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BIOCHEMICAL STUDIES OF PROTEIN CONTENT IN THE HAEMOLYMPH, FAT BODY AND SILK GLAND OF IV AND V INSTARS OF *BOMBYX MORI* LARVAE FED WITH CONTROL AND *MORUS INDICA* COPPER NANOPARTICLES (MICUNPS) TREATED V-1 MULBERRY LEAVES

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ABSTRACT
Silk, which has been utilized widely as an excellent textile fiber, has also been used as a good material for research, as a typical protein. Numerous studies on silk, carried out continuously over a period of more than one century, have contributed not only to the improvement of production process and quality of raw silk and silk products that has supported the development of sericulture and silk industry, but also to the progress of research on protein and fiber. The present study was carried out to analyze the variations in the biochemical constituents of protein in different body tissues viz., haemolymph, silk gland and fat body in the healthy and bacterial infect fourth and fifth instar larvae of the *Bombyx mori* silkworm. The feed efficacy of mulberry leaves such as V-1 and leaves treated with Copper nanoparticles on the protein contents in the haemolymph, fat body and silk gland of silkworm, *Bombyx mori* are fragmentary. The *Bombyx mori* larvae fed with different concentrations (25%, 50%, 75% and 100%) of Copper nanoparticles treated V-1 Mulberry leaves. The 25% is effective's dose of silk worm. Hence, it has been programmed in the present study to find out the feed efficacy of mulberry leaf variety (V-1) and leaves treated with Copper nanoparticles on protein content in the silkworm *Bombyx mori*. It may be concluded that V-1 leaf variety treated with copper nanoparticles elicits some favourable responses in silkworms with regard to the commercial improvement as evidenced from the results of the present study.

***Keywords**: Bombyx mori, Copper Nanoparticles, V-1 Leaf Variety, Haemolymph, Silk Gland, Copper Nanoparticles, Protein Content*

INTRODUCTION
Generally silkworm *Bombyx mori* L. is monophagous in character and feeds solely on mulberry leaves. Silkworm larva obtains different amino acids from the mulberry leaves and uses them to synthesize silk proteins secreted during spinning. Proteins play an important physiological role in growth and development of silkworm and silk proteins synthesis. Necessary amino acids are derived from the amino acids present in body fluid in a free state and from the amino acids formed in the posterior silk gland cells. Silk worm requires all the ten essential amino acids for growth and development (Ito, 1978).
The silkworm larvae feed only on mulberry leaves; all the amino acids required for fibroin and sericin and body proteins of silkworm are derived from mulberry leaf proteins. The most significant components of the leaf, which determines the quality of the nutrition supplied to the silkworm, its protein content. Proteins are the building blocks for cellular and sub-cellular structures. They are the important organic constituents of the animal's body cells, which provide the chief structural elements of the glands and other tissues. The protein content tends to decrease as the leaf become old. The leaf protein was also found to be indispensable to the development of the silk gland (Quader, 1987).

Silk is made up of two proteins such as fibroin and sericin. Fibroin forms the core and is surrounded by sericin. These two proteins differ in their characteristics and secreted from different parts of silk gland. Fibroin is secreted from the posterior part and sericin is secreted from middle part of silk gland. Fibroin is formed from the amino acids of posterior silk gland cells. Sericin quality is one of the important features of cocoon. Sericin is classified in various ways, but generally as α-sericin and β-sericin. α-sericin being presents in the inner layer of cocoon and differs from β-asericin present in the outer layer. Amount of sericin in cocoon varies in different strains of *Bombyx mori* L. By knowing the economic importance and convenience of study, silkworm has almost become an important tool for several biochemical, physiological and genetic studies in insects. Physiological and biochemical studies include general metabolism and morphogenesis in insects, digestion and digestive enzyme, protein synthesis and their metabolism, hormones and their mechanisms of action, structure and function of chromosomes etc., for better productivity. Major biomolecules such as carbohydrates, lipids, proteins, hormones and chromosomes etc., play an important role in biochemical process underlying growth and development of insects (Wyatt, 1967). Mulberry silkworms produce the precious material in the world. Silkworm, *Bombyx mori* L., is monophagase insects (Tazima Y, 1978). The growth and development of silkworm are dependent on the mulberry leaves components. Mulberry leaf quality is dependent on the variety, agronomic practices, maturity, position of the leaf, and season. Feeding with good quality mulberry leaves that meet the requirements of silkworm larvae throughout the growing period results in high silk productivity (FAO, 1990). A mulberry field with fertile soil, high planting density, luxuriantly growing branches and leaves with high productivity requires greater quantities of manure and fertilizers (Ting-Xing Z *et al.*, 1980). The quality of mulberry leaves alone contributes to the tune of 38.2 % (Miyashita V, 1986). Silk production affected by the quality of mulberry leaves. Many researchers over all the world attempt to increase the quality of mulberry leaves through food additives or using different kinds of fertilizers (Shruti *et al.*, 2019). Silkworms as all insects need macro and micronutrients. As well as larvae needed appreciable amounts of potassium, phosphate and magnesium (Muniandy S *et al.*, 2001). Larval nutrition and nutritive value of mulberry leaves is very essential for silk production which producing good quality cocoons (Hanson B *et al.*, 2004). Many of the heamolymph proteins are enzymes. It is play important role in the economy of insects (Laufer H, 1960). Many experiments are to improve the quality of mulberry leaves. Find best solution for traditional rearers who depended on spread trees over channels and road sides. Also, when the lack of common fertilizers (Tahia A Fouad and Ghada M Ahmed, 2020).

