**IJPSR (2016), Vol. 7, Issue 2** **(Research Article)**

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 12 August, 2015; received in revised form, 23 September, 2015; accepted, 21 November, 2015; published 01 February, 2016

# EFFICACY OF SODIUM HYPOCHLORITE IN CONTROLLING BACTERIAL INFECTIONS IN FIFTH INSTAR LARVAE OF *ANTHEREAE MYLITTA DRURY (DABA TV)*

Lakshmi Marepally * and G Benarjee

Department of Zoology, Kakatiya University, Warangal, Telengana, India.

**Keywords:**

Anthereaemylittadrury, Bacterial Diseases, Sodium Hypochlorite, Protein Carbonyl Content, Haemocyte Count, Alanine Aminotransferase, Aspartate Aminotransferase.

**Correspondence to Author:**
**Lakshmi Marepally**

Post Doctoral Fellow - UGC Department of Zoology, Kakatiya University, Warangal, Telangana, India-506009, India

**Email:** lakshmi.velide@gmail.com

**ABSTRACT:** Bacterial diseases are highly virulent and cause significant crop loss in *Anthereae mylittadrury (Daba TV*).Therefore an attempt has been made to evaluate the efficient concentration of sodium hypochlorite in controlling bacterial infection through studies on mortality, Total haemocytes, Protein carbonyl content, Alanine transaminase, Aspartate transaminase in the fifth in star larvae. Results reveal that supplementation of 0.05% sodium hypochlorite concentration had significantly reduced the mortality rate due to bacterial infections. In comparison with the control (14788±123), the haemocyte count in 0.05% sodium hypochlorite supplemented larvae were recorded low as14125±146 whereas 0.1% and 0.01%concentration supplementation resulted in an increase of 15025±158 and 14965±185 haemocytes. In comparison with the control results also show that protein carbonyl content was doubled with 0.05% supplementation whereas an increase of 33.3% and 16.6% was recorded with 0.01% and 0.1% respectively. Alanine aminotransferase had shown significant increase of 138.7% with 0.05% sodium hypochlorite supplementation followed by 39% increase with 0.01% concentration in its activity. Supplementation with 0.1% did not show much variation in Alanine aminotransferase from the control. Aspartate aminotransferase activity levels found increased by 106.2% in fifth in star larvae supplemented with 0.05% sodium hypochlorite whereas 30.2% and 21.75% increase was recorded with 0.01%, 0.1% respectively. Based on the results obtained from present study 0.05% sodium hypochlorite is found efficient in controlling bacterial infections in *Anthereae mylitta drury (Daba TV).*

**INTRODUCTION:** *Antheraea mylitta Drury (Daba TV)* is a commercially important tropical Tasar silkworm produces the important vanya silk [1]**.** It suffers from climatic hazards, erratic emergence and high bacterial infections during first and second crops leading to quantitative and qualitative crop loss [2]. In India 40% of cocoon crop loss of *Anthereae mylitta* is due to bacterial diseases with symptoms like chain type excreta, rectal protrusion and sealing of anal lips [3].

QUICK RESPONSE CODE

**DOI:** 10.13040/IJPSR.0975-8232.7(2).703-07

**Article can be accessed online on:** www.ijpsr.com

**DOI link:** http://dx.doi.org/10.13040/IJPSR.0975-8232.7 (2).703-07

Infected larvae stops feeding, does not respond to external stimuli and body becomes darker [4]. Studies on biochemical changes during various stress like coldness and pebrine disease in *Anthereae mylitta* has been carried out [5, 6].

