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DETERMINATION OF MINIMUM EFFECTIVE DOSE OF ZINC CHLORIDE FOR MODULATION OF METABOLISM AND SILK PRODUCTION IN THE SILKWORM, *BOMBYX MORI*

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ABSTRACT

The minimum effective dose (MED) of zinc chloride for modulation of metabolism and silk production in *Bombyx mori* is determined by analyzing the changes in the protein profiles of silk gland, haemolymph, fat body and muscle. The step-down method with zero-dose control was employed to achieve the desired objective. The experimental samples included the silkworm larvae, fed with mulberry leaves soaked in different concentrations $ZnCl_2$ solution (300μg, 200μg, 100μg, 50μg, 20μg, 10μg, 5μg, 2μg and 1μg) in distilled water, while the zero-dose control included those fed with normal mulberry leaves. The impact of zinc was analyzed in terms of day-to-day effect (changes on alternative days) and overall effect (fifth instar-end changes) on protein profiles. The dose-response analysis showed that the effect of $ZnCl_2$ on proteins is more pronounced at lower doses (1μg) in the silk gland and haemolymph and at higher doses in fat body (200μg) and muscle (20μg). Keeping in view, the impact of $ZnCl_2$ on silk gland proteins, a minimum dosage @ 1μg /100 worms is suggested for positively modulating the metabolism and silk production in the silkworm.

INTRODUCTION

The role of nutrients, minerals and a variety of exogenous modulators on the growth and metabolism of silkworm and its impact on the economic parameters of sericulture has been well documented. For instance, the modulatory effect of zinc chloride (Hugar *et al.,* 1998), ascorbic acid (Javed and Gondal, 2002), magnesium, phosphorous and calcium (Waseem *et al.,* 2002), nickel chloride (Islam *et al.,* 2004), potassium and magnesium chlorides (Bhattacharya and Kaliwal, 2005), potassium bromide (Kochi and Kaliwal, 2006), folic acid (Rahmathulla *et al.,* 2007), soyabean protein (Raman *et al.*, 2007), thyroxine (Ramakrishna and Bhaskar, 2009) and digoxin (Vitthalrao and Sarwade, 2009), on the silkworm metabolism, immune response and silk production has been extensively studied.

The determination of minimum effective dose (MED) of such exogenous nutrients and minerals that could elicit response greater than the zero- dose control assumes greater significance in such investigations. The dose - response studies are often employed to identify the MED and to obtain useful gains in metabolism and the products thereof (Ruberg, 1989). One of the exogenous modulator that attracted the attention of investigators is the zinc, a micronutrient that bioaccumulates in the tissues of silkworm and its food, the mulberry leaves (Ashfaq *et al.*, 2010). Though the zinc is essential and harmless at low concentrations, it turns out to be toxic at higher concentrations, adversely affecting metabolism, metamorphosis, nucleic acid synthesis, hormonal activity, appetite and neuronal activity often with deleterious consequences (Wright, 1984; Neto *et al.*, 1995). The present investigation was taken up with a view to suggest the minimum effective dose of zinc chloride that could effectively modulate metabolism and silk production and the safe dosage that could be allowed to accumulate in the *Bombyx mori* tissues (silk gland, haemolymph, fat body, and muscle) without causing any adverse effect on its growth and metabolism.

MATERIALS AND METHODS

The minimum effective dose (MED) of zinc chloride was determined by examining its impact on the total protein content in the Pure Mysore x CSR_2 hybrid variety of the silkworm *Bombyx mori,* reared under standard environmental conditions of 28°C, 85 % RH, as per Krishnaswami (1986). After hatching, the worms were reared under normal 12 hr light and 12 hr dark conditions and fed with M_5 variety of mulberry leaves 5 times a day, at 6AM, 10AM, 2PM, 6PM and 10PM. The step down process given by Williams (1971) and modified by Li Jan (2005) with zero-dose control was adopted to determine the MED of zinc chloride. After the third moult, the larvae were divided into control and one to four experimental samples each with 100 larvae. The control sample was given normal feedings 5 times a day as stated above, while the larvae of experimental samples were fed with mulberry leaves soaked in different concentrations (300μg, 200μg, 100μg, 50μg, 20μg, 10μg, 5μg, 2μg, 1μg) of zinc chloride dissolved in 100 mL of distilled water, from fourth instar onwards. Before feeding, the zinc-treated mulberry leaves were dried under cool dry weather in the laboratory and given to the larvae of experimental samples, once in a day (at 6PM), while continuing normal feeding process at other timings. In all, the impact study was carried out on the silkworm larvae reared separately in four different batches at different timings.

