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Efficacy of Botanical extracts with incidence of tukra mulberry on histopathology of Silkworm, (*Bombyx mori L.*)

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ABSTRACT

Present study was conducted to find out the effect of various botanical extract on the tissue, cellular and sub cellular level and histopathology of silkworm, findings of the present study gives useful data concerning the changes in the insect. Three plants extract viz Azadirachta indica, Ocimum sanctum and Parthenium hysterophorus were used as experimental while untreated leaves consider as control. These botanicals were sprayed on the tukra (Pink mealy bug) infected mulberry leaves and fed to silkworm. Findings of the study suggested no change in the fad body of the silkworm feed on the botanical sprayed leaves and it was with normal columnar epithelium had striated border (microvillus) covered by the peritrophic membrane. While epithelial cells slightly sloughed fat body was reported in the silkworm fed on the tukra leaves. The outer layers of the nucleolus were reported somewhat hypertrophied and cytoplasm was reported vacuolated with mild degeneration of cell in silkworm fed on the tukra infected leaves. Silkworm fed leaves revealed almost similar changes to that of normal and there was no change in botanical sprayed fed larvae. The impact in tissue of the silkworm when fed with normal and crude botanical extracts against mealy bugs shows normalcy, but in the tukra mulberry leaves fed by silkworms the tissues shows slight degenerative with nutritional impact upon them.

Keywords: Tukra (pest), Botanical extracts, fat body, silk gland, *Bombyx mori*

INTRODUCTION

Histological study is also one of the very important tools used in evaluating the level of a tissue from the toxicant. The architectural dynamics of a tissue is very essential for maintaining the tissue integrity for effective physiological, biochemical and metabolic functions. The cellular and sub cellular constitutions of it in terms of size, shape, number and position play an important role in the physiological and metabolic responses. Therefore the histological structure of a tissue in an insect has a profound influence on its function. Histology the study of microanatomy of specific tissues has been successfully employed as a diagnostic tool in medical and veterinary sciences since the first cellular investigations carried out in the nineteenth century (Virchow, 1858). Diagnosis and prediction of physiological consequences in the animal can be obtained through histopathology (Fanta 1997; Silva *et al*., 1993). Tadasu Mori *et al*., (1990) reported that the biochemical and physiological function of the fat body in the silkworm, *Bombyx mori* electron microscopic observations have been carried out on the fatbody fed with normal mulberry leaves and starved larvae that the lipodial bodies decreased in number and became less electron dense in the fat body cell of starved larvae.In most insects the midgut shows no sign of external anatomical differentiation but there are many signs in the internal and functional differentiation commonly associated with histological and ultra structural differences in the insect (Chapman, 1985). Shiva Kumar (1995) reported that the microscopic examination of a midgut when the insect is enhanced food conversion food digestion, resulting the increased body weight in the silkworm fed with normal leaves compared to infected mulberry leaves. Aruga and Tanaka (1968) studied the ressistance of silkworms from feeding with contaminated mulberry leaves. Tandada *et al.*, (1982) studied the

cytology of larvae of the silkworm such as the fatbody, trachea hypodermis and blood causes death in about 5 to 7 days after feeding with highly contaminated leaves. Silkgland is the second largest organ in the sikworms which occupies most of the ventro-lateral side of the body. It has a pair of tubular structures of 20-40cms in length situated on either side of the insect and fills the entire coelomic cavity. The cell of the silk gland synthesis by its silk proteins, fibroein, sericin, and the fluid is surrounded by later.

MATERIALS AND METHODS

Experimental Animals

The disease free laying (dfls) of popular pure Bivoltine breed, $PMxNB_4D_2$ was obtained from the Directorate, Government Sericulture Grainage Center, Shahupuri, Kolhapur, for laboratory rearing. The larvae were reared as per the method of Krishnaswamy (1978, 1979) and experiments were conducted during January to August 2011 during which four rearing were done.