Haemocytes are the important components of the insect immune system. Cellular responses are direct interactions between haemocytes and non-self materials. The interactions results in responses like nodulation, phagocytosis and encapsulation [7]. In insects several types of haemocytes are observed in the haemolymph with various functions like mechanization and immobilization of invading organism by encapsulation and phagocytosis, wound repair and coagulation [8]. Studies on the susceptibility of three eco-races of *Anthereae mylitta drury* against AmCPV reported that

ecoraces showing reduced number of haemocytes are tolerant to pathogen [9]. Recently, some work has been carried out on the haemocytes and protein changes in Tasar silkworm and reported that total haemocyte counts indicate the susceptibility status of insect [10]. Increase in the number of haemocytes was recorded in *Anthereae mylitta drury* infected with *Nosema* species [11].

Silkworm growth is correlated with the synthesis of carbohydrates, proteins, amino acids and enzymes like proteases, glutamate dehydrogenases and aminotransferases and changes in these biochemical constituents are correlated with the events of histogenesis and histolysis [12]. Infection causes many biochemical changes in the silkworm larvae with a change in metabolic pathways to defend against pathogen invasion [13]. Nosema infection in silkworm causes a decrease in enzyme activity [14].

Disease control in sericulture can be affected by application of natural and synthetic chemicals [15]. Information on suitable chemicals required to control bacterial infection in *Anthereae mylitta drury* is scanty. Hence, the present work was carried out to understand the efficient concentration of sodium hypochlorite in controlling bacterial infection in *Anthereae mylitta drury (Daba TV)* based on mortality rate, Total haemocyte count, Aspartate transaminase, Alanine transaminase and Protein carbonyl content.

## MATERIALS AND METHODS:

Third instar larvae of *Daba TV* with bacterial infection (chain type excreta) were collected during first crop from the forest patches of Jakaram, Warangal, Telangana, India. Isolation of bacteria was done by dissecting the hind gut of infected larvae in distilled water, homogenisation and followed by serial dilution agar plating method [16]. Isolated bacteria were cultured in liquid broth made up of beef extract and peptone and stored at-20°C for further research.

To estimate the efficient concentration of sodium hypochlorite in controlling bacterial infections in *Daba TV*, healthy first in star larvae were divided into two groups. One group fed with untreated *Terminalia arjuna* leaves (T1-Control). Other group fed on the *Terminalia arjuna* leaves supplemented with inoculum dosage of $1X10^5$/ml and sodium hypochlorite. Based on sodium hypochlorite concentrations this group was subdivided as S1 (0.01%), S2 (0.05%) and S3 (0.1%). Three different concentrations of sodium hypochlorite solution were sprayed by foliar sprayer 3 to 4 times on foliage two days before brushing and two times on foliage at $2^{nd}$, $3^{rd}$,$4^{th}$and $5^{th}$ in star stages. Treated and untreated groups with 100 larvae each were reared till cocooning separately to avoid contamination.

Fifth in star larvae were selected from all the groups separately. Haemolymph was collected in Eppendorf tubes of 1.5ml by bending the thoracic legs. Phenyl thiourea was mixed with collected haemolymph to prevent melanisation [17]. Samples were centrifuged at 20000rpm for 10 min. The supernatant was used to estimate Aspartate transaminase (AST) and Alanine transaminase (ALT) according to [18] whereas Protein carbonyl content by [19]. For haemocyte estimation, haemolymph was drawn into a Thoma white blood cell pipette up to 0.5 mark and diluted up to the 11 mark with Tauber–yeager fluid [20]. The pipette was then shaken for several minutes and the first three drops were discarded. A double line with improved Neubauer ruling Haemocytometer was filled with diluted haemolymph and the haemocytes counted in its four corner and one central ($1mm^2$) squares. The number of circulating haemocytes per cubic millimeter was calculated using the following formula [21].

Haemocytes in five $1mm^2$ x Dilution × Depth factor of chamber / No. of squares counted

Where dilution = 20 times, Depth factor of the chamber = 10 (constant) and No. of squares counted = 5.

### Statistical analysis:

Each assay was replicated 3 times. Values were expressed as mean ± SE of replication and Student's t-test was applied to locate significant ($P$ <0.05) differences between supplemented and control larvae. Critical differences (CD5%) was analysed by Tukeys post hoc procedure [22].