The first batch included four experimental samples fed with mulberry leaves soaked in $ZnCl_2$ solutions of 300μg, 200μg, 100μg and 50μg, while the second (20 and 10μg treated) and third (5 and 2μg treated) batches comprised two experimental samples each and the fourth batch one experimental sample (1μg treated). The total protein content of control and experimental samples was assayed as per the methods given by Lowry *et al.,* (1951) in the silk gland, fat body, haemolymph and muscle tissues, isolated by dissecting the silkworm larvae in ice-cold Silkworm Ringer (Yamaoka *et al.,* 1971), on alternative days, *i.e.,* day-1, day-3, day-5 and day-7 of fifth instar.

RESULTS AND DISCUSSION

The minimum effective dose (MED), the lowest dose level with a response greater than that of the zero-dose control is a powerful tool for detecting the existence of a dose-response relationship (Stewart and Ruberg, 2000; Li Jan, 2005). The dose-response relationship between protein levels and different doses of zinc chloride ($ZnCl_2$), observed in four tissues of the silkworm, viz., silk gland, haemolymph, fat body and muscle is presented in Tables1, 2, 3,4 and in the Fig. 1(A, B, C, D). The effect of zinc is analyzed in two ways;

1. Its day-wise impact on protein levels as reflected in the form of deviations from the zero dose control on each day of experimentation.
2. Its overall impact on protein levels of the last day (i.e., day 7) of fifth instar, represented as deviations from the first day zero-dose control.

Effect of $ZnCl_2$ on silk gland proteins

The silk gland is a repository of over 93 proteins (Jin *et al.,* 2004; Zhang *et al.* 2006; Hou *et al.,* 2007a) that primarily includes two silk proteins, fibroin and sericin and a vast array of other proteins such as chaperones, metabolic enzymes, heat shock proteins, immuno proteins, serpins, transport proteins cytoskeleton proteins and those involved in gene expression (Nirmala *et al.,* 2001; Takasu *et al.,* 2005; Kyung *et al.,* 2006). The impact of $ZnCl_2$ on the protein profiles of the

Table 1: Silk gland total proteins in the fifth instar larval of *Bombyx mori* under the influence of different concentrations of zinc chloride. Each value, expressed as mg protein / g wet weight of tissue, is the mean ± standard deviation (SD) of four separate observations. For each observation tissue from 10 to 15 larvae was pooled. The percent change (PC) from the control value, is calculated day-wise and batch-wise separately

Days		Batch - I					Batch - II			Batch - III			Batch-IV	
		Control	300μg	200μg	100μg	50μg	Control	20μg	10μg	Control	5μg	2μg	Control	1μg
	Mean	51.3	44.8	52	77.2	74.7	34.3	17.8	20.6	51.5	96.3	110	34.6	34.3
DAY-1	P.C	-	-12.7	1.4	50.5	45.6	-	-48.1	-39.9	-	87	113.6	-	-0.9
	S.D	±5.5	±8.4**	±8.2**	±7.2*	±4.2*	±3.9	±2.0*	±1.9*	±2.5	±3.4*	±3.0*	±1.3	±2.5**
	Mean	56.3	68.5	54.4	84.2	74.8	55.8	50.1	34.3	98.5	77	65.8	76.5	80.5
DAY-3	P.C	-	21.7	-3.4	49.6	32.9	-	-10.2	-38.5	-	-21.8	-33.2	-	5.2
	S.D	±3.2**	±16.6**	±2.9**	±12.9*	±4.4*	±3.9*	±4.0**	±3.5*	±2.4*	±2.5*	±3.2*	±3.7*	±1.6**
	Mean	118	78	108	130	108	88.4	59.4	81.8	140	181	102	85.4	84.8
DAY-5	P.C	-	-33.9	-8.5	10.2	-8.5	-	-32.8	-7.5	-	29.3	-27.1	-	-0.7
	S.D	±2.4*	±8.7*	±7.6*	±11.5**	±7.9**	±2.1*	±0.5*	±5.5**	±1.3*	±1.6*	±1.7*	±1.5*	±2.6**
	Mean	286	82.3	150	167	231	96.4	69.1	93	245	267	258	144	182
DAY-7	P.C	-	-71.2	-47.6	-41.6	-19.2	-	-28.3	-3.5	-	40.5	35.8	-	26.4
	S.D	±5.7*	±6.3*	±19.3*	±3.7*	±11.8*	±6.5**	±4.2*	±2.4**	±4.22*	±5.3*	±5.7*	±6.2*	±6.0*