In our laboratories, we observed that some silkworm larvae cannot spin their cocoons and complete their life cycle. Our prefeeding trials conducted with the leaves obtained from different regions showed that larvae could not spin their cocoons based on the source of mulberry leaves used for feeding. Given that the metabolite composition of silkworm hemolymph is highly influenced by nutrient composition of the mulberry leaves, it seems likely that mulberry leaves used to feed silkworms probably caused this problem (Sahan *et al.*, 2020). Silkworms depend on the biomolecules viz., protein, carbohydrate, lipid etc. for the growth and development. During the larval feeding stages, energy reserves are accumulated in the fat body, later to be utilized for their growth and sequence of metamorphic events as well as to provide energy for the developing adult moth to drive copulation and egg laying (Sivaprasad and Bhuvaneswari, 2018).

In the life cycle of silkworm, the fifth larval instar is considered as the silk synthesizing phase, where the silk gland grows tremendously and the insects prepare its body with the reserved metabolic compounds for its future metamorphic events. The amount of nutrients stored in the form of biochemical reserve during the larval stages accommodates stage specific morphogenetic changes as well as pupal and adult metamorphic events due to its non-feeding nature. The health status of the larva determines the spinning behaviour, size of the cocoon, survivability of pupa and moth. If the larvae are exposed to unfavourable condition, it results in non-spinning or partial spinning and even if spun, the cocoon size will be smaller. In such conditions, silkworms adopt for survival rather spinning cocoon by depending on accumulated reserves, such as carbohydrates and amino acids. Hence, the present study enlightens the comparative

analysis of the alteration of a few biochemical events associated with the energy metabolism of healthy spinning and unspun larvae (E. Bhuvaneswari, *et al.*, 2022). Silkworm eats only mulberry leaves to make its cocoon, producing the silk. Mulberry leaves are rich in proteins and amino acids; therefore, an increase in proteins level of mulberry leaves may lead to improvements in silk productivity (Eman M. Hassan, *et al.*, 2014). The production of high quality and quantity of natural silk depends mainly on larval feeding, suitable environmental conditions and protection from diseases (Dechu *et al.*, 1997). The nutritional value of the mulberry leaves can be improved by enriching them with extra nutrients like glucose, glycine, egg albumin, molasses, etc., was found to increase the larval growth and improve cocoon characteristics (Sengupta *et al.*, 1992).

Metabolism and accumulation of these biomolecules in insect tissues during their development in different stages of life cycle was studied by many workers (Bhattacharya and Kaliwal, 2004). The concentrations of these biomolecules mainly depend on mulberry leaf quality. Proteins in haemolymph are at higher concentration during development and are useful in silk proteins synthesis. Keeping this in view, in the experiment an attempt has been made to study the proteins level during silkworm larval development in different silkworm races (V.N.Yogananda Murthy, 2015). The natural cocoon spinning process in silkworms involves the conformation transition of the liquid silk protein in the silk gland to a fiber. Silk quality not only depends on the breed but also on the physio-biochemical status of the silkworm during the spinning stage and care taken by the farmers. The factors, such as nutrition, temperature, light and hygienic conditions maintained in the silkworm rearing house play a crucial role in the cocoon spinning process (Rahmathula, 2012).