## RESULTS AND DISCUSSION:

**Table 1** indicate the mortality rate and haemocyte count in the control and sodium hypochlorite supplemented fifth in star larvae (P<0.05). Haemocyte count in the larvae supplemented with 0.05% sodium hypochlorite are less than the control whereas highest number were recorded in 0.01% concentration followed by 0.1%. The eco-races showing reduced number of haemocytes are tolerant to pathogen [9]. Out of 100 larvae supplemented with 0.01% sodium hypochlorite concentration 28 larvae found dead because of bacterial infection whereas 0.05% concentration caused death of 8 larvae and 0.1% concentration caused 14 larvae death(P<0.05).The application of sodium hypochlorite was found to reduce the bacterial and viral diseases in *Muga silkworm* [23]. Forty percent of control larvae found dead because of chain type excreta.

**TABLE 1: MORTALITY RATE AND TOTAL HAEMOCYTE COUNT IN FIFTH INSTAR LARVAE OF *ANTHEREAE MYLITTADRURY (DABA TV)***

| Larvae type | Mortality number/100 larvae due to bacterial infection | Number of haemocytes |
|---|---|---|
| Larvae fed on 0.01% sodium hypochlorite treated foliage | 28 | 14965±185 |
| Larvae fed on 0.05% sodium hypochlorite treated foliage | 08 | 14125±146 |
| Larvae fed on 0.1% sodium hypochlorite treated foliage | 14 | 15025±158 |
| Larvae fed on untreated foliage(Control) | 35 | 14788±123 |
| CD 5% | 0.07 | 0.5 |

CD: Critical difference. All the values are the mean values of three replications

Data presented in **Table 2** and **Fig. 1** depicts a significant increase (P<0.05) in protein carbonyl content in sodium hypochlorite supplemented larvae than control larvae (0.06nol m/L of serum, SE=±0.005). In comparison with the control, larvae supplemented with 0.05% concentration of sodium hypochlorite have doubled the carbonyl content and 0.01% concentration increased the carbonyl content by 16.6%.Dexatrol and peppermint oil reduced the protein carbonyl content in haemolymph of infected *Bombyx mori* and decrease in protein content can be correlated with decrease in protein carbonyl content in infected larvae [24]. Present results in **Fig. 2** and **Table 2** shows an increase (P<0.05) of Alanine transaminase in S1(0.01%), S2(0.05%) and S3 (0.1%) batch larvae treated with sodium hypochlorite in comparison with the control (T1) (3.05µg Pyruvate/ mL SE= ±0.08). Larvae supplemented with 0.05% sodium hypochlorite concentration have shown a significant increase of Alanine transaminase of 138.7% over the control whereas 0.01% concentration by 4.24% and 0.1%concentration by 3.25% respectively. It has been reported that silkworm larvae under stress conditions like cold stress, parasitism, treatment with pesticides and hormone analogues show increase in the enzyme activities [5, 14].

**TABLE 2: EFFECT OF SODIUM HYPOCHLORITE ON PROTEIN CARBONYL CONTENT, ALANINE TRANSAMINASE, ASPARTATE TRANSAMINASE OF HAEMOLYMPH OF FIFTH INSTAR LARVAE OF ANTHEREAE MYLITTA DRURY**

| Treatment | Protein carbonyl content (nmol m/L of serum) | Alanine transaminase (µg pyruvate/mL) | Aspartate transaminase (µg pyruvate/mL) |
|---|---|---|---|
| S1(0.01%) | 0.08 (+33.3%) | 4.24 (+39%) | 11.25 (+30.2%) |
| S2(0.05%) | 0.12 (+100%) | 7.28 (+138.7%) | 17.82 (+106.2%) |
| S3(0.1%) | 0.07 (+16.6%) | 3.25 (+6.5%) | 10.52 (+21.75%) |
| T1(Control) | 0.06 | 3.05 | 8.64 |
| CD 5% | 0.08 | 0.06 | 0.14 |

