre, TLC and lymphocyte in PRWS + CPF group as compared to control.

Ashwagandha (*Withenia somnifera*) is known since the ancient age in our country for its medicinal values. Medicinal values of root, leaves and oil of *Withenia somnifera* in treatment of goiter, insomnia, to improve semen quality and as a hepatoprotective in organophosphate toxicity has been recorded (Gupta *et al.*, 2008). *Withenia somnifera* caused significant increase in blood cell counts, haemoglobin and haemolytic antibody responses towards human erythrocytes (Ziauddin *et al.*, 1996 and Tiwari *et. al.,* 2011). There are little literatures are available for herble remadies in pesticides intoxication. In view of these facts, the present investigation was carried out to assess the effect of simultaneous administration of pesticides and *Withania somnifera* on haematological parameters and to know the ameliorative efficacy of Ashwagandha in pesticide intoxicated cockerels.

MATERIAL AND METHODS

Experimental animals

Eight week old, white leghorn cockerals, procured from Singh hatchery and poultry farm, Faizabad were used in this study. The animals were kept in uniform managemental conditions and offered feed and water *ad libitum* till the completion of the study. All the birds were dewormed once before the study, using fenbendazole at the dose rate of 5 mg/kg body weight

Chemical

Pure salt of endosulfan, chlorpyrifos, deltametnrin and fenvalerate were procured from the Crystal Phosphate Ltd, GI-17, GT Karnal Road (Behind Lalbagh Masjid) Azadpur, Delhi-33. Roots of Ashwagandha (*Withania Somnifera*) were procured from Herbal Garden of Department of Horticulture & Aromatic Plants, N. D. University of Agriculture and Technology, Kumarganj, Faizabad.

Experimental design

Ninety, eight week old male white leghorn, cockerels were randomly divided into 9 groups of 10 birds each. Group I was considered as control and group II to IX were considered as treated groups as in Table 1 and 2. They were fed medicated ration for 24 weeks. Blood samples (4-6 ml) were collected at 12 and 24 week interval from wing vein of cockerels using sterilized disposable syringes in a heparinised test tube. Haematological studies were carried out on the day of collection. Packed cell volume (PCV, %) and haemoglobin (Hb, g/L), total leukocyte count (TLC, x 10^3/cumm) and total erythrocyte count (TECx10^6/cumm) were calculated as per the method described by Jain (1986) by using 0.015% Toludine blue as a diluting fluid. Differential leucocyte count (DLC%) was done by preparing thin blood smear from a drop of blood without anticoagulant. The smear was air dried, fixed in methanol for 2 min and stained with 1:10 diluted Giemsa stain for 30 min (Lucas and Jamroz, 1961) and by standard method. All the values were expressed as mean ± SEM. Statistical analysis was done by one-way and two way analysis of variance test. Complete randomized design was used. Inter group comparisons were made by least significant difference. Statistically significant difference was recorded at 1% and 5% level of significance (Das, 2000).

RESULTS AND DISCUSSION

On haematological examination, there were significant (P <0.01) decrease in TEC, TLC, PCV, Hb and Lymphocytes in pesticides intoxicated cockerel at 12 and 24 weeks interval (Kuttan, 1996) and simultaneously feeding of aswagandha subsides the levels in comparison to control as in Table 1 and 2. Similarly Significantly (P <0.01) increases the hetrophil, monocytes, eosinophil and basophil in pesticides intoxicated cockerel at 12 and 24 weeks interval (Rawat, 2002) and simultaneously feeding of PRWS subsides the levels in comparison to control as in Table 1 and 2. This might have resulted due to the effect of pesticide on haemopoetic system (Kumar *et al.,* 2010). These results are in agreement with the findings reported by (Choudhary and Joshi, 2002) and Withania Somnifera have hemopoitic property repoted by Mishra *et. al.,* 2005.

Increase in haematogram of ashwagandha medicated cockerels and no change in chlorpyrifos + ashwagandha medicated cockerels indicated haematinic action of ashwagandha. An improvement in haematopoiesis characterised by stem cell proliferation

Table 1.