The seasonal differences in the environmental components considerably affect the genotypic expression in the form of phenotypic output of silkworm crop, such as cocoon and shell weight and cocoon shell percentage. The quality of the cocoon determinesthe quality of the fiber and fabric. Greater the quality of silk produced, higher the grade. In insects, the inbuilt physio-biochemical mechanisms cope with the outer environmental factors and thus enable them to withstand the adverse conditions while completing its life cycle (Sinclair *et al.*, 2003). Silkworms depend on the biomolecules viz., protein, carbohydrate, lipid etc. for the growth and development. During the larval feeding stages, energy reserves are accumulated in the fat body, later to be utilized for their growth and sequence of metamorphic events as well as to provide energy for the developing adult moth to drive copulation and egg laying (Sivaprasad and Bhuvaneswari, 2018). Silkworm larvae converts mulberry leaf proteins into its constituent amino acids on one hand and utilizes these amino acids for its growth and synthesis of fibroin and sericin proteins on the other. So, silkworm larvae feed on mulberry leaves for three purposes,

1) Development of body
2) Synthesis of fibroin protein and
3) Synthesis of sericin protein

It has been reported that 75% of mulberry leaf protein is directly converted into silk protein and the rest 25% of silk protein comes from body tissue of silkworm larva and are collected in silk glands (Gupta and Gupta, 1998 and Centhilnayaki, 2004). Insects in addition to sugar and lipids, use free amino acids as the readily available source of respiratory fuels (Candy and Kilby, 1975). From the above cited literature, the works in relation to feed efficacy of mulberry leaves such as V-1 and leaves treated with Copper nanoparticles on the protein contents in the haemolymph, fat body and silk gland of silkworm, *Bombyx mori* are fragmentary. Hence, it has been programmed in the present study to find out the feed efficacy of mulberry leaf variety (V-1) and leaves treated with Copper nanoparticles on protein and amino acid content in the silkworm *Bombyx mori*.

MATERIALS AND METHODS

Silkworm V instar of popular Indian bivoltine hybrid (CSR_2 x CSR_4) silkworm *Bombyx mori* (Local Bivoltine) race were collected from silkworm culture centre at Vatharayanthethu, Melbhuvanagiri,

Cuddalore distict in Tamilnadu, and they were maintained up to cocoon. The larvae transported from Vatharayanthethu, Melbhuvanagiri, were transferred to bamboo baskets of size 26 cm diameter and 5 cm height as described by Govindan *et al.*, (1981). The bamboo baskets were covered with paraffin paper and placed in an iron stand with ant wells. The larvae were reared simultaneously both in control and experimental groups separately on mulberry leaves dipped in copper nanoparticles solution in the laboratory. The V instar larvae placed at ambient temperature of 25+ 27℃ and relative humidity of 70 to 80%. The larvae were reared in card board boxes measuring 22×15×5 cms covered with nylon net and placed in an iron stand with ant wells. The control and copper nanoparticles treated V-1 mulberry (*Morus indica)* leaves were fed to silkworm, *Bombyx mori.*

Preparation of Morus indica leaf extract

Fresh leaves of *Morus indica* were collected from faculty of Agriculture, Vatharanthethu, Melbhuvanagiri, Tamilnadu, India. Thoroughly cleaned *Morus indica* leaves were then dried in the sun for 5-7 days. Fine powder was made by grinding dried leaves. 30 gm of leaf powder and 300 ml of distilled water were refluxed in a soxhlet device at 100°C for five hours to create the aqueous extract. The extracts were then gathered and stored for later use in a deep freeze in an airtight bottle (G.Valli and S.Geetha, 2016).

Green synthesis of Copper nanoparticles from Morus indica leaf extract

A 40 ml aqueous solution of 10 mM Copper sulphate was combined with 10 ml of *Morus indica* leaf extract for reduction into Cu^{+} ions, which was then stored at room temperature for incubation (in the dark). For 10 mM of $CuSo_4$, the filtrate here serves as a reducing and stabilize agent. Throughout the studies, appropriate controls (40 ml of distilled water plus 10 ml of plant extract in a different test tube) were kept. The extract's color changed from bright yellow to dark brown after the reduction of copper sulphate to copper ions. Additionally, a control setup was kept in place without copper sulphate being added to the plant extract. Further research was done on the creation of copper nanoparticles (G.Valli and S.Geetha, 2016).