* Statistically significant: ** statistically not significant:

Table 2: Haemolymph total proteins in the fifth instar larval of *Bombyx mori* under the influence of different concentrations of zinc chloride. Each value, expressed as mg protein / g wet weight of tissue, is the mean ± standard deviation (SD) of four separate observations. For each observation tissue from 10 to 15 larvae was pooled. The percent change (PC) from the control value, is calculated day-wise and batch-wise separately

Days		Batch - I					Batch - II			Batch - III			Batch-IV	
		Control	300μg	200μg	100μg	50μg	Control	20μg	10μg	Control	5μg	2μg	Control	1μg
	Mean	**12.1**	**15.7**	**15.6**	**10.6**	**16.7**	**7.9**	**7.1**	**6.4**	**11.4**	**14**	**16.4**	**7.4**	**5**
DAY-1	P.C	-	29.8	28.9	-12.4	38	-	-9.7	-18.5	-	22.8	43.9	-	-32.5
	S.D	±0.7	±0.4*	±0.3*	±0.7*	±0.5*	±0.3	±0.06*	±0.9*	±0.08	±5.0**	±0.06*	±0.4	±0.2*
	Mean	**11.7**	**14.7**	**18.9**	**14.7**	**10**	**16**	**14.3**	**15.3**	**18.6**	**13.3**	**12.9**	**9.3**	**9.9**
DAY-3	P.C	-	25.6	61.5	25.6	-14.5	-	-10.6	-4.4	-	-28.5	-30.7	-	5.9
	S.D	±0.4**	±0.4*	±0.5*	±0.8*	±0.6*	±0.1*	±0.7*	±0.2*	±0.4*	±0.08*	±0.3*	±0.6*	±0.3**
	Mean	**19.5**	**24.3**	**20.6**	**25.3**	**25.2**	**21.7**	**17**	**20.9**	**22.6**	**10.9**	**14.4**	**10.6**	**10.7**
DAY-5	P.C	-	24.6	5.6	29.7	29.2	-	-21.7	-3.7	-	-51.8	-36.3	-	0.9
	S.D	±1.5*	±0.7*	±0.5**	±0.6*	±0.6*	±0.2*	±0.4*	±0.1*	±0.5*	±0.2*	±0.2*	±0.2*	±0.7**
	Mean	**28.8**	**26.9**	**22.7**	**21.4**	**23**	**27.7**	**19.6**	**23.3**	**24.8**	**20.1**	**21**	**16.5**	**19.6**
DAY-7	P.C	-	-6.6	-21.2	-25.7	-20.1	-	-29.2	-15.9	-	-19	-15.3	-	18.8
	S.D	±0.4*	±0.6*	±0.5*	±0.6*	±0.6*	±0.8*	±0.6*	±0.4*	±0.3*	±0.5*	±0.04*	±1.1*	±0.2*

* Statistically significant: ** statistically not significant:

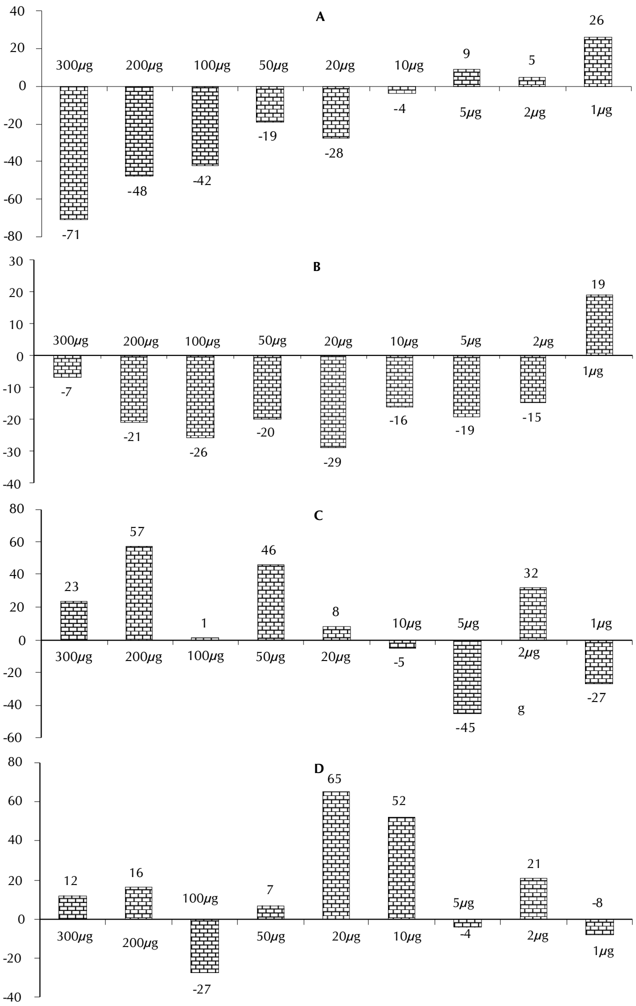

Figure 1: The overall impact of $ZnCl_2$ on the total protein profiles of *B. mori*, represented as per cent change from the first day zero-dose control of the fifth instar larva. A. Silk gland: B. Haemolymph: C. Fatbody: D. Muscle

The protein values, expressed as mg protein / g wet weight of tissue or 1 mL of haemolymph represent the mean ± S.D of four separate observations (p values: < 0.001). Source: Tables 1 to 4.

silk gland is largely negative at higher doses and positive at lower doses (Table 1 and Fig. 1A). The day-wise trends in protein levels showed a mixed response to different doses of $ZnCl_2$ with characteristic falls and elevations. The inhibitory effect of $ZnCl_2$ ranged from ~13 to 71% at 300µg dosage, ~3 to 48% at 200µg, ~42% at 100µg, 9 to 19% at 50µg, ~10 to 48% at 20µg, ~4 to 40% at 10µg, 22% at 5µg dosage. The positive impact of $ZnCl_2$ started at a dose of 2µg, wherein the protein levels were elevated by ~114% on day 1 and ~36% on day 7 of fifth instar (Table 1). Further, the overall increase in the protein levels by the end of fifth instar is also not encouraging at higher concentrations of $ZnCl_2$. On the 7th day of fifth instar, the silk gland proteins declined from the zero-dose control by ~71% at 300µg, ~48% at 200µg, ~42% at 100µg, ~19% at 50µg, ~28% at 20µg and by 4% at 10µg dosages. Though, the positive impact of $ZnCl_2$ was reflected at dose of 5µg (+9% rise) and 2µg (+5%) dosages, maximal effect (~26% rise) was recorded at 1µg dosage (Fig. 1A). Evidently, a dose of 1µg of $ZnCl_2$ or even a lesser dose seems to favour silk gland growth by stimulating protein synthesis during fifth instar development. Obviously, the silk production in *B. mori* could be modulated profitably for the sericultural industry by feeding the larvae with the mulberry leaves dipped in $ZnCl_2$ at dosages lower than 1µg / 100mL / 100 worms. Similarly, a dosage of about 1 to 5µg / 100mL / 100 worms seems to be tolerable range of $ZnCl_2$ that could be safely accumulated in the silk gland of *B. mori*.