Effect of feeding pesticides and PRWS **at different dietary levels for 12 weeks on haematological parameters in cockerels (n=10).**

Groups	Dietary levels (ppm)	Parameters(Mean± SE; n=10)								
		TEC (10^6/ cumm)	TLC (10^3/ cumm)	PCV (%)	Hb (gm/L)	hetrophil (%)	Lymphocytes (%)	monocytes (%)	eosnophil (%)	Basophil (%)
I-Control	0	3.332± 0.008^{e,A}	13.066± 0.018^{h,A}	38.580± 0.135^{f,A}	139.300± 1.220^{f,A}	44.100± 0.363^{c}	44.365± 0.141^{d}	4.215± 0.054^{a,b}	3.630± 0.066^{a}	1.303± 0.015^{a}
II-Endosulphan	100	3.180± 0.009^{d,B}	11.074± 0.007^{c,A}	36.700± 0.260^{d,B}	131.500± 0.307d,B	44.920± 0.290^{d}	43.070± 0.090^{c}	5.439± 0.043^{e}	7.928± 0.021^{g}	2.214± 0.006^{f}
III-Chlopyriphos	250	3.088± 0.012^{b,c,B}	11.055± 0.005^{c,A}	34.350± 0.150^{c,B}	132.300± 0.472d,A	45.800± 0.335^{e}	40.380± 0.118^{a}	6.250± 0.050^{f}	8.420± 0.029^{h}	2.503± 0.007^{g}
IV-Deltamethrin	100	3.079± 0.006^{b,B}	10.830± 0.021^{b,A}	32.900± 0.481^{b,B}	124.700± 0.366b,c,B	44.283± 0.132^{c,d}	42.320± 0.145^{b}	4.500± 0.033^{c}	7.550± 0.016^{e}	1.911± 0.032^{d}
V-Fenvalerates	250	2.928± 0.025^{a,B}	10.632± 0.032^{a,A}	31.400± 0.221^{a,A}	121.400± 0.305a,B	43.050± 0.320^{a}	43.160± 0.129c	4.530± 0.021^{c}	7.400± 0.021^{d}	2.060± 0.022^{e}
VI-Endosulphan + PRWS	100 + 100	3.215± 0.004^{d,A}	11.959± 0.054^{g,A}	37.800± 0.133^{e,B}	135.200± 0.200e,B	46.750± 0.200^{f}	44.120± 0.05^{d}	5.29± 0.031^{d}	7.670± 0.024^{f}	2.020± 0.013^{e}
VII-Chlorpyriphos + PRWS	250 + 100	3.189± 0.005^{d,A}	11.850± 0.021^{f,A}	34.600± 0.179^{c,B}	134.500± 0.372e,A	44.801± 0.214^{d}	42.06± 0.089^{b}	5.39± 0.027^{e}	8.275± 0.040^{i}	2.210± 0.027^{f}
VIII-Deltamethrin + PRWS	100 + 100	3.190± 0.004^{d,B}	11.226± 0.007^{d,A}	32.600± 0.163^{b,B}	125.900± 0.526c,B	43.351± 0.116^{a,b}	44.03± 0.107^{d}	4.300± 0.014^{b}	7.225± 0.010^{c}	1.760± 0.0124^{c}
IX-Fenvalerates + PRWS	250 + 100	3.123± 0.027^{c,B}	11.249± 0.024^{a,A}	31.00± 0.365^{a,A}	123.500± 0.223b,B	43.910± 0.126^{b,c}	44.200± 0.185^{d}	4.510± 0.016^{c}	7.235± 0.013^{c}	1.720± 0.027^{c}

- Different small alphabets differ significantly (P<0.01) between rows within a column.
- Different capital alphabets differ significantly (P<0.01) between column within a row.

Table 2.
Effect of feeding pesticides and PRWS at different dietary levels for 24 weeks on haematological parameters in cockerels(n=10).

