# Effect Of Atrazine On Expression Of Matrix Metalloproteinase, Blood Smear, Reproductive Hormone And Histological Studies Of Reproductive System Of Albino Rat

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## ABSTRACT

Over the years, use of triazine herbicide atrazine have increased many folds, simultaneously harboring a persistent environmental presence disrupting many developmental processes and reproductive well-being in non-target animals. It is concerning to flag that researches having found bioaccumulation of atrazine in food chains, said to pose serious threats on various organ systems in humans including digestive, and immune systems. On the contrary, existing data couldn't sufficiently claim to prove the potential toll on reproductive health. This study aims to elucidate the recent findings on altered activity of Matrix-metalloproteinase, with respect to exposure to varying concentrations of Atrazine. Five-week Wister rats of both sexes were administered atrazine via food pellets to detect changes in blood cell count, gonadal histology, hormonal matrix, and pronounced up regulation of matrix metalloproteinase (MMPs). On top of that, our findings reveal, prolonged exposure to atrazine leads to histomorphological changes at levels of epithelial tissue and underlying layers. Exposure to high doses of atrazine caused the test animals to lose body weights and displayed external morphological changes such as changes in body colour and loss of body hairs. We might draw conclusion based on the results obtained, that atrazine exposure revealed hormonal imbalances in both the sexes, and reduced follicular populations in male testes and female ovaries alike.

**Key Words:** Atrazine, albino mice, MMPs, Estrogen, Testosterone.

# INTRODUCTION

Prolonged exposure to chlorinated triazine group of selective, systemic herbicide atrazine poses a serious threat to non-target organisms like humans and leads to bioaccumulation in the ecosystem. According to the World Health Organization report in 2012, pesticides negatively impact the reproductive system of humans and wildlife around the world. Atrazine (ATR), an herbicide of the chrolo-s-triazine class, is one of the most extensively utilized pesticides worldwide, which is used in agriculture for its effectiveness in controlling broadleaf weeds and enhancing crop yields. ATR exhibits slow degradation in the environment, leading to its widespread presence as a persistent pollutant in various environmental mediums, including soil, surface water, and groundwater across numerous countries and regions. Its long-term residue and extensive use may be harmful to ecological environment and human health. Possible adverse effects on nervous system, endocrine system, and immune systems have been reported.

The immune system is a tight-knit network, which is a solid defense against effects of chemical exposure. A long-term immunotoxicity study of atrazine showed that perinatal atrazine exposure altered the T-independent, but not the T-dependent, antibody response to a bacterial antigen in male offspring, and significant decreases in IFN-γ and a decreasing trend in IL-10 were observed in splenocytes from atrazine-exposed mice.

In a series of studies in rodents, it has been shown that exposure to ATZ alters reproductive function in males and females. Specifically, chronic exposure to ATZ during adulthood impairs folliculogenesis in female rats and leads to increased follicle atresia and the production of multi-oocyte follicles (MOF). The changes in follicular morphology were associated with abnormal endocrine function. It is suggested that the effects of ATZ on ovarian

function in rats are mediated through Luteinizing Hormone (LH) and prolactin (PRL), which are known to control the secretion of steroid hormones. High doses of ATZ inhibit the LH surge and reduce the number of ovulated follicles in rats. Exposure to ATZ significantly increases the reproductive cycle length in rats. Exposure to ATZ in other animal species similarly has endocrine-disrupting effects.

It is also believed that the morphological changes in the testes of rats caused by atrazine might be irreversible after a time longer than the duration of spermatogenesis. It is known that atrazine decreases testosterone level, making it a weak anti-androgen for tissues requiring testosterone. In this case, estradiol level was increased causing testicular atrophy, estrogen-mediated toxicities and inappropriate sexual differentiation. This appears to support the hypothesis that aromatase enzyme might be a direct potential target for atrazine in the testis of vertebrates.
The aim of this study was to investigate the effect of ATZ on expression on Matrix Metalloproteinase, Blood Smear, Reproductive Hormone and Histology of Reproductive system of Albino Rats. Matrix metalloproteinases (MMPs) compose a family of Zinc and Calcium dependent endopeptidases responsible for the remodelling and degradation of ECM proteins, such as collagen, elastin, laminin, fibronectin and proteoglycans. Results showed, atrazine up regulated MMP-2.