Experimental group

The *Bombyx mori* larvae were divided into four experimental groups as shown in **Table 1**, those are (i) Control, (ii) Treated, (iii) Disease Control and (iv) Disease Treated.

Table. 1. Experimental groups of *Bombyx mori* larvae

Group 1	Control	*Bombyx mori* larvae fed with normal V-1 Mulberry leaves.
Group 2	Treated Subgroups (T_1, T_2, T_3 &T_4)	*Bombyx mori* larvae fed with different concentrations (25%, 50%, 75% and 100%) of Copper nanoparticles treated V-1 Mulberry leaves (Select the effectives dose).
Group 3	Diseased Control	Bacterial infected *Bombyx mori* larvae fed with control V-1 Mulberry leaves.
Group 4	Diseased Treated	Bacterial infected *Bombyx mori* larvae fed with MiCuNps (effective dose) treated V-1 Mulberry leaves

Each groups contains 50 larvae except group 2 (200 larvae) fed with V-1 Mulberry (*Morus indica*) leaves, five feedings / per day.

Estimation of protein

The protein contents in the fat body, haemolymph and silk gland were determined by adopting the procedure of Lowry *et al*., (1951). For the estimation, 20 mg of tissue and 10 µl of haemolymph were used. The tissues were, transferred to 1 ml of 10 per cent deproteinizing agent, trichloro acetic acid (TCA) and homogenized. The solution was centrifuged at 3000 rpm for 15 minutes. The supernatant was removed and the residue was dissolved in 1 ml of in NaOH. To 0.5 ml of extract 4 ml of reagent D (carbonate copper solution) and 0.4 ml of Folin′s phenol reagent was added and the tubes were gently shacked and were kept at laboratory temperature (29° C + 1° C) for 30 minutes. The transparent blue colour developed was read at 600 nm against a reagent blank in Grating Linear Readout Spectrophotometer (CECIL, Model CE373). The

standard graph was constructed using Bovine serum albumin. The amount of protein present in the tissue was expressed in µg / mg wet weight of tissue.

RESULTS

(Quantitative analysis of protein (control and MiCuNPS (25%) treated groups)

The protein content of haemolymph, fat body, and silk gland of IV and V instar larvae fed with control V-1 mulberry leaves and MiCuNps (25%) treated V-1 mulberry leaves are presented in **Table 2**.

The protein content of haemolymph was found to be increased steadily from 0.236 ± 0.0066 µl/ml to 0.582 ± 0.0047 µl/ml in IV and V instar larvae fed with control V-1 mulberry leaves. Similarly, protein content of haemolymph also found to be steadily increased in IV and V instar larvae fed with MiCuNps (25%) treated V-1 mulberry leaves such as 0.556 ± 0.0032 µl/ml and 0.988 ± 0.0066 µl/ml, respectively. In these two observations, MiCuNps (25%) treated IV and V instar larvae protein content of haemolymph was highly significant at 1% level (P<0.01) than control. Significant was indicated by the t-value **(Table 2a)** such as 34.577 and 218.057 of IV and V instar larvae (control and MiCuNps (25%)), respectively.

Table 2. Quantitative analysis of protein content in the haemolymph, fat body and silk gland of IV and V instars of *Bombyx mori* larvae fed with control and MiCuNps treated V-1 mulberry leaves

Larval stage	Groups	Haemolymph (µl/ml)	Fat body (µg/mg)	ASG (µg/mg)	MSG (µg/mg)	PSG (µg/mg)
IV instar	**Control**	0.236± 0.0066	0.184± 0.0049	0.206± 0.0038	0.373± 0.0081	0.265±0. 0052
	MiCuNps (25%)	0.556±0.0032	0.253±0.0065	0.254±0.0 041	0.844±0.0 053	0.451±0. 0046
V instar	**Control**	0.582±0.0047	0.289±0.0044	0.234±0.0 058	0.879±0.0 055	0.736±0. 0059
	MiCuNps (25%)	0.988±0.0066	0.490±0.0078	0.398±0.0 062	1.552±0.0 086	1.322±0. 0075

Values are Mean ± Standard Deviation.