CD: Critical difference. All the values are the mean values of three replications

Data represented in **Fig. 3** and **Table 2** shows a significant increase (P<0.05) in Aspartate transaminase in S1 (0.01%), S2 (0.05%) and S3 (0.1%) batch larvae than control (T1) (8.64µg Pyruvate/ mL SE= ±0.58). Especially 0.05% sodium hypochlorite concentration batch larvae

have shown a tremendous increase of 106.2% over the control whereas 0.01%concentration by 30.2% and 0.1concentration by 21.75% respectively. Increase in enzyme activity can be correlated with the stimulation of enzymes which influences the biochemical components of haemolymph of silkworm. Aspartate aminotransferase activity is a sign for entrance of amino acids to glucogenesis [25]. The increase in activity of aminotransferases decreases the amino acids because of their conversion to pyruvate in turn increases the supply of precursors to Krebs cycle [26].

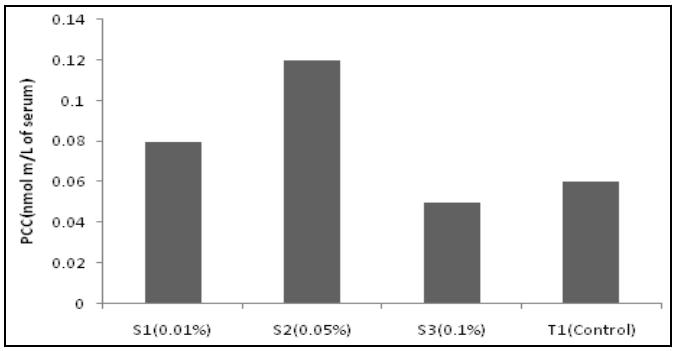


**FIG 1: EFFECT OF SODIUM HYPOCHLORITE ON PROTEIN CARBONYL CONTENT (PCC) OF INFECTED FIFTH INSTAR LARVAE OF *ANTHEREAE MYLITTA DRURY (DABA TV)***

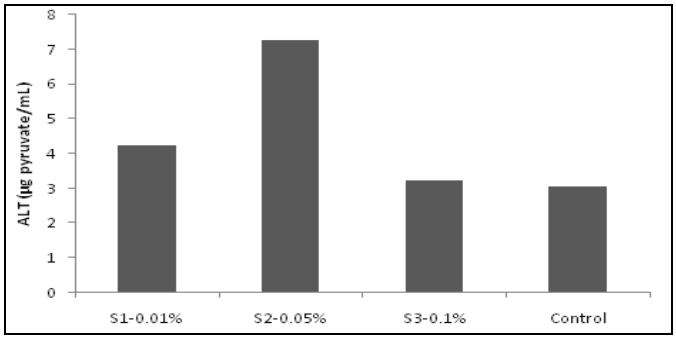


**FIG 2: EFFECT OF SODIUM HYPOCHLORITE ON ALANINE TRANSAMINASE OF INFECTED FIFTH INSTAR LARVAE OF *ANTHEREAE MYLITTA DRURY (DABA TV)***

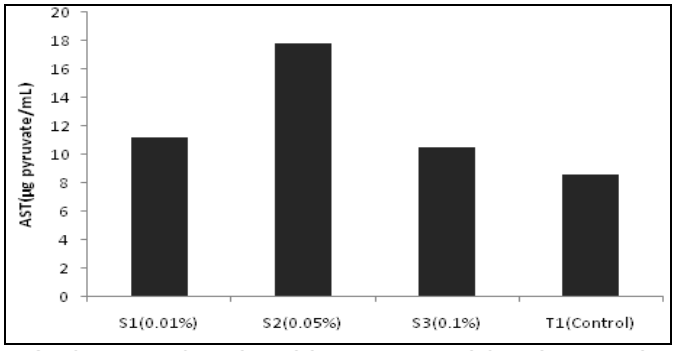


**FIG 3: EFFECT OF SODIUM HYPOCHLORITE ON ASPARTATE TRANSAMINASE OF INFECTED FIFTH INSTAR LARVAE OF *ANTHEREAE MYLITTA DRURY* (*DABA TV*)**