# AIMS AND OBJECTIVES

1. **To blood smear study**, we can observe the blood cell shape and type by studying blood smear of Control mice and mice treated with Atrazine.

2. **To analyse serum FSH and LH and Testosterone levels**, in the "control" as well as in the "Atrazine Treated" serum sample using ELISA. In an ELISA, an antigen must be immobilized to a solid surface and then complexes with an antibody that is linked to an enzyme detection is accomplished by assessing the conjugated enzyme activity via incubation with a substrate to produce a measurable product. The most crucial element of the detection strategy is a highly specific antibody – antigen interaction.

3. **Protein Measurement through spectrophometry**. It is done through Bradford Assay which is a calorimetric method.

4. **To examine the effect of Atrazine on developmental enzyme studies**, the objective of our study was to observed the role of MMPs in mice after treatment. Zymography is defined as an electrophoretic technique, that is used to study hydrolytic 9 enzymes. Gelatin zymography is used for the detection and analysis of gelatinase (MMP – 2).

5. **Histological studies**, we could observe the effect of Atrazine on the structure of Ovary and Testis after an experimental administration.

# METHODS AND MATERIALS USED

## 1. CULTURE AND HANDLING OF ALBINO RATS:

A total of 12 Winstar albino rats were used in this study, which were divided into 3 groups. 1 "control" group with 3 females and 3 males and 2 other "treatment" groups consisting of 3 males in one and 3 females in another. The average weight of the rats was about 30 grams. Sufficient amount of Atrazine was taken for feeding the rats except for those in the "control" group.

## 2. CARE AND TREATMENT:

The animals were arranged into small "treatment" groups separating male and female, where each group consisted of 3 rats so the experiment can be carried out efficiently and there is no breeding among the specimens. Thus, total 2 small "treatment" groups of 3 males and 3 females were created. Then they were kept in a controlled environment (25.2°C temperature
,15% humidity and typical photoperiod of 12hrs dark and 12 hrs. light) for the duration of the experiment.

After that, each cage was provided with food and water.

## 3. FOOD MATERIALS AND OTHERS:

For food, small dough of gram flour mixed with milk powder and water (as per requirement). Each cage was provided with 3 no. of dough's every day and 125 ml of water in a laboratory water feeding bottle. Thus, fresh food and water were served each and every day and was continued for about **7 days in** the day shift. Cage cleaning was obviously necessary for good animal health and hygiene. Cleaning of cages were done everyday as they urinated and defecated on a frequent manner.

## 4. ATRAZINE TREATMENT:

**For 7 days**, we divided them into "Control" group and "Treatment" group. For this we took two "Control" cages: Control cage A- consisting of 3 female rats and Control cage B -consisting of 3 male rats, respectively.

We took 2 "treatment" cages (which were treated with atrazine): Treatment cage C and D - each of them include 3 treated female rats and 3 treated male rats, respectively. They were treated accordingly to their weights and size (**For Male- 5mg/ individual and for Females- 3mg/ individual**).

## 5. SAMPLE COLLECTION:

**On the 8th day**, all the control and treated mice were ready for dissection. They were placed on dissecting tray and are cut ventrally. After that, the testes (from males) and ovaries (from females) as well as blood from heart were collected from both control and treated mice. We took a few Eppendorf tubes and labeled some of them as CONTROL and rest of them as TREATED. Then we poured blood from control mice into the control tube and atrazine treated blood into those tubes labelled as treated.

## 6. COLLECTION OF BLOOD FOR BLOOD SMEAR:

Blood collection from the experimental animals is one of the important events in biomedical research. Here, blood is collected from the heart of mice by using 1ml Tuberculin syringe. During blood sample collection, the mice were terminally anaesthetized. We obtained about 0.1-1ml of blood from the mice depending on their size of hearts. Blood is preferably taken slowly from the LEFT VENTRICLE to avoid the collapsing of heart (Andjelkovic et. al., 2019). To prepare a blood smear, a small blood droplet from the syringe is immediately placed on a clean slide, a second clean slide is used to spread the blood into a thin film, and then the slide is stained with Leishman stain and examined under a microscope.

## 7. COLLECTION TESTIS AND OVARY FOR HISTOLOGY:

For histological purpose, we had cut out the target organs(ovary and testis), removed fat bodies from them, sliced them transeversely by using a sharp blade and put those sections in Bouin's solution.Ovary and testis of each mouse was removed and fixed in 10% formalin solution and prepared using routine techniques for histology. Samples underwent dehydration by alcohol, clearing with xylol, embedding in paraffin wax, sectioning under 8 μm thicknesses, and staining with hematoxylin-eosin. Finally, photos were taken from theprepared slides under ZEISS microscope (Monsefi et. al., 2013).