Table 2a. 't' - test

Larval stage	Haemolymph (µl/ml)	Fat body (µg/mg)	ASG(µg/mg)	MSG (µg/mg)	PSG (µg/mg)
IV instar	34.577**	16.028**	185.903**	123.549**	98.951**
V instar	218.057**	73.558**	35.549**	171.126**	117.514**

**Highly Significant at 1% level (P<0.01).*

The protein content of the fat body seems to be increased steadily from 0.184 ± 0.0049 µg/mg to 0.289 ± 0.0044 µg/mg in IV and V instar larvae fed with control V-1 mulberry leaves. Similarly, protein content of fat body also found to be steadily increased in IV and V instar larvae fed with MiCuNps (25%) treated V-1 mulberry leaves such as 0.253 ± 0.0065 µl/ml and 0.490 ± 0.0078 µg/mg, respectively. In these two observations, MiCuNps (25%) treated IV and V instar larvae protein content of fat body was highly significant at 1% level (P<0.01) than control. Significant was indicated by the t-value such as 16.028 and 73.558 of IV and V instar larvae (control and MiCuNps (25%)), respectively. The protein content of the

anterior silk gland (ASG) seems to be increased steadily from 0.206 ± 0.0038 µg/mg to 0.234 ± 0.0058 µg/mg in IV and V instar larvae fed with control V-1 mulberry leaves. Similarly, protein content of anterior silk gland (ASG) also found to be steadily increased in IV and V instar larvae fed with MiCuNps (25%) treated V-1 mulberry leaves such as 0.254 ± 0.0041 µg/mg and 0.398 ± 0.0062 µg/mg, respectively. In these two observations, MiCuNps (25%) treated IV and V instar larvae protein content of anterior silk gland (ASG) was highly significant at 1% level (P<0.01) than control. Significant was indicated by the t-value such as 185.903 and 35.549 of IV and V instar larvae (control and MiCuNps (25%)) respectively.

The protein content of the middle silk gland (MSG) seems to be increased steadily from 0.373 ± 0.0081 µg/mg to 0.879 ± 0.0055 µg/mg in IV and V instar larvae fed with control V-1 mulberry leaves. Similarly, protein content of middle silk gland (MSG) also found to be steadily increased in IV and V instar larvae fed with MiCuNps (25%) treated V-1 mulberry leaves such as 0.844 ± 0.0053 µg/mg and 1.552 ± 0.0086 µg/mg, respectively. In these two observations, MiCuNps (25%) treated IV and V instar larvae protein content of middle silk gland (MSG) was highly significant at 1% level (P<0.01) than control. Significant was indicated by the t-value such as 123.549 and 171.126 of IV and V instar larvae (control and MiCuNps (25%)) respectively.

The protein content of the posterior silk gland (PSG) seems to be increased steadily from 0.265 ± 0.0052 µg/mg to 0.736 ± 0.0059 µg/mg in IV and V instar larvae fed with control V-1 mulberry leaves. Similarly, protein content of posterior silk gland (PSG) also found to be steadily increased in IV and V instar larvae fed with MiCuNps (25%) treated V-1 mulberry leaves such as 0.451 ± 0.0046 µg/mg and 1.322 ± 0.0075 µg/mg, respectively. In these two observations, MiCuNps (25%) treated IV and V instar larvae protein content of posterior silk gland (PSG) was highly significant at 1% level (P<0.01) than control. Significant was indicated by the t-value such as 98.951 and 117.514 of IV and V instar larvae (control and MiCuNps (25%)) respectively.

Quantitative analysis of protein (diseased and diseased treated groups)

The protein content of haemolymph, fat body and silk gland of IV and V instars of diseased *B. mori* larvae fed with control and MiCuNps (25%) treated V-1 mulberry leaves are presented in **Table 3**.

The protein content of haemolymph was found to be increased steadily from 0.119 ± 0.0022 µl/ml to 0.348 ± 0.0128 µl/ml in IV and V instar of diseased larvae fed with control V-1 mulberry leaves, respectively. Similarly, protein content of haemolymph also found to be steadily increased in IV and V instar of diseased larvae fed with MiCuNps (25%) treated V-1 mulberry leaves, such as 0.220 ± 0.0037 µl/ml and 0.547 ± 0.0018 µl/ml, respectively. In these two observations, MiCuNps (25%) treated IV and V instar of diseased larvae protein content of haemolymph was highly significant at 1% level (P<0.01) than diseased larvae (fed by control leaves). Significant was indicated by the t-value **(Table 3a)** such as 52.746 and 36.132 of IV and V instar larvae (diseased and treated diseased), respectively.