Groups	Dietary levels	Parameters(Mean± SE; n=10)								
		TEC (10^6 cumm)	TLC (10^3 cumm)	PVC (%)	Hb (gl/L)	hetrophil (%)	Lymphocyte (%)	Monocytes (%)	Eosinophil (%)	Basophil (%)
I-Control	0	3.366± 0.014^{e,B}	13.269± 0.019^{h,B}	38.590± 0.162^{g,A}	151.600± 3.088d,B	47.950± 0.089^{h}	42.850± 0.098^{i}	2.97± 0.026^{a}	4.265± 0.014^{a}	1.2± 0.011^{a}
II-Endosulphan	100	3.059± 0.033^{c,A}	9.763± 0.076^{e,B}	34.800± 0.200^{e,A}	122.300± 0.213b,A	45.250± 0.134^{f}	34.550± 0.189^{b}	6.723± 0.010^{g}	10.98± 0.111^{g}	2.47± 0.011^{h}
III-Chlopyriphos	250	3.002± 0.047^{c,A}	9.430± 0.013^{d,B}	31.300± 0.213^{b,A}	132.200± 0.133c,A	44.130± 0.065^{d}	32.730± 0.142^{a}	7.172± 0.023^{h}	11.595± 0.105^{h}	2.790± 0.017^{i}
IV-Deltamethrin	100	2.799± 0.020^{b,A}	8.798± 0.014^{b,B}	30.500± 0.307^{a,A}	121.800± 0.249b,A	42.400± 0.163^{b}	36.140± 0.088^{d}	6.660± 0.030^{g}	9.425± 0.021^{f}	2.130± 0.021^{e}
V-Fenvalerates	250	2.690± 0.025^{a,A}	8.450± 0.016^{a,B}	31.700± 0.152^{b,c,A}	118.300± 0.213a,A	41.250± 0.20^{a}	37.04± 0.063^{f}	6.520± 0.023^{f}	8.950± 0.040^{e}	1.910± 0.031^{e}
VI-Endosulphan + PRWS	100 100	3.250± 0.018^{d,B}	10.819± 0.013^{f,B}	35.400± 0.163^{f,A}	123.700± 0.152b,A	46.07± 0.026^{g}	36.630± 0.074^{e}	6.066± 0.014^{e}	8.435± 0.022^{c}	2.235± 0.019^{f}
VII-Chlorpyriphos + PRWS	250 100	3.218± 0.004^{d,B}	11.091± 0.003^{g,B}	32.100± 0.100^{c,A}	134.00± 0.365^{c,A}	45.130± 0.042^{f}	35.090± 0.105^{c}	5.497± 0.011^{d}	8.76± 0.025^{d}	2.360± 0.017^{g}
VIII-Deltamethrin + PRWS	100 100	3.025± 0.009^{c,A}	9.114± 0.003^{c,B}	31.600± 0.163^{b,c,A}	123.100± 0.276b,A	44.650± 0.130^{e}	38.180± 0.077^{g}	5.225± 0.017^{c}	8.147± 0.027^{b}	1.760± 0.014^{d}
IX-Fenvalerates + PRWS	250 100	2.771± 0.081^{a,b,A}	8.823± 0.027^{b,B}	33.500± 0.220^{d,B}	122.500± 0.166b,A	43.500± 0.210^{c}	39.090± 0.097^{h}	5.185± 0.061^{c}	8.031± 0.016^{b}	1.565± 0.021^{c}
IX-Fenvalerates + PRWS	250 + 100	2.771± 0.081^{a,b,A}	8.823± 0.027^{b,B}	33.500± 0.220^{d,B}	122.500± 0.166^{b,A}	43.500± 0.210^{c}	39.090± 0.097^{h}	5.185± 0.061^{c}	8.031± 0.016^{b}	0.016^{b} 0.021^{c}

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and increased bone marrow cellularity was also reported in mice and rats following ashwagandha medication (Aphale *et al.*, 1998).

The present study was undertaken to know the protective efficacy of PRWS in pesticides intoxicated cockerels. Ninety, 8-week old male white leghorn cockerels were randomly divided into 9 groups of 10 birds each. Group I was considered as control and group II to IX were considered as treated groups. They were fed medicated ration for 24 weeks. Blood samples (4-6 ml) were collected at 12 and 24 week interval from wing vein of cockerels using sterilized disposable syringes in a heparinised test tube and in other test tube without anticoagulant for estimation of different haematological parameters. There were significant (P <0.01) decrease in TEC, TLC, PCV, Hb and Lymphocytes in pesticides intoxicated cockerel at 12 and 24 weeks interval and simultaneously feeding of aswagandha subsides the levels in comparison to control. Similarly Significantly (P <0.01) increases the hetrophil, monocytes, eosinophil and basophil in pesticides intoxicated cockerel at 12 and 24 weeks interval and simultaneously feeding of aswagandha subsides the levels in comparison to control. From the present study it was concluded that ashwagandha have the ameliorating efficacies against the pesticide induced toxicity in cockerels and have haematinic property also.

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