## 8. COLLECTION OF SERUM FOR HORMONAL ASSAYS: (MEASUREMENT OF ESTRADIOL, FSH, LH AND TESTOSTERONE LEVELS):

After blood collection, it is allowed to be clotted by leaving it undisturbed at room temperature for 15-30 minutes.Then the clots are removed by centrifuging it at 10000 RPM for 10 minutes in a refrigerated centrifuge machine.The resulting supernatant is designated as the SERUM. The estradiol concentrations were measured by ELISA based on Competitive enzyme immunoassay.

**PROCEDURE:** i. Competitive ELISA, also known as inhibition ELISA, is a surface/plate based assay, where the plate is coated with capture antibodies reactive to the molecule of interest.

ii. Here, the sample (containing native molecule of interest) and enzyme conjugated recombinant protein (the competing molecule) are added to the coated wells. Since the amount of enzyme conjugated molecule in each well is constant, the level of native molecule in the sample will determine the binding ratio of enzyme conjugated molecule vs. native molecule.

iii. After an incubation period, any unbound antibody is washed off. Enzyme substrate (for example, TMB for HRP) is added to each well and will be transformed into a blue precipitate, the amount of which is linearly proportional to the amount of enzyme in the well.

iv. The precipitate is then turned into yellow by adding the acid stop solution and the concentration of yellow precipitate is read at 450nm for light absorbance (O.D. value). The

O.D. is then used to calculate the amount of molecule of interest in each well, by comparing each sample well against the standard curve.

## 9. SPECTROPHOTOMETRY:

Tissues of the testis and ovary of both control and treated group were collected and were stored. A small section of the tissue samples were manually homogenized with the help of a homogenizer. After homogenization of tissue sample (testis, ovary), supernatant was collected. After that it was mixed with 2ml of phosphate buffer solution (PBS) in an eppendorf. Then, it was centrifuged at 10,000 rpm for 10 minutes. After that, supernatant was collected.20µl of that sample and 1ml of Bradford reagent were mixed and collected in cuvette and incubated for 2-3 minutes. After that, protein samples were measured against BLANK (Bradford + double distilled water) in spectrophotometer.

## 10. TO DETERMINE MMP LEVEL (ZYMOGRAPHY):

- Matrix metalloproteinases (MMP) are zinc – containing metalloproteinases that can cleave any type of extracellular matrix (ECM) proteins (Verma et. al., 2007; Visse et. al., 2003).

- Two classes of MMP include – secreted MMP and membrane – bound MMP (Page – McCaw et. al., 2007).

- MMP1 is primarily a secreted collagenase, cleaving collagen. Whereas, MMP2 is primarily a membrane – bound gelatinase breaking down the gelatin.

Zymography is defined as an electrophoretic technique that is used to study hydrolytic enzymes. We have performed gelatin zymography.

**GELATIN ZYMOGRAPHY**: Gelatin zymography is used for the detection and analysis of gelatinase (MMP2).

**MATERIALS:** Glassplates, Combs, Electrophoretic system, Spatula, Power supply, Incubator.

**CHEMICALS REQUIRED:**

| Sl. No. | Chemicals | Components |
|---|---|---|
| I. | Resolving Gel (8%) | i. Tris Buffer pH 8.8 |
| | | ii. 30% polyacrylamide gel |
| | | iii. Casein |
| | | iv. 10% Sodium Dodecyl Sulphate (SDS) |
| | | v. 10% Ammonium persulfate (APS) |
| | | vi. N,N,N′,N′-Tetramethylethylenediamine |
| II. | Stacking Gel (4%) | i. Tris Buffer pH 6.8 |
| | | ii. 30% polyacrylamide gel |
| | | iii. Distilled water |
| | | iv. 10% Ammonium persulfate (APS) |
| | | v. N,N,N′,N′-Tetramethylethylenediamine |
| III. | Non-reducing sample buffer | i. Tris HCl, pH 6.8 |
| | | ii. Gylcerol |
| | | iii. SDS |
| | | iv. Distilled water |
| | | v. Bromophenol blue |
| IV. | 1X Running Buffer Ph 8.3 | i. Tris – based solution |
| | | ii. Glycin |
| | | iii. SDS |
| | | iv. Distilled water |
| V. | Washing buffer | i. Triton X-100 |
| | | ii. Distilled water |
| VI. | 1X Running Buffer pH 8.3 | i. Tris – HCl (pH 8.0) |
| | | ii. $CaCl_2$ |
| | | iii. NaCl |
| | | iv. $NaN_3$ |
| | | v. Distilled water |
| VII. | Destaining solution | i. Methanol |
| | | ii. Acetic acid |
| | | iii. Distilled water |