The protein content of fat body was found to be increased steadily from 0.127 ± 0.0019 µg/mg to 0.173 ± 0.0062 µg/mg in IV and V instar of diseased larvae fed with control V-1 mulberry leaves, respectively. Similarly, protein content of fat body also found to be steadily increased in IV and V instar of diseased larvae fed with MiCuNps (25%) treated V-1 mulberry leaves, such as 0.246 ± 0.0046 µg/mg and 0.370 ± 0.0162 µg/mg, respectively. In these two observations, MiCuNps (25%) treated IV and V instar of diseased larvae protein content of fat body was highly significant at 1% level (P<0.01) than diseased larvae (fed by control leaves). Significant was indicated by the t-value such as 92.177 and 27.442 of IV and V instar larvae (diseased and treated diseased), respectively.

The protein content of anterior silk gland (ASG) was found to be increased steadily from 0.074 ± 0.0019 µg/mg to 0.0198 ± 0.0029 µg/mg in IV and V instar of diseased larvae fed with control V-1 mulberry leaves, respectively. Similarly, protein content of anterior silk gland (ASG) also found to be steadily increased in IV and V instar of diseased larvae fed with MiCuNps (25%) treated V-1 mulberry leaves, such as 0.216 ± 0.0043 µg/mg and 0.412 ± 0.009 µg/mg, respectively. In these two observations, MiCuNps (25%) treated

Table 3. Quantitative analysis of protein content in the haemolymph, fat body and silk gland of IV and V instars of diseased *Bombyx mori* larvae fed with control and MiCuNps treated V-1 mulberry leaves

Larval stage	Groups	Haemolymph (µl/ml)	Fat body (µg/mg)	ASG (µg/mg)	MSG (µg/mg)	PSG (µg/mg)
IV instar	**Diseased Control**	0.119 ±0.0022	0.127 ±0.0019	0.074 ±0.0019	0.034 ±0.0017	0.039±0.0013
	MiCuNps (25%)	0.220 ±0.0037	0.246 ±0.0046	0.216 ±0.0043	0.056 ±0.0008	0.065±0.0028
V instar	**Diseased Control**	0.348 ±0.0128	0.173 ±0.0062	0.198 ±0.0029	0.037 ±0.0006	0.066±0.0006
	MiCuNps (25%)	0.547 ±0.0018	0.370 ±0.0162	0.412 ±0.0009	0.068 ±0.0032	0.126±0.0015

Values are Mean ± Standard Deviation.

Table 3a. 't' - test

Larval stage	Haemolymph (µl/ml)	Fat body (µg/mg)	ASG (µg/mg)	MSG (µg/mg)	PSG (µg/mg)
IV instar	52.746**	92.177**	82.910**	28.066**	19.748**
V instar	36.132**	27.442**	146.516**	21.920**	87.831**

***Highly Significant at 1% level (P<0.01).*

IV and V instar larvae protein content of anterior silk gland (ASG) was highly significant at 1% level (P<0.01) than diseased larvae (fed by control leaves). Significant was indicated by the t-value such as 82.910 and 146.516 of IV and V instar larvae (diseased and treated diseased) respectively. The protein content of middle silk gland (MSG) was found to be increased steadily from 0.034 ± 0.0017 µg/mg to 0.037 ± 0.0006 µg/mg in IV and V instar of diseased larvae fed with control V-1 mulberry leaves, respectively. Similarly, protein content of middle silk gland (MSG) also found to be steadily increased in IV and V instar of diseased larvae fed with MiCuNps (25%) treated V-1 mulberry leaves such as 0.056 ± 0.0008 µg/mg and 0.068 ± 0.0032 µg/mg, respectively. In these two observations, MiCuNps (25%) treated IV and V instar larvae protein content of middle silk gland (MSG) was highly significant at 1% level (P<0.01) than diseased larvae (fed by control leaves). Significant was indicated by the t-value such as 28.066 and 21.920 of IV and V instar larvae (diseased and treated diseased) respectively.

