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**RESEARCH ARTICLE**
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# HISTO-MORPHOMETRIC ANALYSIS OF UTERINE TISSUE IN ALBINO MICE FOLLOWING EXPOSURE TO THE ANTHROPOGENIC CHEMICAL THIAMETHOXAM

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**ABSTRACT**: The use of Thiamethoxam (Brand name Actara 25 WG) is commonly used as insecticide mostly in tea gardens and paddy field as well to destroy the bugs and beetles and eliminates a broad range of soil pests. The present study was conducted to analyze the effect of thiamethoxam on uterine endometrial lining of Swiss albino mice. Six adult mice were divided into three groups of which Group-1 consists of two untreated mice maintained in laboratory condition, the Group-2 consists of two mice given daily Actara at the dose of 2mg/gm body weight and the Group-3 consists of two mice given daily Actara at the dose of 4mg/mg body weight for a period of 14 days administered via oral gavage. Histopathological study was performed following the processes of microtomy per the method of (Luna et al., 1968), and histomorphometric study was performed as per method of (Girija et al., 2015). Histopathological changes like endometrial edema, endometrial epithelial hyperplasia of the mucosa or glands, hyperplasia/edema of the myometrium and the result is significant at $p < 0.005$. when morphometric analysis of the epithelial layers of the uterus study were done by one-way ANOVA between the control and treated groups.

**Keywords:** Histology, Histomorphometry, Actara, ANOVA.

## INTRODUCTION:

These days pollution of the environment by pesticides is a problem of great importance and is of every body's concern. There is a great concern that like other chemicals, these pesticides may modify the normal functioning of human and wild animal's endocrine system. Organophosphate (OPs) chemicals are commonly used worldwide to kill or repel pests in agriculture and horticulture and are also used in the home. Pesticides are natural or synthetic chemicals used to control pests. In order to support an expanding population there is a continuous need for pesticides (such as insecticides, herbicides, fungicides and acaricides). Worldwide, there are thousands of pests including insects, weeds, fungi, bacteria, viruses, mycoplasma and nematodes that destroy crops, transmit diseases and compete for resources. Thiamethoxam (TMX) is a synthetic organic insecticide included in the class of neonicotinoids, the most important new class of insecticides developed in the last three decades. Since their introduction onto the market in 1991, neonicotinoids

have been the fastest growing class of insecticides, due to their expected moderate toxicity to mammals and their advantage in combating insects that are resistant to other pesticide classes (Bingham et al., 2008). Thiamethoxam uses for control of aphids, whitefly, thrips, rice hoppers, rice bugs, mealy bugs and some lepidopterous species (Maienfisch, *et al.,* 2001). Although these chemicals have many beneficial purposes, they can also cause adverse effects in both humans and animals. Pesticides have chronic health effects both as the sequel of acute poisonings and through chronic exposure. Many studies have documented the adverse health effects of pesticide exposure on humans. There is also convincing evidence that pesticides play a role in human cancers. There are several areas of concern that need to be extensively investigated. These chemicals are widely used in industry, agriculture, home and gardens for many different purposes, including the protection of seed grain during storage and germination. Individuals who are exposed to these chemicals include agricultural workers, those living in proximity to farms/orchards or consumers who are exposed to pesticide residues in food.

Thiamethoxam and thiacloprid are the only two neonicotinoids shown to have carcinogenic effects (Green *et al.,* 2005, Green *et al.,* 2005 and Tomizawa and Casida., 2005). Thiamethoxam is a hepatotoxicant and hepatocarcinogen in mice for long day's exposure. The mode of action was determined to initially involve a reduction in plasma cholesterol, then single cell necrosis and increased apoptosis followed by an increase in hepatic cell replication rates. The higher toxicity of Thiamethoxam can be known as more bio accumulative (Guillen J *et al.,* 2013). Since the major discovery of DDT, advances have continued with the synthesis and commercialization of hundreds of pesticides including five major neuroactive insecticide classes all with unique toxicity profiles (in both insects and mammals) and target sites. DDT has low acute toxicity to mammals, but is persistent in the environment which ultimately led to it being banned. Other problems from DDT include its potential carcinogenicity, thinning of bird eggshells and fish death. Neonicotinoids, the most important class of insecticides, have favourable mammalian and environmental toxicology and now account for approximately 25 percent of the worldwide insecticide market value.