**CONCLUSION:** Thus in conclusion 0.05% sodium hypochlorite is efficient in reducing the mortality rate of *Anthereae mylitta drury* by bacterial infections. It also reduced the haemocyte count but significantly increased the protein carbonyl components and activities of alanine aminotransferases and aspartate aminotransferases.

**ACKNOWLEDGEMENTS**: The author, Dr. Lakshmi Marepally would like to thank UGC for providing financial assistance in the form of Postdoctoral fellowship.

## REFERENCES:

1. Suryanarayana N, Srivastava A-K: Monograph on Tropical Tasar Silkworm. Central Tasar Research and Training Institute 2005; Ranchi, India, pp: 1-87.
2. Lakshmi M: Studies on the genome stability in Andhra local ecorace Tassar silkworm, *A.mylitta. D* with special reference to environmental factors. Ph.D Thesis, Department of Zoology, Kakatiya University, 2011; Warangal, Telangana.
3. Sahay D-N, Roy D-K, Sahay A: Diseases of Tropical Tasar Silkworm, *Antheraea mylitta D.,* Symptoms and Control Measures, In: Lessons on Tropical Tasar, Thangavelu, K. (Ed.). Central Tasar Research and Training Institute, Piska-Nagri, Ranchi, 2000; 104.
4. Singh G-P, Sinha A-K, Roy D-K, Sahay A, Madhusudhan K-N, Kumar K-P, Prasad B-C: Cellular and biochemical changes of *Antheraea mylitta D.* on immunization with attenuated *Antheraea mylitta* cytoplasmic polyhedrosis virus (Am CPV). International Journal of zoological research 2011; 7(3), 263-271.
   Lakshmi Velide: Studies on biochemical components of the larval haemolymph, fat body and silkgland of tropical tasar silkworm, *Anthereaemylitta Drury (Daba T.V )*under cold stress condition. European Journal of experimental biology 2012; 2(6):2238-2242.
5. Lakshmi Marepally, Benarjee G: Studies on metabolic alterations in pebrinised fifth instar larvae of tropical tasar silkworm *Antheraea mylitta drury (Daba TV).* Journal of biological engineering research and review 2014;1(1): 23-27.
6. Singh G-P, Sinha A-K, Madhusudhan K-N, Kumar K-P, Prasad B-C, Roy D-K, Sahay A: Attenuation of *Antheraeamylitta* cytoplasmic polyhedrosis virus (AmCPV) and its potential as an oral vaccine against virus diseases in tasar silkworm, *Antheraea mylitta D.* International journal of virology 2011;7(1):33-38.
7. Saran S-K, Mishra P-K, Kumar D, Singh B-M-K, Sinha B-R-R-P,RaiS,PandeyP-N:Effect of total and differential haemocyte counts due to infection of Nosema species in *Antheraeamylitta D.* (Lepidoptera: Saturniidae) larvae. Proceedings of the 19th Congress of the International Sericultural Commission, 2002; Sep. 21-25, Bangkok, Thailand.pp, 308-311.
8. Singh G-P, Zeya S-B, Srivastava A-K, Prakash B, Ojha N-G, Suryanarayana N:Susceptibility of three Eco-races of tropical tasar silkworm to *Antheraea mylitta* cytoplasmic polyhedrosis virus (AmCPV). Caspian Journal of environmental science 2008; 6:161-165.