Effect of $ZnCl_2$ on haemolymph proteins

The haemolymph of *B. mori* is the chief circulating fluid and flowing reservoir of about 241 to 298 proteins (Lix *et al.,* 2006) that promote larval growth, metamorphosis, silk production, apoptosis, chitin and haemocyte formation, growth of salivary glands and reproductive organs, ecdysis (Chai *et al.*, 2008; Nakahara *et al.,* 2009; Naga Jyothi *et al.*, 2010). Though, the response of haemolymph proteins to $ZnCl_2$ is fluctuating at higher doses (50 to 300µg), it is significantly positive at 10 and 1µg dosages (Table 2). Notwithstanding occasional elevations in the levels of total proteins, the day- wise effect of $ZnCl_2$ presented in Table 2, shows ~7% inhibition at 300µg dosage, ~21% at 200µg, ~12 to 26% at 100µg, ~15 to 20% at

Table 3: Fat body total proteins in the fifth instar larval of *Bombyx mori* under the influence of different concentrations of zinc chloride. Each value, expressed as mg protein / g wet weight of tissue, is the mean ± standard deviation (SD) of four separate observations. For each observation tissue from 10 to 15 larvae was pooled. The percent change (PC) from the control value, is calculated day-wise and batch-wise separately

Days		Batch - I					Batch - II			Batch - III		Batch - IV		
		Control	300µg	200µg	100µg	50µg	Control	20µg	10µg	Control	5µg	2µg	Control	1µg
	Mean	58.1	78.7	75.6	76.9	67.8	37.5	37.5	42.4	50.8	49.5	46.9	26.6	35.2
DAY-1	P.C	-	35.5	30.1	-40.4	16.7	-	0	13.1	-	-2.6	-7.7	-	32.3
	S.D	±1.4	±2.5*	±3.1*	±3.1*	±3.7*	±4.2	±4.4**	±0.1**	±2.9	±1.3**	±3.1 **	±1.7	±2.4*
	Mean	76.8	57.7	45.2	71.5	82	46.3	65.6	58.1	52.7	98.9	77.8	55.6	50.8
DAY-3	P.C	-	-25.3	-41.2	-6.9	6.8	-	41.7	25.5	-	87.7	47.6	-	-8.6
	S.D	±2.5*	±2.9*	±1.3*	±3.2*	±4.1**	±1.5*	±2.7*	±2.3*	±1.9**	±4.9*	±2.2*	±1.5*	±2.2*
	Mean	97.4	82.4	120	102	118	51.6	48.5	40.3	66.7	40.6	51.2	56	53.4
DAY-5	P.C	-	-15.4	23.2	4.7	21.2	-	-6	-21.9	-	-39.1	-23.2	-	-4.6
	S.D	±4.6*	±4.3*	±3.6*	±3.3**	±3.4*	±2.1*	±2.8**	±3.9*	±4.0*	±0.5*	±3.01*	±2.8*	±1.3**
	Mean	54.1	66.3	84.9	54.4	78.9	37	40	35.3	38.2	21	50.6	26.1	19.1
DAY-7	P.C	-	22.6	56.9	0.6	45.8	-	8.1	-4.6	-	-45	32.5	-	-26.8
	S.D	±1.2*	±5.2*	±4.7*	±1.1**	±2.3*	±4.6*	±4.5**	±1.0**	±4.6*	±1.1*	±2.1*	±3.3*	±2.7*

* Statistically significant: ** statistically not significant

Table 4: Muscle total proteins in the fifth instar larval of *Bombyx mori* under the influence of different concentrations of zinc chloride. Each value, expressed as mg protein / g wet weight of tissue, is the mean ± standard deviation (SD) of four separate observations. For each observation tissue from 10 to 15 larvae was pooled. The percent change (PC) from the control value, is calculated day-wise and batch-wise separately