## MATERIALS AND METHOD:

**Animal: -** The examined animals in this study were adult of Balb/c mice of 20±1g were obtained from the Animal Breeding House of Gauhati University. Mice were kept under the laboratory conditions of 20-25 °c and 12hrs dark/light cycle in plastic cages for one month as an acclimatization period. They were housed in special healthy standard cages and a standard mice diet was given. The maintenance and handling of the animals were done according to the guidelines of the Committee for the Purpose of Control and Supervision of Experimental Animals, Ministry of Environment and Forests, Government of India. The experimental protocols were approved by the Institutional Animal Ethical Committee of the University.

**Experimental design: -** The experimental animals were grouped into three groups-

- Controlled group- consisted of 2 untreated mice, maintained in laboratory conditions in mice box/containers with tap water which was changed daily.
- Treated group 1- consists of 2 mice treated with Actara at the dose of 2mg/kg body weight.
- Treated group 2- consists of 2 mice treated with Actara at the dose of 4mg/kg body weight.

## RESULTS: -

**Histopathological findings**- After sacrificing the animals followed by the experimental procedures. Uterine tissue were removed quickly. The organs were dissected and fixed in 10 % neutral formalin, dehydrated in ascending grades of alcohol and imbedded in paraffin wax. Paraffin sections (5µm thick) were stained for routine histological study using Hematoxylin and Eosin stain (H&E) (Bancroft and Stevens, 1982).The slides are then observed under the microscope (LABOMED Lx400). In histomorphometric analysis the tissue cross section area and total scanned area were measured in millimeter scale by using software AmScope (version 3.7). The determination of the length of the perimetrium, myometrium & endometrium were done by taking the measurement

from six random regions. Then the image data analysis were done by one-way ANOVA between the control and treated groups.

Microscopic examination of uterine section of control mice showed normal structure endometrium comprises a lamina propia and a simple columnar epithelial extending tubular endometrial glands into the lamina propia. After the treatment with Thiamethoxam the histological structure shows several alternations. Each Hematoxylene and Eosin (H&E) stained tissue section was studied for histopathological changes observed in the uterus (endometrial edema, endometrial epithelial hyperplasia of the mucosa or glands, hyperplasia/edema of the myometrium). Small focal patches of abnormal mucosal cells, degeneration of the epithelial lining and few of the endometrial glands.

**Morphometric data of Treated group :-( Table-1)**

| Group | Perimetrium | | Myometrium | | Endometrium | | Lumen | |
|---|---|---|---|---|---|---|---|---|
| | Length | Average | Length | Average | Length | Average | Length | Breadth |
| Group 1 | 13.77 | 8.82 | 4.03 | 8.12 | 70.04 | 40.31 | 68.40 | 14.74 |
| | 6.55 | | 5.03 | | 21.00 | | | |
| | 7.71 | | 10.56 | | 55.04 | | | |
| | 4.23 | | 11.42 | | 57.64 | | | |
| | 6.23 | | 7.06 | | 11.95 | | | |
| | 14.44 | | 10.62 | | 26.24 | | | |
| Group 2 | 9.17 | 9.011 | 8.99 | 10.54 | 74.61 | 42.70 | 77.92 | 15.14 |
| | 9.82 | | 4.94 | | 16.19 | | | |
| | 7.12 | | 7.89 | | 38.95 | | | |
| | 10.32 | | 12.53 | | 77.27 | | | |
| | 10.06 | | 9.20 | | 29.93 | | | |
| | 7.58 | | 19.70 | | 19.30 | | | |
| Group 3 | 9.79 | 10.13 | 11.60 | 10.68 | 51.98 | 44.47 | 67.02 | 15.88 |
| | 6.29 | | 16.40 | | 69.30 | | | |
| | 12.96 | | 8.00 | | 20.66 | | | |
| | 8.08 | | 7.62 | | 13.50 | | | |
| | 11.48 | | 10.50 | | 67.23 | | | |
| | 12.23 | | 9.96 | | 44.19 | | | |
| Group 4 | 8.99 | 10.03 | 7.94 | 9.10 | 47.55 | 43.11 | 74.37 | 15.82 |
| | 5.45 | | 10.71 | | 69.58 | | | |
| | 13.23 | | 9.63 | | 10.47 | | | |
| | 12.24 | | 7.95 | | 51.83 | | | |
| | 10.11 | | 11.26 | | 58.78 | | | |
| | 10.21 | | 7.14 | | 20.47 | | | |