Mulberry Plantation:

Mulberry crop was maintained by following standard agronomic practices. Treatments were imposed on 15th day of pruning in each plot, five plants were randomly selected and the population of pink mealy bug was counted. In each plant population was counted on three leaves (top, middle and bottom). The total number leaves per plant were also counted and the population was expressed as number per leaf. Observations were made just before spraying (pre-treatment count), 3, 5 and 7 days after spraying. The following plant extracts with naturally existing insecticidal properties were chosen for spray of mealybug infection in mulberry plants.

Preparation of aqueous plant extract:

Plants having insecticidal properties like *Azadirachata indica*, *Ocimum sanctum* and *Parthenium hysterophorus* were taken from the department of Botany, University College of sciences, Acharya Nagarjuna University, Guntur, AndhraPradesh. The leaves of plants were collected, washed thoroughly with distilled water the fresh leaves were homogenate with the help of mechanical device. Further 200 gm of crude selected plants were subjected to extraction through soxhlet apparatus with 500 ml methanol solvent for 24 hrs. After 24 hrs given extract was filtered and filtrate was evaporated completely. Evaporated extract material was dissolved in distilled water and diluted to 2.5 % concentration and used for spray at the identified plot with earlier infection of mealy bug in mulberry plants. Botanical extracts sprayed to tukra leaves of various concentrations were fed to third instar larvae with four feeding per day. The feeding was maintained up to the earlier end of cocoon stage of the silkworm.

Microscopic examination used in Silkworm fed with botanical-Sprayed Mulberry leaves:

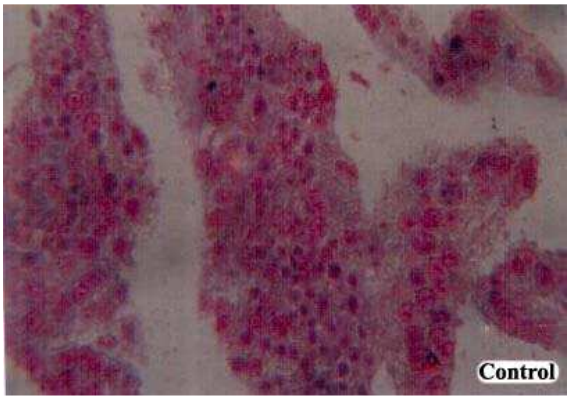
Microscopic examinations were carried out to find the effect of feeding healthy and botanical sprayed leaves on rearing silkworm hybrid. Leaves were collected from the experimental plots 0, 2, 5, 7, 10, 15 and 20 days after spray and were fed to second instar larvae. From 3 to 6 days of silkworm tissues were taken for the present study. Morphological changes in silkworm exposed to different percentages of tukra infected and healthy mulberry leaves fed to silkworm and were analyzed and changes in tissue were photographed to observe the external symptoms and the histological sections of fat body and silk gland were taken and fifth instar of day 6 were isolated from normal and experimental batch. They were fixed in Bouin's fluid (75ml saturated aqueous picric acid, 25ml 40% formaldehyde and 5ml glacial acetic acid) for 24 hours. The sections were stained with Harris hemotoxylin (Harris, 1900) and counter stained with eosin, dissolved in 95% alcohol. After dehydration and cleaning, the sections were mounted in Canada balsam. Photomicrographs of the sections preparation were taken using Olympus (PM – 6 models) photomicrography equipment.