Days		Batch - I					Batch - II			Batch - III			Batch-IV	
		Control	300µg	200µg	100µg	50µg	Control	20µg	10µg	Control	5µg	2µg	Control	1µg
	Mean	52.3	49.1	60.6	61.3	53.5	36.1	35.3	25.6	52.6	95	87.9	37.7	24.3
DAY-1	P.C	-	-6.1	15.9	17.2	2.3	-	-2.2	-29.1	-	80.6	67.1	-	-35.5
	S.D	±3.1	±4.1**	±3.5*	±4.8*	±2.2**	±3.1	±2.7**	±0.4*	±1.6	±2.5*	±3.6*	±4.1	±2.0*
	Mean	90.7	89.6	80.1	65.6	59.2	71.1	56.9	67.6	88.9	67.4	68.5	61.6	55.3
DAY-3	P.C	-	-1.2	-11.7	-27.7	34.7	-	-20	-4.9	-	-24.2	-23	-	-10.2
	S.D	±5.2*	±3.2**	±2.5*	±4.1*	±5.0*	±3.5*	±0.7*	±1.5**	±2.9*	±2.8*	±3.6*	±2.2*	±1.6*
	Mean	131	132	120	132	121	53.5	62.6	70	75.2	49.4	69	62.6	48.7
DAY-5	P.C	-	0.8	-8.4	0.8	-7.6	-	17	30.8	-	-34.3	-8.2	-	-22.2
	S.D	±3.5*	±8.9**	±5.2*	±15.2**	±9.7**	±2.6*	±2.0*	±2.8*	±1.2*	±0.9*	±2.8*	±0.9*	±3.2*
	Mean	106	119	123	77.3	113	25.6	42.3	38.8	53.9	51.6	65.4	34.5	31.7
DAY-7	P.C	-	12.3	16	-27.1	6.6	-	65.2	51.6	-	-4	21	-	-8.1
	S.D	±3.7*	±11.7**	±5.8*	±7.6*	±6.5**	±3.4*	±2.1*	±2.2*	±2.3*	±3.7**	±1.5*	±2.8*	±1.7**

* Statistically significant: ** statistically not significant

50µg, ~10 to 29% at 20µg, ~4 to 18% at 10µg, 19 to 52% at 5µg and ~15 to 22% at 2µg dosage. Significantly, the actual positive impact of $ZnCl_2$ on protein synthesis (~1 to 19% increase) was recorded at 10 and 1µg dosages (Table 2). The overall impact of $ZnCl_2$ on haemolymph proteins is almost similar to that of silk gland, being negative at higher doses and positive at lower doses (Fig. 1 B). The protein levels declined by ~7% at 300 µg dosage, ~21 % at 200 µg, 26% at 100 µg, ~20 % at 50 µg ~29 % at 20 µg, ~16 % at 10 µg, ~19 % at 2 µg concentration. But the response is positive at 1 µg dosage, wherein the protein levels recorded an increase of about 19% from the zero-dose control (Fig. 1B). Obviously, $ZnCl_2$ could be comfortably maintained at this lower dosage in the circulating medium of haemolymph.

Effect of zinc chloride on fat body proteins

The insect fat body plays a metabolic role similar to that of the mammalian liver and adipose tissues (Scott *et al*., 2004). In *B. mori* it synthesizes and stores over 177 proteins that constitute nine glycolysis related proteins, several metabolically related proteins like diacylglycerol binding protein, triacylglycerol lipase, putative hydrolases, cytoskeleton proteins and defence proteins, heat shock proteins (Hou *et al*., 2007b). The impact of $ZnCl_2$ on fat body protein profiles is opposite to that of silk gland and haemolymph. Barring a few exceptions, the total protein content of the fat body recorded an elevation under the influence of zinc chloride at higher doses (300µg, 200µg , 50µg) compared to lower doses examined (Table 3). The elevations ranged from ~23 to 36% under 300µg, ~23 to 57% at 200µg, ~7 to 46% at 50µg, 8 to 42 % at 20µg and 13 to 25% at 10µg dosage. From 5µg dosage downwards, the impact of $ZnCl_2$ is negative with inhibitions ranging from 3 to 45% at 5µg, 8 to 23% at 2µg and 5 to 27% at 1µg dosage (Table 3). The overall impact of $ZnCl_2$ on fifth instar-end protein profiles is relatively positive at higher doses and negative at lower doses (Fig. 1C). Their levels recorded an increase of ~23 % at 300µg, 57 % at 200µg, 46 % at 50µg, 8 % under 20µg and 32 % at 2µg dosage from the zero-dose control (Fig. 1C). But conversely, the protein levels were declined at 10µg (~5 %) 5µg (~45%) and at 1µg (~27%) dosages of $ZnCl_2$. Evidently, the response of protein profiles of the fat body were declined at lower dosages, but increased at higher doses (50 to 300µg) showing maximum response at 200µg of $ZnCl_2$. Obviously, $ZnCl_2$ at a concentration of about 200µg / 100mL / 100 worms seems to have a positive impact on protein synthesis in the fat body and at this dosage it could be conformably stored in this tissue.