**Morphometric data of Control group :-( Table-2)**

| Group | Perimetrium | | Myometrium | | Endometrium | | Lumen | |
|---|---|---|---|---|---|---|---|---|
| | Length | Average | Length | Average | Length | Average | Length | Breadth |
| Control Group | 24.74 | 27.37 | 36.80 | 31.72 | 92.96 | 126.38 | 351.37 | 28.43 |
| | 27.20 | | 20.00 | | 133.42 | | | |
| | 30.00 | | 29.15 | | 106.63 | | | |
| | 20.88 | | 38.28 | | 160.42 | | | |
| | 41.40 | | 38.08 | | 151.21 | | | |
| | 20.00 | | 28.02 | | 113.65 | | | |

**Analysis of the significance by One-way ANOVA-**

The one-way analysis of variance (ANOVA) is used to determine whether there are any statistically significant differences between the means of three or more independent (unrelated) groups. The one-way ANOVA compares the means between the groups you are interested in and determines whether any of those means are statistically significantly different from each other. Specifically, it tests the null hypothesis. (www.statistics.laerd.com)

$$H0: u1 = u2 = u3 = \cdots = uk$$

The morphometric analysis of the epithelial layers of the uterus were done to study whether any significance between and within the groups of treated and control mice were found or not. The phomicrograph image data analysis were done by one-way ANOVA between the control and treated groups. (Where P value <0.05 was considered statistically significant). By putting the value in ANOVA calculating website www.socscistatistics.com. The result of One-way ANOVA found that the *f*-ratio value is 20.38642. The p-value is < 0.0001. The result is significant at p < 0.005.

**Treatments**

| | 1 | 2 | 3 | 4 | 5 | Total |
|---|---|---|---|---|---|---|
| N | 6 | 6 | 6 | 6 | 6 | 30 |
| ∑X | 164.42 | 52.93 | 54.07 | 60.83 | 60.23 | 392.48 |
| Mean | 27.4033 | 8.8217 | 9.0117 | 10.1383 | 10.0383 | 13.0827 |
| $\sum X^2$ | 4809.882 | 557.1789 | 496.3781 | 650.0195 | 641.8293 | 7155.2878 |
| Std. Dev. | 7.8003 | 4.2485 | 1.3504 | 2.5809 | 2.7284 | 8.3472 |

**Result Detail**

| Source | SS | df | MS | Total |
|---|---|---|---|---|
| Between Treatments | 1546.4863 | 4 | 386.6316 | F= 20.3864 |
| Within Treatments | 474.1165 | 25 | 18.9647 | |
| Total | 2020.6028 | 29 | | |

**Table-3 One-way ANOVA showing statistically significant in Uterine Perimetrium.**

**Treatments**

| | 1 | 2 | 3 | 4 | 5 | Total |
|---|---|---|---|---|---|---|
| N | 6 | 6 | 6 | 6 | 6 | 30 |
| ∑X | 190.33 | 48.72 | 63.25 | 64.08 | 54.63 | 421.01 |
| Mean | 31.7217 | 8.12 | 10.5417 | 10.68 | 9.105 | 14.0337 |
| $\sum X^2$ | 6304.5277 | 446.0998 | 797.2067 | 735.036 | 511.4543 | 8794.3245 |
| Std. Dev. | 7.3067 | 3.1778 | 5.1078 | 3.1831 | 1.6762 | 9.9759 |

**Result Details**

| Source | SS | df | MS | Total |
|---|---|---|---|---|
| Between Treatments | 2373.4182 | 4 | 593.3545 | F= 28.93891 |
| Within Treatments | 512.5923 | 25 | 20.5037 | |
| Total | 2886.0105 | 29 | | |

**Table- 4. -One-way ANOVA showing statistically significant in uterine Myometrium.**

**Treatments**

| | 1 | 2 | 3 | 4 | 5 | Total |
|---|---|---|---|---|---|---|
| N | 6 | 6 | 6 | 6 | 6 | 30 |
| ∑X | 758.29 | 241.91 | 256.25 | 266.86 | 258.68 | 1781.99 |
| Mean | 126.3817 | 40.3183 | 42.7083 | 44.4767 | 43.1133 | 59.3997 |
| $\sum X^2$ | 99327.7779 | 12529.7129 | 14584.8185 | 14586.125 | 13772.458 | 154800.8923 |
| Std. Dev. | 26.4342 | 23.564 | 26.9845 | 23.3113 | 22.8906 | 41.085 |

