**ORIGINAL RESEARCH ARTICLE**

**ASSESSMENT OF PESTICIDE INDUCED FOOD TOXICITY ON PROGESTERONE LEVELS IN FEMALE ALBINO RATS**

**ABSTRACT**

Stress is considered to be an unavoidable phenomenon that is experienced by individuals of all ages and from different walk of life. It is the body’s response to any real or imagined danger that leads to a number of physical and emotional changes in an individual. Stress is caused by stressors there are several types of stressors present in nature like food toxicants, prolonged exposure of light, high intensity of light and contaminated water. Among all of this food toxicity is the key stress for every organism. This study focusses on the effect of acute stress of food toxicity on progesterone hormone to check the impact of toxicity of pesticides on food using malathion as a representative of toxicants. The study utilizes malathion as an organophosphate pesticide. The study was conducted in two phases where acute stress in one phase was followed by withdrawal of stress in the second phase. ELISA, t-test and ANOVA were applied to compare significant.

**KEYWORDS-** Food Toxicity, Stress, Malathion, Pesticide, Progesterone, Reproductive Health

**INTRODUCTION**

Stress is considered to be an unavoidable phenomenon experienced by individuals of all ages and from different walks of life. It is the body’s response to any real or imagined danger that leads to a number of physical and emotional changes in an individual (Attia *et al.*, 2022). Stress is also a motivation for action and sometimes can be positive depending on the situation (Li *et al.*, 2016). Chronic stress is always negative and affects health and wellness in an adverse manner (Stress., n.d.).

There are several types of stressors present in nature and amongst all of this food toxicity is one of the major stresses for every organism, as food is a basic requirement for all. There are several stressors that can increase the toxicity of the food such as chemical stressors, physiological toxicants, and biological toxicants (Capozzi *et al.*, 2009).

New ways have been developed to preserve food and also increase the flavour of food and so every organism directly or indirectly gets exposed to chemical substances which may either cause allergy or leave an adverse effect on fertility in organisms (Lekhi, 2024). On the other side there are several chemical agents being used in agriculture fields which can disrupt the endocrine system (Mnif *et al.,* 2011).

Various studies done support the idea that chemicals that are being used worldwide affect the reproductive system of women (Piazza and Urbanetz, 2019, Peivasteh-Roudsari, 2023). A chemical substance namely Monosodium glutamate (MSG) is very common in food industries to enhance the flavour of food is also a substance which is found to alter the average level of progesterone and estrogen in organisms (Abdulghani *et al.*, 2022).

The potential for exposures due to atmospheric transport and deposition of pesticides and related contaminants may pose risks to humans and wildlife also (Woodrow *et al.,* 2019).

In a similar manner chemicals used in agricultural fields include different pesticides. They increase the lifespan of the crop but accumulation of these pesticides into the organism’s body leaves a negative impact. Some of them cause alteration in the level of gonadal hormones and may further cause adverse effects on the reproductive system (Bretveld *et al.,* 2006).

Out of the several types of pesticides including insecticides, herbicides, fungicides, rodenticides, etc. insecticides are the one of the most prevalent types used in the agricultural sector. For our study we have used Malathion as a representative of these pesticides. Organophosphates include malathion, parathion and diazinon among them malathion has low toxicity level as compared to the other two (Horsak *et al.,* 1964). The studies on malathion support adverse effects when used for a long period of time.

Inspite of endocrine-disrupting effects it is used worldwide in agriculture and public premises such as offices, hospitals and health programs to keep away pests but not without causing harm (Storm *et.al.*, 2000). Malathion can be handled in various ways, such as in the form of spray, or as dust, or liquid to control the wide range of pests and insects including fruit flies, wasps, mosquitoes etc. (Arab *et al*., 2018).

It can also cause severe health risk after continuous exposure to humans and other non-targeted organisms. Many studies have well documented the function and adverse effect of Malathion on the reproductive health of mammals including humans (Koca *et al.,* 2018).

Studies have demonstrated that malathion exposure inhibits progesterone secretion, leading to reduced plasma progesterone concentrations (Elham *et al.*, 2015). Additionally, it also leads to adverse effects on wildlife and beneficial insects of society (Wankhad, 2012). Pesticides lead to residual toxicity in food and are transferred to humans on consumption. Malathion is rapidly absorbed by mammals, including humans (Jadhav *et al.,* 1992 ). Adverse effects on the level of estrogen and progesterone have been interpreted by various studies on malathion treated female albino rats (El-Sayed *et al.,* 2015).

This paper aims to check the effect of pesticides namely malathion as a  representative of food toxicity on gonadal hormone secretion of female albino rats. The results may help us understand the effect of pesticide laden food consumption and its impact on the hormone related to pregnancy and child bearing.