RESULTS

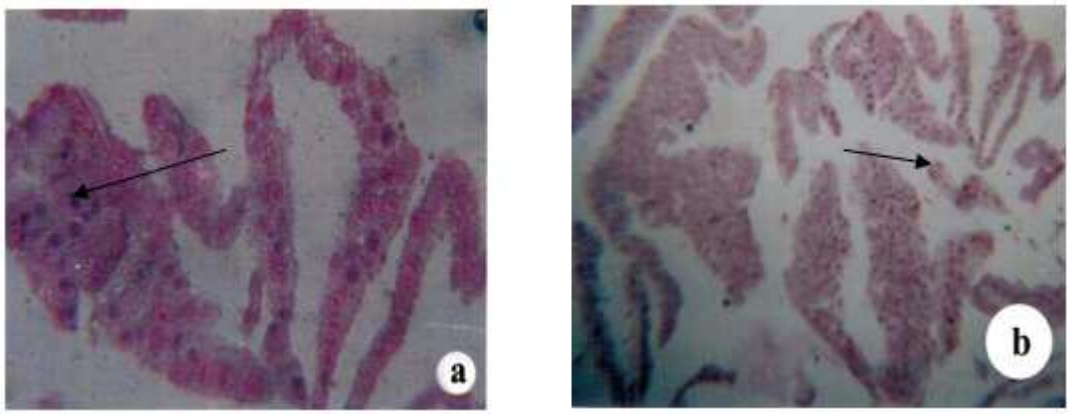
Microscopic examination was identified in tissues of silkworm fed with botanicals sprayed batch and there was no changes observed in fat body with normal columnar epithelium had striated border (microvillus) covered by the peritrophic membrane the fatbody of silkworm is organized into thin lobes of highly tracheated tissue suspended in the haemolymph. It presents in many locations within the body cavity. It is composed of adipocytes (also called trophocytes) which are prominent metabolic–storage cells contains filamentous mitrochondria, free ribosomes and glycogen and urocytes that sequester uricacid for storage and excretion. The metabolically active adipocytes are outer most in the lobe with urocytes situated within the lobe. The more exterior position of adipocytes gives them maximal exposure to the haemolymph for metabolic exchange. (PlateI-C&D). In the silkworm fat body, the silkworms fed with tukra infected chawki leaves, at day 5^{th} the nucleus of fat body cells were shown larger i.e. hypertrophied that in the normal cells. Vacuoles appeared in the cytoplasm were somewhat less and the membranous sheath surrounding the fat cells were slightly destructed (Plate I-A& B). In the silkworm the silk gland when fed with normal and botanical sprayed mulberry fed leaves there was no significant damage to the inner and middle

layers (Plate II-C) and normallacy of epidermal layer and vacuolization in the cells of outer layer was observed (Plate II-D). In the tukra affected leaves fed to silkworms the nuclei of the outer layer of the silkgland were slight by somewhat hypertophied, vacuolization appeared in the cytoplasm with mild degree of degeneration of cells (Plate II-A&B)were formed in the nuclei and vacuolization appeared in the cytoplasm of the epithelial cells appeared.

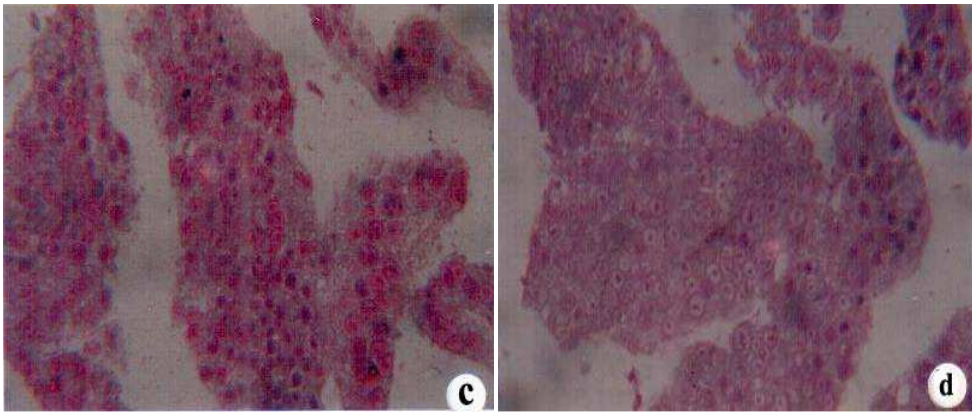
PLATE – 1



Transverse Section of fatbody of Fifth instar day 5th PMXNB4D2 hybrid Silkworm, *Bombyxmor*i when fed with normal mulberry leaves H&E, (100X&400X)