**Result Details**

| Source | SS | df | MS | Total |
|---|---|---|---|---|
| Between Treatments | 33703.3608 | 4 | 8425.8402 | F= 13.81474 |
| Within Treatments | 15247.9195 | 25 | 609.9168 | |
| Total | 48951.2803 | 29 | | |

**Table- 5. One-way ANOVA showing statistically significant in uterine Endometrium.**

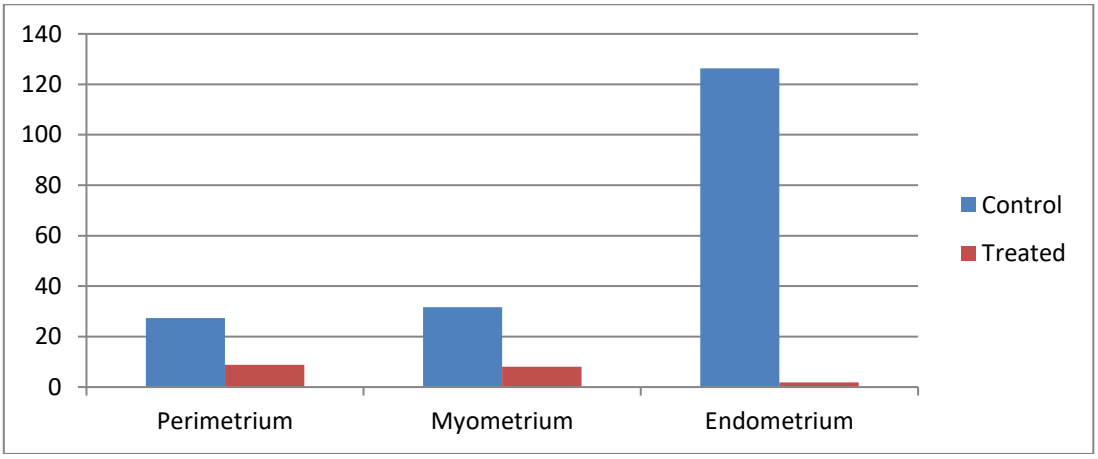


**Table 6- Bar graph showing the relative changes in the uterine epithelial layers between control and treated groups. (Adverse effect on endometrial layer)**

## DISCUSSION AND CONCLUSION:

The present study was done to investigate the toxic effect of Thiamethoxam on the histological structure of Uterus in Swiss albino mice. To meet the human need on agro-based products the agricultural industries need to increase the productivity by 50%, the damages caused by the pests is of major concern. To eradicate the pest damages by the use of pesticides is increased by a maximum level. Among several commercial pesticides available on market, Thiamethoxam is one among them, Thiamethoxam (TMX) is one of seven neonicotinoid insecticides currently sold in the market. Neonicotinoids are the most important new class of insecticides that acts selectively as agonists of the insect nicotinic acetylcholine receptors (AChRs) (Matsuda K *et al.*, 2001). The findings of the present study clearly shows that exposure to Thiamethoxam in Swiss albino mice resulted in uterine endometrial edema, endometrial epithelial hyperplasia of the mucosa or glands, hyperplasia/edema of the myometrium. Small focal patches of abnormal mucosal cells, degeneration of the epithelial lining and few of the endometrial glands. There is increasing evidence that environmental exposures, specifically to pesticides, should also be considered potential risk factors for uterine as well as other organs.(Akshay *et al*, 2017) Certain insecticides, herbicides, and fungicides have been previously reported to be endocrine disruptors which acts on gonadal organs and causes effects to some extent, like the degeneration of the uterine endometrium (endometrial hyperplasia). Exposure to endocrine disturbing (ED) chemicals during critical periods of development as prenatal or early postnatal period could result in adverse effects to wildlife and humans. (Essam Eldin Salama *et al*, 2015). Uterus undergone atrophic condition in treated group