The protein content of posterior silk gland (PSG) was found to be increased steadily from 0.039 ± 0.0013 µg/mg to 0.066 ± 0.0006 µg/mg in IV and V instar of diseased larvae fed with control V-1 mulberry leaves, respectively. Similarly, protein content of posterior silk gland (PSG) also found to be steadily increased in IV and V instar of diseased larvae fed with MiCuNps (25%) treated V-1 mulberry leaves, such as 0.065 ± 0.0028 µg/mg and 0.126 ± 0.0015 µg/mg, respectively. In these two observations, MiCuNps (25%) treated IV and V instar larvae protein content of posterior silk gland (PSG) was highly significant at 1% level (P<0.01) than diseased larvae (fed by control leaves). Significant was indicated by the t-value such as 19.748 and 87.831 of IV and V instar larvae (diseased and treated diseased) respectively.

DISCUSSION

Biochemical studies on the silk gland of *Bombyx mori* have shown that the secretory production of the gland is protein complex substances and it serve as a source of energy for the process of spinning and also

for the synthesis of silk fiber (Centhilnayaki, 2004). It is well known that the energy is derived from three major sources namely carbohydrate, protein and fat when they are oxidized (Gilmour, 1965). Earlier studies on fat body of *Bombyx mori* have established the existences of a close relationship between fat body and the silk gland with regard to the spinning activity which involves an expenditure of energy (Centhilnayaki, 2004 and Balasundaram, 2008).

Many functions like protein synthesis, cocoon construction and energy production for flight are attributed to amino acids (Sacktor, 1953). Shiegmatsu , (1958 & 1960a) has identified the blood globulin and three different protein components in the haemolymph of silkworm has shown further that some new proteins, in addition to these are synthesized in the fat body and released into the blood during development.

Proteins have always been an interesting biochemical food for insect biochemists because of their pertinent role in the development, morphogenesis and almost in all the intermediary metabolic pathways of the insects. The first ever observations on insect haemolymph protein was made by Lauffer, (1943) on silkworm, *Bombyx mori*. Afterwards, a series of detailed physic-chemical studies on *Bombyx mori* haemolymph proteins appeared (Wyatt, 1961; Wyatt and Pan, 1978; Ogawa and Tojo. 1981). Tojo *et al.*, (1980) have scanned a detailed account of storage proteins in *Bombyx mori.*

Protein metabolism is important in the silkworm because of it's vital role in the determination of chemical characteristics of silk protein like fibroin and sericin (Shigematsu *et al.,* 1978). In general, breakdown of proteins dominated over their synthesis due to the enhanced proteolytic activity (Harper *et al.,* 1979). The rate of protein breakdown was higher in haemolymph than that of fat body. The depletion of proteins may constitute a physiological and compensatory mechanism under stress to provide intermediate metabolites to the Kreb's cycle or to maintain the osmoregulation by releasing the free amino acids into the haemolymph (Rajeswari, 1986). In the present study, it has been observed that the amount of protein contents in the haemolymph of IV instar larvae were decreased when fed with V-1 than copper nanoparticles treated mulberry leaves. Similar results have also been observed in the fat body and also in all the regions of silk gland comparatively fed with two feeds of mulberry leaves such as V-1 and treated with copper nanoparticles mulberry leaves.

Further, it has been shown that the amount of amino acid contents in the haemolymph of IV instar larvae were comparatively less than V instar larvae, suggested that the occurrence of this decrement may be due to the mobilization of less amounts of protein component from the fat body via haemolymph into the silk gland. Similar trends have also been observed in the fat body, suggested that the less amount of amino acid comparatively in IV and V instar larvae of *B. mori* when fed with V-1 and copper nanoparticles treated mulberry leaves. These changes may be attributed due to the synthesis and sequestration of more quantity of both amino acid and protein for the enhancement of breakdown and synthesis of proteins which are required for silk production

In addition, remarkable changes in the quantity of protein content in the fat body of IV and V instar larvae has been observed in the present study. The less amount of protein content during these stages of late larval period may probably be due to utilization of these proteins for the synthesis of silk proteins by the silk glands. Further, it may be suggested that the silk gland of IV and V instar larvae have shown the acceleration of high quantity of proteins in all the regions of the silk gland of IV and V instar larvae, comparatively more in the V instar larvae of *Bombyx mori* may be due to an active involvement of the silk gland for the silk production during pre-spinning period for cocoon construction.

The present study is in parallel with the works of Lakshmi Kumari *et al.,* (1997) who have also opined that the high activity of the amylase and protease in haemolymph and midgut tissue might be due to a greater utilization of exogenous proteins resulting in the production of more silk.