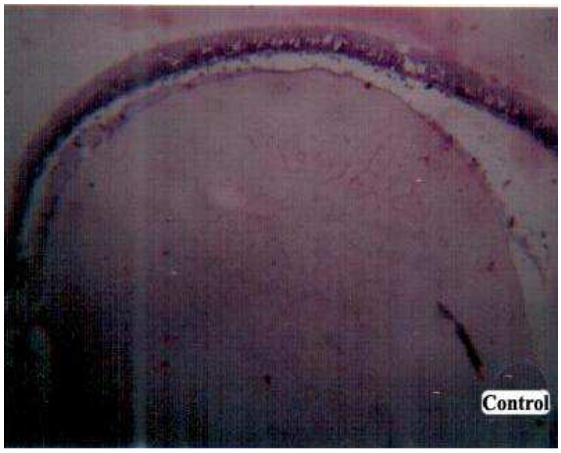


Transverse Section of fatbody of Fifth instar larvae at day 3 & day 5 of PMXNB4D2 hybrid Silkworm, *Bombyxmor*i when fed with Tukra diseased mulberry leaves H&E, (100X&400X)

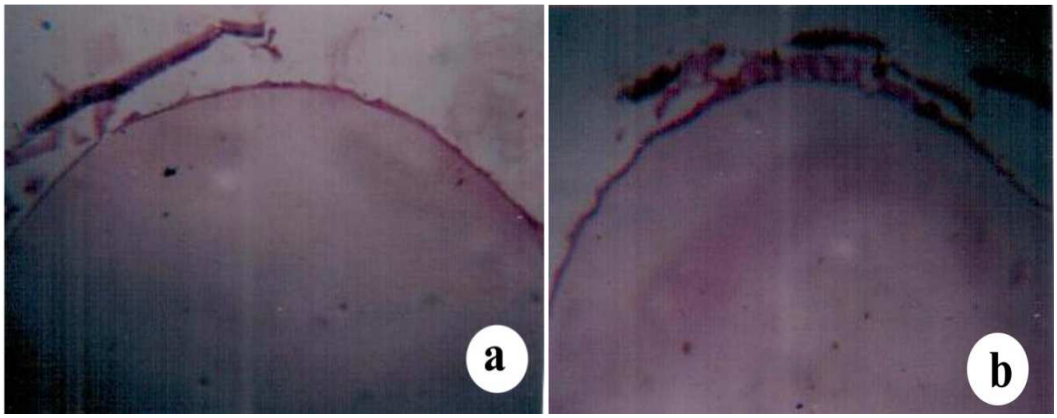


Transverse Section of fatbodyof Fifth instar at day 3 & 5 of PMXNB4D2 hybrid Silkworm, *Bombyxmor*i when fed with botanical extracts sprayed mulberry leaves H&E, (100X&400X)

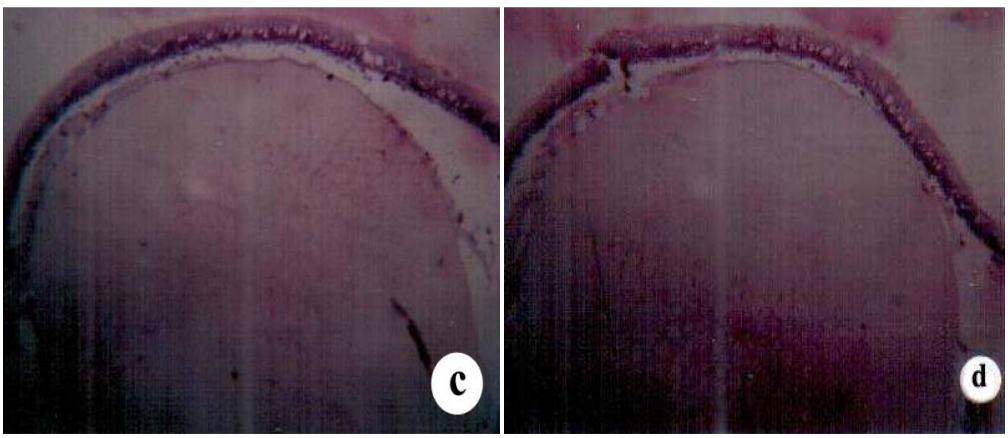
PLAT – 2



Transverse Section of silkgland of Fifth instar larvae at day 5th of PMXNB4D2 hybrid Silkworm, *Bombyxmor*i when fed with normal mulberry leaves H&E, (100X&400X)