**TABLE 1 :Showing the chemical required and their components**

**PROCEDURE:** i. Both control and treated ovaries and testes are homogenized and centrifuged for 10 minute in 2 ml of Phosphate Buffered Saline (PBS).

ii. Non reducing sample buffer added to each supernatant collected.

iii. Prepare 8% resolving gel containing gelatin and 8% resolving gel is prepared containing Casein.

iv. After 15 to 20 minutes, 4% stacking gel is prepared and the comb placed on the stacking gel.Wait for 20-25 minutes for the stacking gel to polymerize.

v. Load the samples in each well after removing the comb and the gel was run at 160-170 V.

vi. Wash the gel in washing buffer for 30 minutes and then keep it in incubation buffer for 24 hrs at 370 C.

vii. The gel is washed with distilled water and stained with Coomassie Blue (R-250) solution for 1 hr to 3 hrs at room temperature.

viii. Lastly, Place the gel in destaining solution for 10-15 minutes at room temperature.

# RESULTS:

## I. Comparative study of blood smear:

In our study, we observed that there is slight decrease of RBC in the treated group compared to the control group though the shape was not changed. Increase in neutrophils in the treated group is observed with probable signs of phagocytosis. It is an inflammatory response which proves increase in the functioning of the immune system against atrazine treatment. But no extreme morphological abnormalities found on the stained blood smear of the treated group.

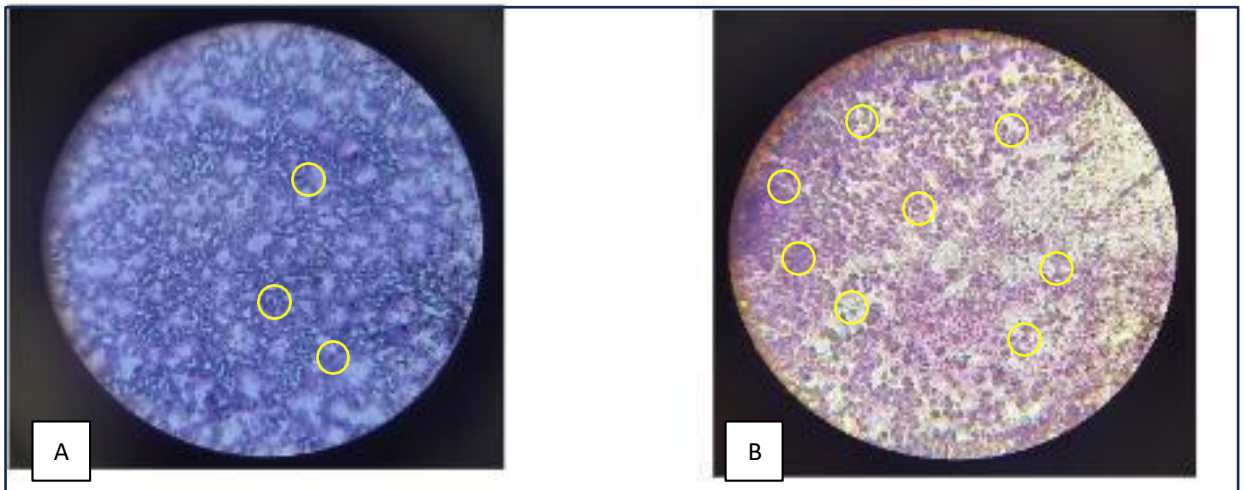


**Fig 1:** Microscopic view of blood smears of control and treatment rat. The WBCs are shown in yellow circles.

**Picture A** shows the blood smear of control. It shows that RBC is uniformly distributed throughout smear without any major clustering. A few WBC are visible and appear normal. Neutrophils are present; no abnormal increase in number. No clear sign of active phagocytosis is observed.