The productivity of silkworm interms of cocoon crop depends on several factors that operate within and outside the body of silkworm (Benchamin and Jolly, 1986). The high levels of free amino acid in haemolymph indicate the synthesis of proteins. Padmaja, (1995) has reported that this will be received by the haemolymph after digestion of the leaf. Proteins are probably absorbed after digestion by proteases and

then amino acids are intracellulary liberated into the gut epithelium by aminopeptidases as shown by Horie *et al.*, (1982) in *Bombyx mori* as in other insects (Law *et al.*, 1977). However, on the day before spinning, haemolymph protein was found to be decreased (Tojo *et al.*, 1980). Another possibility is that the increased proteolytic activity arises due to a rate of degradation of secreted proteases in the presence of proteins. Proteins and amino acids are of particular importance for the silkworm larvae because of their active utilization even for the synthesis of silk protein (Centhilnayaki, 2004).

In the present study, the amount of free amino acid and protein content have been found to increase concomitantly more in the fat body, haemolymph and silk gland of Bombyx *mori* from III, IV and V instar larvae fed with copper nanoparticles treated mulberry leaves than V-1 leaf variety. From this result, it has been suggested that the active proteolytic and protein synthesis have been taken place for the elaboration of protein for the synthesis of silk fiber as well as immediate formation of amino acid have been found to be increased for the synthesis of proteins. These results are in conformity with the works of Sundaramurthy and Ahmed, (1978) and Kajiura and Yamashita (1989). Davis *et al.*, (1990) and Centhilnayaki (2004) for *Spodoptera litura, Bombyx mori, Cymantia dispar* and *Bombyx mori* respectively, when these insects were treated with methoprene and V-1 mulberry variety induced increase in the protein content along with feed. It is evident from the present study that the nutritional quality of mulberry leaf plays a vital role in silkworm growth. These results have given a clear indication that V-1 leaves treated with copper nanoparticles triggers off certain positive metabolic changes in the tissues directly or indirectly involved in silk protein synthesis which ultimately resulted in the improvement of the economic characters of the silkworm, *Bombyx mori*. It has been revealed from the present study that the contents of protein and amino acids were comparatively more and also concomitantly increased in all the target tissues and haemolymph of III to V instar larvae may be attributed due to the contents in V-1 leaf variety treated with copper nanoparticles probably be activated the fat body glycogen phosphorylase and promotes oxygen consumption by the larvae. These metabolic adjustments add upto the development of the silkworm manifesting an increased silk production. Similar results have been observed by Gade, (1981) in *Bombyx mori* fat body during development and also as reported by Prasad and Mohan, (1990) that the total free amino acid level in the V instar larvae were elevated during the metamorphosis of *Bombyx mori*. These results were correlated with active protein synthesis and silk production in developing *Bombyx mori* in the present study, when the silkworms fed with -1 leaf variety treated with copper nanoparticles than V-1 leaf variety.

Since silk being commercially and economically important, *Bombyx mori* has been the target of research in different countries. In holometabolous insects, the most metabolic fuels and building blocks for pupal and adult development are derived from the materials stored in the haemolymph especially during the V instar larvae. The fat body of the insect is the major site of the insect plasma protein bio-synthesis. Further, the proteins are transported into the haemolymph (Tojo *et al.*, 1980). Similar results have been observed in the present study that the protein synthesized in the fat body was found to be sequestered and mobilized from tissues to the silk gland via haemolymph due to an increased amount of protein contents in the silk gland of *Bombyx mori* during the period of spinning. This inference gain support from the works of several authors, that the mobility of certain protein from fat body into the haemolymph has been reported for the male *Melanoplus sangainipes*, (Elliott and Gillott, 1979a) and *Odontopus varicornis* (Selvisabhanayakam, 1995), respectively. In the light of these observations and the observation reported in earlier chapter, it may be suggested that the secretory activity of certain new secretory substances which inturn activated the fat body to synthesis of certain specific protein to be released into the haemolymph for their incorporation into the silk gland was evident. The existence of certain receptor sites in the cells of fat body and their role responding to JH of corpus allatum, the mechanism of the synthesis of specific protein in the fat body and its incorporation into the silk gland warrant further investigation in *Bombyx mori*, with reference to silk production and also in other insects by using modern techniques like RNA Recombinant Technology, DNA sequencing and PCR. It may be concluded that V-1 leaf variety treated with copper nanoparticles elicits

some favourable responses in silkworms with regard to the commercial improvement as evidenced from the results of the present study.

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