9. Kumar D, Pandey J-P, Jain J, Mishra P-K, Prasad B-C: Qualitative and quantitative changes in protein profile of various tissue of tropical tasar silkworm, *Antheraea mylitta drury*. International journal of zoological research 2011; 7:147-155.
10. Lakshmi velide, Bhagavanulu M-V-K: Estimation of efficient concentration of bavistine in controlling microsporidiasis and improving cocoon chracters in *Anthereae mylitta drury (Andhra local ecorace*). International journal of plant animal and environmental sciences 2012; 2(1):177-182.
11. Naga Jyothi P, Suneetha Y, Praveen kumar K:Ultrasound induced enhancement of protein metabolism and enzyme activities in the fat body of fifth instar silkworm, Bombyxmori L. International journal of pharmacy and life sciences 2014;5(5):3546-3553.
12. Gao L, Chen K, Yao Q, and Chen H: BmNPV infection enhances ubiquitin conjugating enzyme E2 expression in the midgut of BmNPV susceptible silkworm strain. International journal of industrial entomology 2006; 13: 31-35.
13. Madhusudhan K-N, Iresh Kumar, Nungshi Devi C, Singh G-P, Sinha A-K, Kirankumar K-P, Prasad B-C: Impact of pebrine infection on catalase activity in tropical tasar silkworm (Antheraeamylitta D.) Interanatioal Journal of Science and Nature 2012; 3(1): 212-213.
14. Saad M-I-S, Eman H-M, Zannoon A-A: Effect of some antibiotics on the biological and silk production of mulberry silkworm, *Bombyxmori L*. Egyptian journal of agricultural sciences 2012; 90:537-545.
15. Nataraju B, Satyaprasad K, Manjunath D, Kumar C-A: Silkworm crop protection, Central silk board, 2005; Bangalore, India, pages: 310.
16. Mahmoud S-M: Activation of silk secretion by silkworm, *philosamiaricini* and *Bombyxmori* after applying antibiotics. Ph.D. Thesis 1988; Faculty of Agriculture, Cairo University, Egypt.
17. Thomas L: Clinical laboratory diagnostics. First edition TH Books 1998; Verlasgesellschaft, Frankfurt.
18. Levine R-L, Garland D, Oliver C-N, AmiciA, Climent I: Determination of carbonyl content in oxidatively modified proteins. Methods of Enzymology 1990; 186:464-478.
19. Tauber O-E, Yeager J-E: On the total blood counts of insects Orthoptera, Odonata, Hemiptera and Homoptera. Annals of Entomological Society America 1935; 28:229-240.
20. Jones J-C: Current concepts concerning in secthemocytes: American Zoology 1962; 2:209-246.
21. John Wilde Tukey, Brillinger D-R, Cox D-R, Braun H-I: The collected works of John W. Tukey. Wadsworth advanced books and software 1984; Belmont, California.
22. Ibotombi Singh N, Dulal Goswami, Mushtaq Ahmed, Giridhar K: Efficacy of sodium hypochlorite in controlling viral and bacterial diseases in muga silkworm, *Antheraeaassamensis Helfer*. Journal of Applied Biology and Biotechnology 2014; 2 (2):12-15.
23. Eman Hassan M: Effect of Dexatrol and peppermint oil as disinfectants on some biochemical characters of infected silkworm *Bombyxmori*. Journal of Entomology 2015; 12(1):12-20.
24. Lehninger A-L: Principles of biochemistry. Worth publishers 1982; Newyork, 1982.
25. Singh Anitha, Sharma Ratnesh K, Sharma Bechan:Low temperature induced alterations in certain biochemical constituents of 5th instar larvae of *philosamiaricini* (Lepidoptera: Saturnidae). Insect Physiology 2010; 3:11-16.


**How to cite this article:**
Marepally L and Benarjee G: Efficacy of Sodium Hypochlorite In Controlling Bacterial Infections In Fifth Instar Larvae of *Anthereae Mylitta Drury (Daba Tv)*. Int J Pharm Sci Res 2016; 7(2): 703-07.doi: 10.13040/IJPSR.0975-8232.7(2).703-07.