**Picture B** shows the blood smear of treated group. In this field, RBC distribution appears irregular; many cells show signs of distortion and crowding. A slight high concentration of WBC is visible. Increase in number of Neutrophils suggests activation. Some cells appear to engulf particles indicating mild phagocytic activity or cellular stress.

## II. Comparative Histological studies:

### A. CONTROL GROUP:

I. Histological section of ovary:

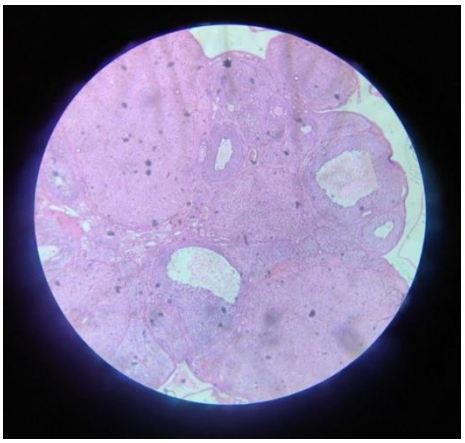

**Fig 2:** Histological section of ovary of female control rat (40X magnified)

**Key observations:**

- Primary, secondary and Graafian follicles seems to be healthy.
- In developing follicles clear zona pellucida and antrum is seen.
- The supportive tissue of the ovary (stroma) looks even, consistent and the number of cells present in the tissue is normal and equally distributed.
- Presence of corpora lutea shows normal ovulatory cycle.

**Interpretations:**

- This section shows a normal oestrous cycle, as it is seen in a healthy mammal.
- The ovary shows follicles at different stages: indicating normal follicle maturation and ovary is responding well to hormones (especially FSH as LH) .

II. Histological section of testis:

**Key observations:**

- The section shows that the shape of Seminiferous tubules varies from round to oval, they are tightly packed, and uniform in size.
- The germinal epithelium lining is well arranged, showing clearly defined layers of:

1. Spermatogonia lies in the periphery
2. Primary and secondary spermatocytes, spermatids, and spermatozoa toward the lumen.

- Sertoli cells are visible, which forms the lining of germ cells.
- The shape of the interstitial (Leydig) cells between tubules appears normal and intact.

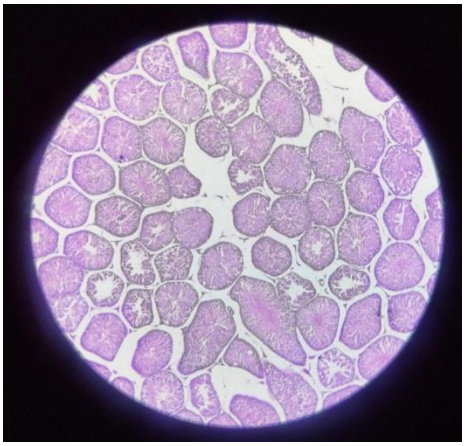

**Fig 3:** Histological section of testes of male control rat. (40X magnified)

- The cells do not show abnormal empty spaces or bubbles inside them as well as there are no dead or dying tissues present indicating that the tissue is healthy with no signs of damage, infection or poor blood supply.

**Interpretations:**

The histological section represents sperm formation process is happening properly and the tissue structure is intact with no damage or abnormalities.

- The presence of all germ cell stages indicates that the process of spermatogenesis is active and healthy.
- Normal Leydig cells suggest adequate testosterone production.
- Overall, the histological section of control testis shows a functionally normal and structurally healthy gonad.

## B. TREATMENT GROUP:

### i. Histological Section of Ovary

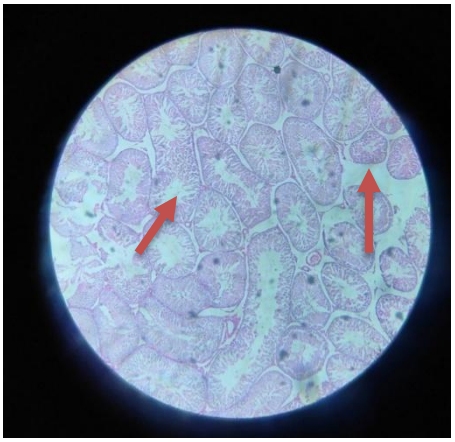

**Fig 5:** Histological section from of testes of atrazine treated male rat (40X magnified)

**Key Observations**

- Healthy follicles (primordial, primary, secondary) are reduced in number.
- Degenerating follicles has increased in number depicting abnormal folliculogenesis.
- The corpus luteum is few in number or not seen at all which means ovulation may not have occurred.
- The structure of Stromal tissue appeared loose, reflecting overall disorganization of ovarian histology.