animals which was confirmed by the endometrial thickness and the endometrial glands readings. (Lee,R.A., 1969). The present study also showed that a significant ($P \leq 0.05$) decrease in the uterine epithelial layers, decrease in average length of endometrial epithelial layer as well as myometrial layer comparable with the controlled group. The exposure to DMPA induces endometrium undergoes atrophy. The endometrial thickness found to be reduced. The number of endometrial glands and the size of the endometrial glands also found to be reduced. (Girija *et al*, 2013). The morphometric analysis on the present study shows that the changes were significant. The present study shows some relative similarity with the work done by other workers on exposure to Thiamethoxam may cause several adverse effects on visceral organs as well as gonadal organs. The present study was attempt to find the toxicity of Thiamethoxam on Swiss albino mice and conclusively shows that oral gavage of Thiamethoxam established an adverse effect on uterus and constituted a toxicity sign in Swiss albino mice, and one-way ANOVA shows the significance at $p < 0.005$. The use of all insecticides especially Thiamethoxam should be limited and be under designed program.

**REFERENCES:-**


1. Haloi, A. K., Barman, T., Kalita, E., & Deka, P. (2017). Actara 25 WG Induced Histopathological Changes in Thyroid and Intestine of Swiss Albino Mice.
2. Luna et al,. (1968) "Manual of histologic staining methods of the armed forces institute of pathology". Third edition, McGraw Hill, New York, 1968, pp. 106-107
3. Bingham et al., (2008) "A susceptibility locus for lung cancer maps to nicotinic acetylcholine receptor subunit genes on 15q25." NATURE Vol 452.( 3 April 2008).
4. Maienfisch, *et al.,* (2001) The discovery of thiamethoxam: a second-generation neonicotinoid. Pest Manag.
5. Green *et al.,* (2005) a, Thiamethoxam induced mouse liver tumours and their relevance to humans. Part 1: Mode of action studies in the mouse. Toxicol. Sci
6. Green *et al.,* (2005) b, Thiamethoxam induced mouse liver tumours and their relevance to humans. Part 2: Species differences in response. Toxicol. Sci.
7. Tomizawa and Casida, (2005), Neonicotinoid insecticide toxicology: mechanisms of selective action. Annu. Rev. Pharmacol. Toxicol, 2005; 45: 247-268.
8. Guillen, J. (Ed.). (2013). *Laboratory animals: regulations and recommendations for global collaborative research*. Academic Press.
9. Sultan, M., Alrasheed, A. A., Zaydan, M. A., Al-Bayati, H. H., Jumaa, Q. S., Noomi, B. S., & Abdulhussain, N. J. (2024). The pathological changes associated with the genus of Streptococcus in immune modified model of rats. *SVU-International Journal of Veterinary Sciences*, *7*(2), 43-54.
10. Colborn, T., Vom Saal, F. S., & Soto, A. M. (1993). Developmental effects of endocrine-disrupting chemicals in wildlife and humans. *Environmental health perspectives*, *101*(5), 378-384.
11. Palanza, P., Morellini, F., Parmigiani, S., & Vom Saal, F. S. (1999). Prenatal exposure to endocrine disrupting chemicals: effects on behavioral development. *Neuroscience & Biobehavioral Reviews*, *23*(7), 1011-1027.
12. Choudhary, N., Goyal, R., & Joshi, S. C. (2008). Effect of malathion on reproductive system of male rats. *Journal of environmental biology*, *29*(2), 259.
13. Sharma, A., Shukla, A., Attri, K., Kumar, M., Kumar, P., Suttee, A., ... & Singla, N. (2020). Global trends in pesticides: A looming threat and viable alternatives. *Ecotoxicology and Environmental Safety*, *201*, 110812.
14. Girija et al,. (2015) Effect of DMPA and A Polyherbal Drug on Histomorphometry of Uterus in Adult Female Rats. IJSR, Volume 4 Issue 3
15. El-Din, M. A. E. D. S., Ghareeb, A. E. W. E., El-Garawani, I. M., & El-Rahman, H. A. A. (2023). Induction of apoptosis, oxidative stress, hormonal, and histological alterations in the

reproductive system of thiamethoxam-exposed female rats. *Environmental Science and Pollution Research*, *30*(31), 77917-77930.

16. Auwal, M. S., Kumar, V., Salihu, S. I., & Sein, A. B. (2020). Subchronic oral exposure of male rats to thiamethoxam, quercetin and their combination on oxidative stress and antioxidant parameters in testes (a). *IJSN*, *11*, 85e94.