**MATERIAL AND METHOD**

**MATERIAL:**

The present study focuses on understanding the effect of stress on reproductive health by using female albino rats (*Rattus norvegicus domestica*). These rats are used as model organism for mammalian studies.

The presence of pesticides in food that is consumed daily is a necessary evil. Of all these varied pesticides we have used Malathion as a representative pesticide for our study. The study is based on in vivo model and use of animals under study was under ethical approval.

**METHOD:**

The rats were bought to the lab and kept for acclimatization. A total of 12 rats were used and they were of similar weight and age. The rats were kept in well-ventilated cages of size 20 inches\* in width 18 inches\* in length. They were fed food and water ad. libitum. They were kept in normal environmental conditions during the whole experiment.

For the study the rats were divided in to two groups, the control group and the experimental group. Both the groups had 6 rats each kept in their respective cages.

The experiment was conducted under two phases:

**Phase I**: Animals would be exposed to Acute Stress.

**Phase II**: Withdrawal of Acute Stress from Experimental Animals for recovery.

Phase I (Acute Stress)

During Phase I the rats in the Control group were fed with normal food including wheat grains, carrots, leafy greens and water *ad libitum*.

The rats in Experimental Group were fed with the same food mixed with 4.71 ml Malathion (in liquid form, Malathion 50% EC insecticide purity, below the lethal concentration value of malathion for Rats which is 5400mg or 5.4mL) (Malathion Technical Fact sheet. n.d.).

The Experiment was conducted for 3 days (72 hrs) to produce acute stress. Following the termination of this Phase I the blood samples were collected from both the groups through the prescribed procedure in Eppendorf tubes with anticoagulant heparin for further testing in the Lab.

Phase II (Withdrawal of Stress)

During Phase II the rats in the Control group were fed with normal food including wheat grains, carrots, leafy greens and water *ad libitum*.

The rats in Experimental Group were also fed with the same food as the Control Group. The Experiment was conducted for 3 days (72 hrs.) to withdraw acute stress and recover the effect of stress.

Following the termination of this Phase II the blood samples were collected from both the groups through the prescribed procedure in eppendorf tubes with anticoagulant heparin for further testing in the Lab.

The blood samples were tested to estimate Progesterone levels using ELISA. The results obtained were tested by One-way ANOVA (one-way analysis of variance) to relate comparison between the levels of gonadal hormones. P < 0.05 considered as significant level.

**RESULTS**

The experiment was conducted on female albino rats (*Rattus norvegicus domestica*) for ascertaining the changes in Progesterone level following acute stress due to food toxicity (malathion) or prevalent presence of pesticides in food.

The control group and the experimental group had 6 rats each (represented by R). These rats were weighed and kept in two cages after acclimatization. After the experiment was terminated the results obtained are as follows:

**TABLE I - Comparative Values of Progesterone in Phase I & Phase II**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **S. No.** | **Control Group**  **Phase I** | **Experimental Group**  **Phase I** | **Control Group**  **Phase II** | **Experimental Group**  **Phase II** |
| **R1** | 3.43 | 6.12 | 3.43 | 4.82 |
| **R2** | 4.90 | 6.67 | 4.90 | 5.78 |
| **R3** | 5.97 | 6.87 | 5.97 | 6.34 |
| **R4** | 6.76 | 6.37 | 6.76 | 6.30 |
| **R5** | 4.49 | 1.45 | 4.49 | 7.30 |
| **R6** | 5.28 | 1.65 | 5.28 | 4.32 |
| Sum of Progesterone value | 30.83 | 29.13 | 30.83 | 34.86 |
| Avg. Progesterone value | 5.13 | 4.85 | 5.13 | 5.811 |
| Variance | 1.34 | 6.63 | 1.34 | 1.18 |

Phase I: t value: 0.24582, p-value: 0.406434.

Phase II: t value: -1.03315, p-value: 0.162934.

The difference of Progesterone level between Phase I and II is 0.771311 which is > p value 0.05 and the F value is 0.375931 whereas the F critical value is 3.098391.

**GRAPH I- Comparative Graphical Representation of Progesterone Values (Phase I and Phase II)**

**GRAPH II- Graphical Representation of Progesterone Values after Phase I**

**GRAPH III- Graphical Representation of Average Progesterone Values after Phase I between the Control and Experimental Group**

**GRAPH IV- Graphical Representation of Progesterone Values after Phase II (Recovery Phase)**

**GRAPH V- Graphical Representation of Average Progesterone Values after Phase II (Recovery Phase) in both Control Group & Experimental Group**

**Phase I (Experimental Group kept under Acute Stress)**

**Difference of Progesterone level with in a group**

Control Group: Among the rats’ present in control group the highest level of progesterone was observed in R4 (6.76 ng/ml) followed by R3, R6, R2, R5 while the lowest level was observed in R1 (3.43ng/ml).