**Interpretations:**

- Indicates ovarian toxicity and abnormal follicular development.
- Atrazine exposure likely alters hormonal balance which is essential for normal folliculogenesis.
- Ovary is showing early changes that indicates declining fertility mainly due to few numbers of active follicles.
- The disorganized tissue pattern reflects improper ovarian function.

### ii. Histological Section of Testis

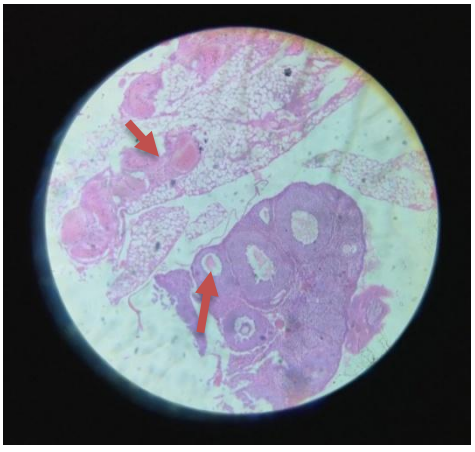

**Fig 4:** Histological section of ovary of atrazine treated female rat. (40X magnified)

**Key Observations**

- Vacuolization and widened intercellular gaps in seminiferous tubules are clearly visible.
- The thinning and degeneration of germinal epithelium shows reduced spermatogenic activity.
- Disrupted arrangement of spermatogenic layers, indicating impaired spermatogenesis.
- Leydig cells are less in number depicting low level of testosterone production.

**Interpretations:**

- Vacuolization and epithelial thinning reflect adverse effects of atrazine exposure.
- Reduced germ cells and disorganized layers indicate suppressed spermatogenesis.
- Damage to Leydig cells may lead to lower testosterone production.
- Overall, the histological section of testes shows clear evidence of toxicity and disrupted function.

**Overall Interpretation from Histological Data:**

**a. Evidence of Gonad Toxicity**

- Both ovary and testis show disorganized tissue degenerated cells.
- Consistent morphological changes depict gonadotoxic effects of atrazine.
- Suggests impaired reproductive structure and function on both males and females.

**b. Disruption of Gametogenesis**

- Degeneration of follicles imply disturbed oogenesis.
- Reduced spermatocytes and spermatids indicate impaired spermatogenesis.

c. **Oxidative Stress and Apoptosis**

- Presence of vacuolization, shrinked nucleus and apoptotic bodies suggests oxidative damage.
- Atrazine can induce ROS generation, which may cause:
  - Lipid peroxidation.
  - DNA fragmentation.
  - Mitochondrial dysfunction in germ cells

**d. Endocrine Disruption**

- Histological changes indicate interference with the HPG axis.
- In males, Leydig cell degeneration may affect the synthesis of testosterone.
- In females, improper follicular development may alter estrogen and progesterone production.
- Hormonal imbalance may influence:

➢ Secondary sexual characteristics.

## III. Comparative study of different hormones levels:

- Follicle Stimulating Hormone (FSH), Luteinizing Hormone and Estradiol levels in control and atrazine treated serum sample from female rat:

**TABLE 2 :Showing FSH, LH and Estradiol levels**

| FSH level | | LH level | | Estradiol level | |
|---|---|---|---|---|---|
| **Control** | **Treatment** | **Control** | **Treatment** | **Control** | **Treatment** |
| **12** | 4.7 | 0.67 | 3.1 | 60 | 34 |
| **12.5** | 5.1 | 0.72 | 0.29 | 63 | 33 |
| **11.5** | 4.8 | 0.76 | 0.3 | 58 | 38 |
| **12** | 4.866667 | 0.71667 | 0.3 | 60.3333 | 35 |
| **0.5** | 0.208167 | 0.04509 | 0.01 | 2.51661 | 2.64575131 |

- Testosterone levels in control and atrazine treated serum sample from male rat:

| Testosterone level | |
|---|---|
| **Control** | **Treatment** |
| **74** | 31 |
| **78** | 36 |
| **75** | 34 |
| **75.6667** | 33.6667 |
| **2.08167** | 2.51661 |