Effect of zinc chloride on muscle total proteins

The silkworm muscle is known to contain many metabolically important proteins that are transported to it from other tissues during metamorphosis and includes those of the cytoskeleton, muscle contraction, feeding, locomotion, respiration and cocoon spinning (Sivaprasad and Muralimohan, 2009a, 2009b; Naga Jyothi *et al*., 2010) apart from those involved in oxidative metabolism and ATP production (David and Ananthakrishnan, 2006). The impact of $ZnCl_2$ on muscle proteins is by and large similar to that of the fat body, with higher doses showing positive impact and lower doses eliciting a negative response (Table 4). The day-wise trends in protein profiles indicated that $ZnCl_2$, in general caused an elevation from the zero-dose control at higher dosages and reduction at lower dosages. At the dose of 300µg, the elevation in protein levels ranged from ~ 1 to 12%, while it is about 15% at 200µg, ~1 to 17% at 100µg, ~6 to 35% at 50µg, ~17 to 65% at 20µg and ~31 to 52% at 10µg dosage. From 5µg dosage downwards, the impact of $ZnCl_2$ is negative with inhibitions ranging from ~24 to 90% at 5µg, ~8 to 88% at 2µg and ~8 to 36% at 1µg dosage (Table 4). The overall impact of $ZnCl_2$ on fifth instar-end proteins is positive and it is reflected in the form of significant elevation in their levels at dosages of 20µg (+65 %) and 10µg (+52 %), while it is less evident at 300µg (+12 %), 200µg (+16 %), 50µg (+7 %) and 2µg (21 %) dosages (Fig. 1D). The protein levels however, showed a declining trend at all other dosages of zinc (100µg, 5µg and 1µg) exposed. Keeping in view the higher protein levels observed at 20 and 10 µg dosages of zinc chloride, it is suggested that these dosages are effective against the muscle tissue and the metabolism in this tissue could be positively modulated at these dosages. Further, it is also evident that the muscle tissue could tolerate higher dosages of $ZnCl_2$ (10 to 300µg) without any adverse affect on its metabolism.

Our study demonstrates that $ZnCl_2$ is a potent modulator of metabolism and silk production in *B. mori*. The modulatory role of $ZnCl_2$ varies as a function of its dose and the type of tissue exposed to it. For instance, the silk gland and haemolymph are sensitive to $ZnCl_2$ at 1μg dosage, while the fatbody and muscle could tolerate higher doses 200μg and 20μg respectively. Our findings are in consistent with similar dose - response studies made on *B. mori*, in which maximal response in respect of the larval growth was elicited at low concentrations (0.1 to 0.3%) and that of biochemical constituents such as the haemolymph proteins, lipids, trehalose at higher concentrations (50 to 150μg) of zinc and other mineral nutrients (Rashid *et al.,* 2001; Javed and Gondal, 2002; Miranda *et al.,*2002; Bhattacharya and Kaliwal, 2005; Rahmathulla *et al.,* 2007; Vittalrao and Sarwade, 2009).

From the sericultural point of view the silk protein synthesis and metabolism in the silk gland could be enhanced at lower doses of $ZnCl_2$, but higher doses are required to elicit similar response in fat body and muscle. Despite minor differences in doses and responses thereof, and in view of the fact that the silk gland is the prime site of silk protein synthesis and contains most of the proteins found in the haemolymph and fat body (Kyung *et al*., 2006; Lix *et al.,* 2006; Hou *et al.,* 2007a, 2007b), it is suggested that the mulberry leaves dipped in 1μg of $ZnCl_2$ @ 100 mL / 100 worms may be fed to the silkworm larvae at least once in a day during both the fourth and fifth instar larval stages for improvements in larval growth, metabolism and the economic traits of cocoon and for realizing the desirable profits from the sericulture industry. Since zinc is toxic at higher concentrations, more practical trials are required before embarking on its large scale utilization in this field.

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