Experimental Group: The highest level of progesterone was observed in R3 (6.87 ng/ml) followed by R2, R4, R1, R6 and the lowest level was observed in R5 (1.45 ng/ml).

**Difference of Progesterone level between control and experimental group**

R1 in control group had lower Progesterone level where as R5 in experimental group had least Progesterone level. R4 of experimental group had higher Progesterone level on the other hand R3 of experimental group had highest Progesterone level.

**Phase II (Withdrawal of Acute Stress from Experimental Group for recovery)**

**Difference of Progesterone level with in a group**

Control Group:Among the rats present in control group the highest level of progesterone was observed in R4 (6.76 ng/ml) followed by R3, R6, R2, R5 and the lowest level was observed in R1 (3.43 ng/ml).

Experimental Group: Among the rats present in experimental group the highest level of progesterone was observed in R5 (7.30 ng/ml) followed by R3, R4, R2, R1 and the lowest level was observed in R6 (4.32 ng/ml).

**Difference of Progesterone level between control and experimental group**

In experimental group highest Progesterone level was found in R5 and in control group R4 had highest Progesterone level. R1 had least level of Progesterone in control group whereas R6 had lowest Progesterone level in experimental group.

**STATISTICAL ANALYSIS:**

As per above observations the obtained difference between Phase I and Phase II in terms of p value is ns= 0.771311 > 0.05. The difference within Phase I is P ns= 0.406434 > 0.05 and Phase II is ns= 0.162934 > 0.05.

t-value obtained between the control and experimental groups in Phase I is 0.24582 t-value for Phase II between the control and experimental group is -1.03315.

**DISCUSSION**

Pesticides can start creating havoc before birth. A study done by Zhang *et al.,* (2014) discusses the effect of exposure to organophosphate pesticides in prenatal conditions and its subsequent effect on neurobehavioral development.

Pesticides are harmful and can lead to severe damage due to oxidative stress. They also disturb the defence mechanism of the body (Abdollahi, 2004 ). Various researches have been done to study the effects of pesticides on the reproductive system. Pesticides in general can cause health related issues in male and female reproductive system as studied by Sifakis *et al.,* (2011). Food toxicity causes a significant threat to reproductive health by altering the hormonal balance and reducing fertility ( Dutta *et al.,* 2023).  It has been found in animal studies relating to malathion that fertility is compromised. Malathion can cause changes in reproductive cells, ovaries, uterus, testes, and sperm, after exposure (Streich *et al.,* 2024).

Considering the seriousness of the issue this study is focused to check the effect of food toxicity on gonadal hormones. The results obtained show that acute exposure of malathion below its lethal dose (LD) does not create any significant difference in progesterone levels. Some researches done to understand the effect of malathion on the reproductive system of male rats as well as female rats have found to have little or no harmful consequences  (Agency for Toxic Substances and Disease Registry, 2003).

On the other hand, some studies mention serious effects post exposure to pesticides (Rupa *et. al.,* 1991). The study states that the pesticide organophosphate causes toxic effect on reproductive health on female albino rats (Kaur and Dhanju, 2005).

There are several studies that interpreted that the food toxicant (malathion) affects organisms in various ways compiled by Toxicological Profile for Malathion - NCBI Bookshelf (2003).

Studies show that women exposed with malathion spray while working in the fields were able to deliver the child and the exposure did not cause any complication in pregnancy (Thomas *et al.,* 1992).

Hence various contradictory studies are available so further research in these issues is needed to come to a conclusion and maintain physiological health. Increasing awareness and implementing protective measures are crucial to minimize the risk and are also necessary for protecting reproductive health (World Health Organization, WHO, 2024).

**CONCLUSION**

Pesticides are known to induce stressful conditions at physiological levels and cause disruptions at endocrine levels further leading to health implications. Studies related to this have provided sufficient evidence of health-related issues especially reproductive health. This study done on the effect of acute stress due to pesticide induced food toxicity has indicated no major change as the results obtained show that the effect was non-significant. This study reveals that acute stresses can be managed by the body and their effects can be reversed if they are withdrawn within a suitable time period. Further studies are needed to conclude this effect. On the other hand, studies on chronic stress also need to be conducted so as to understand long term effects of stress induced toxicity.

**DISCLAIMER (ARTIFICIAL INTELLIGENCE)**

Author(s) hereby declares that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

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