**TABLE 3: Showing different levels testosterone**

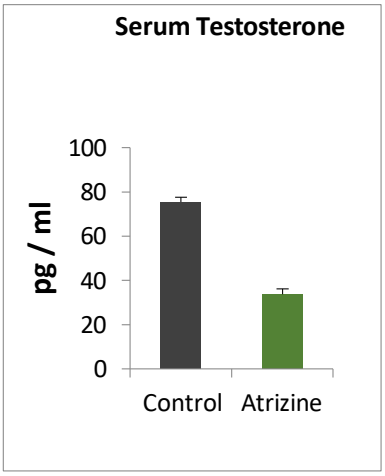


**Fig 6:** Comparative graph showing the difference between testosterone level in control as well as in atrazine treated serum sample from male rat.

## III. Comparative study of estimated protein concentration:

**TABLE 4**: Absorbance of the stock solutions of different concentrations:

| Absorbance of Stock Solution | |
|---|---|
| **Concentration (mg/ml)** | OD Value |
| **1** | 1.2 |
| **0.8** | 1.19 |
| **0.6** | 1.12 |
| **0.4** | 1.08 |
| **0.2** | 1.01 |

**TABLE 5:** Absorbance of the protein solutions from the treated and control group:

| Source of protein sample | | OD value | Conc. (mg/ml) |
|---|---|---|---|
| **Male** | Control | 1.38 | 0.85 |
| | Treatment | 1.32 | 0.81 |
| **Female** | Control | 1.12 | 0.69 |
| | Treatment | 1.20 | 0.74 |

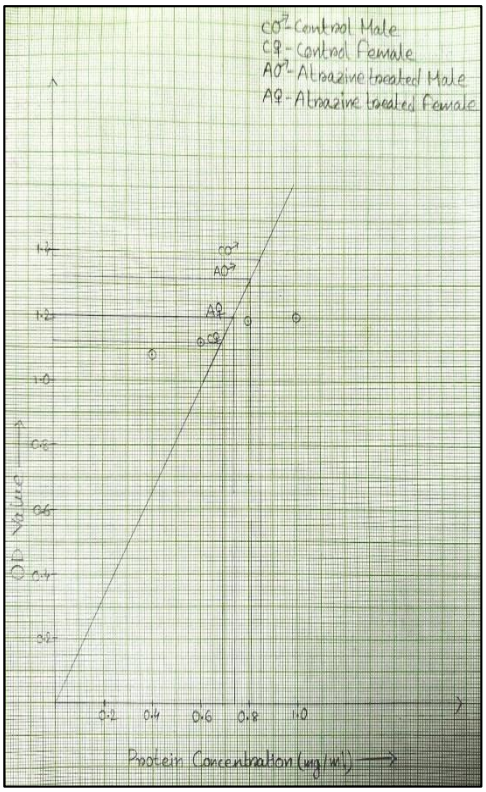


**Fig 7:** Graph showing the different OD values of different samples relying on Beer-Lambert's law. For the first step, i.e. to create the standard curve for protein estimation we prepared a series of known concentrations of a standard protein-BSA. Bradford reagent was added to each, mixed, and incubated for 5–10 minutes. Absorbance [which is the indicator of optical density (OD value)] was measured at 595 nm. Absorbance/ OD value (Y- axis) was plotted against protein concentration (X- axis) to generate the standard curve. For the final step, we plotted the OD values of our sample proteins (from both control and treatment group) in the graph and there measured their corresponding X-axis value to determine the concentrations of the samples.

From the results above we have concluded that after the treatment with atrazine, protein concentration in the gonad of male rats (i.e. testis) is had decreased while in the gonad of female rats (i.e. ovary) had increased.

## IV. Comparative study of Matrix Metalloproteinase:

Experimental study of gonadal tissue samples collected from test animals exposed to atrazine, showed significant overexpression of matrix metalloproteinases (MMPs). Treated samples exhibited darker, more prominent bands in PAGE electrophoresis. This corresponded to the molecular weights of the enzymes compared to vehicle controls, have proven the hypothesis, beyond doubt. These changes were clearly evident in PAGE electrophoresis profiles. We found a significant increase in MMP expression via the densitometric quantification, indicating enhanced proteolytic activity within the gonadal extracellular matrix (ECM). The gel was stained with Coomassie Blue, and gelatinolytic activity appeared as clear band at >72 kDa corresponding to MMP-2. ImageJ software was used to quatify the bands and normalized to total protein loaded, as determined by parallel Coomassie-stained gel.