Transverse Section of silkgland of Fifth instar larvae at day 3 & day 5 of PMXNB4D2 hybrid Silkworm, *Bombyxmor*i when fed with Tukra diseased mulberry leaves H&E, (100X&400X)



Transverse Section of silkgland of Fifth instar larvae at day 3rd & 5th ofPMXNB4D2hybrid Silkworm, *Bombyxmor*i when fed with botanical extracts sprayed mulberry leaves H&E (100X & 400X)

DISCUSSION

The accurate diagnoses of silkworm based on the external symptoms are not specific and it requires microscopic examination of haemolymph and other tissues of the silkworms when it effects with malnutrition. Histological responses of silkworm at different level of feeding of tukra infected show variations in various physiological and biochemical activities. From the present study it is clear that malnutrition and water deficient leaf shows significant histological changes in different tissues of the hybrid silkworm. However, most of these changes occurred in fat

body and some symptoms in the silk gland when fed with tukra normal and botanical extracts administrated on mulberry leaves (or) pest stress to silkworm when insect feeds. On exposure of the hybrid silkworms at different levels with tukra diseased chawki leaves were showing a significant effect on the structure of the silkworm.

The fat body is the principal tissue for intermediary metabolism in insects. It is in reality a tissue of considerable metabolic activity and is the main source for the haemolymph proteins, lipids and carbohydrates; those serve as precursors for various metabolic activities in other tissues. On exposure of the silkworm tissue when fed with normal and sprayed mulberry leaves there was no change in the fatbody when fed to silkworm, normal vaculization appeared in cytoplasm of cells. The magnitude of these changes however, is more at day 5th in tukra affected leaves when fed by silkworm. Valantina Sangamithirai *et al., (*2014) have reported changes in fat bodies and silkgland of silkworm when fed with V1 mulberry leaves treated with nano particles and supplements enhances the feed. Tadasu Mori *et al*., (1990) reported that the biochemical and physiological function of the fat body in silkworm *B. mori* microscopic observations have been carried out on the fat body in both fed with normal mulberry leaves and starved larva that the lipodial bodies decreased in number and become less electron dense in the fat body cell of starved larva. In the silkworm fat body the nucleus of fat body cells were shown larger vacuoles appeared in the cytoplasm were somewhat less and the membranous sheath surrounding the fat cells were slightly destructed when fed with tukra affected chawki leaves. Vail *et al.,* (1973) observed that mealybugs infestation in mulberry is more at tukra affected leaves with lack of biochemical components when fed on mulberry, insect cells is characterised by hypertrophy of the cell nucleus, cell enlargement and tissue dryness occurs. Umesh Kumar *et al*., (1990) reported that the damage of fat body cells shows the decrease of biochemical constituents of total lipids when fed with tukra and healthy mulberry leaves varied from one variety to another.

The silk gland, which is second largest organ in the body which occupies most of the ventro-lateral side of the body. In the botanicals sprayed leaves when fed by silkworm there was no damage in the cells compared to normal silkworm and there was no difference in the epidermal layer of silkgland this sign is due to acceleration of metabolic activity. The inner fibres in and sericin layers however are not affected which indicates that these layers could with stand without infestation of mealy bugs on mulberry at the time of feed to silkworms. The tukra affected leaves when fed with silkworms the damage was restricted only to the outer layer and no marked differences are observed in inner layers depending upon the nutrition and impact of sprayed mulberry upon pests with mulberry feed or nutritionally normal as good healthy leaves taken by silkworm. Aruga *et al*., (1957) observed formation of inclusion bodies in the middle and posterior positions of the silkgland of silkworms. Masatoshi Kobayashi (1956) observed that during V instar the mucus like substance in the silkgland is also observable just after first feeding and gradually increases the amount and there after decreases the mucus with lack of nutritive value present in the mulberry when fed by silkworms.

CONCLUSION

In the present investigation the impact of the crude extract on the silkworms tissues was tested and found that when silkworms fed with normal and crude botanical extracts against mealy bugs shows normalcy, but in the tukra infected mulberry leaves fed by silkworms the tissues shows slight degenerative with nutritional impact upon them.

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