### Interpretations:

The overexpression of MMPs is strongly indicative of dysregulated ECM remodelling, which corresponds closely with the histological abnormalities we documented—namely, fragmentation of seminiferous epithelium and basement membrane thinning in male gonads, and increased follicular atresia, stromal collagen deposition, and granulosa cell detachment in female ovaries. These findings reflect atrazine's potential to disrupt the regulatory balance of tissue remodelling enzymes that are essential for maintaining gonadal structure and function.

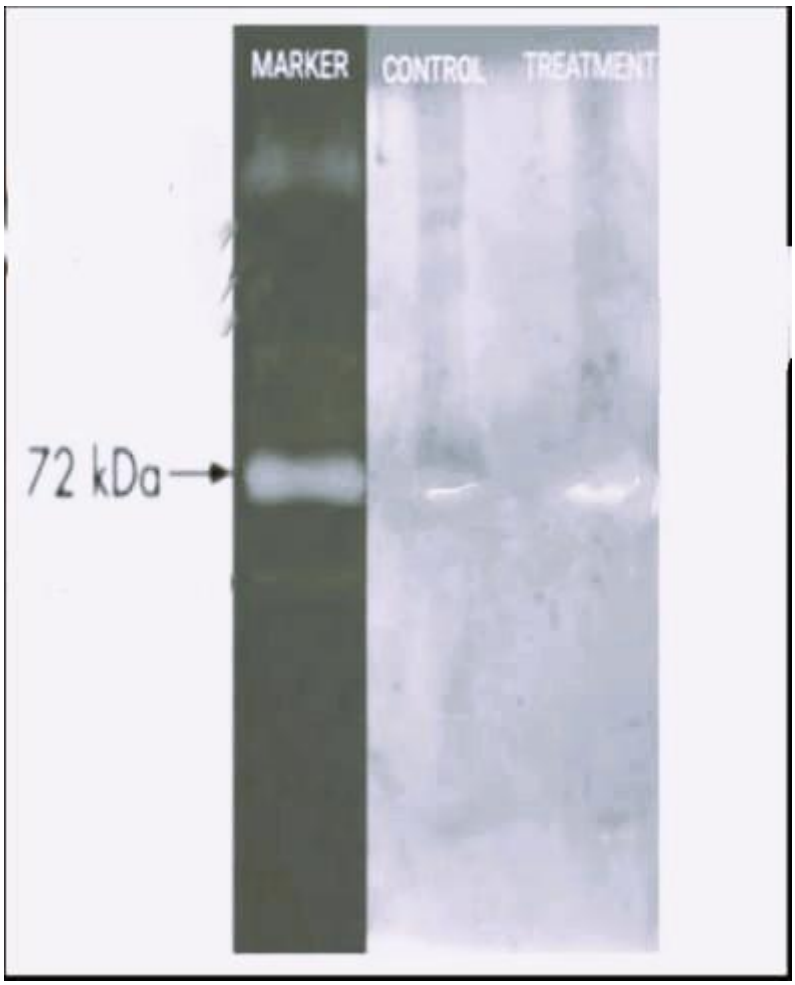


**Fig 8:MMP2 is shown by Gelatin zymography. In this figure there is clearly visible that band appeared in between 72kDa(kDa= kilo Dalton) in treatment group.**

The biochemical environment in which these changes occur may involve alterations in intracellular signalling cascades that respond to environmental toxicants. Notably, atrazine has been shown to increase reactive oxygen species (ROS) generation in reproductive tissues, a factor that can influence several downstream signalling pathways. ROS are known to activate transcription factors such as NF-κB, AP-1, and STAT3, which in turn regulate MMP gene expression. This link is supported by evidence showing that oxidative stress can trigger MAPK pathways—particularly p38 and JNK—which are implicated in MMP regulation (10.3390/cancers12010024). In vitro studies further demonstrate that atrazine exposure leads to increased MMP expression in ovarian epithelial cells through STAT3 activation and VEGF signalling, accompanied by elevated ROS levels.

Within the testis, MMPs contribute to the physiological process of germ cell migration and ECM turnover during spermatogenesis. However, when their expression is dysregulated, as observed in our treated groups, this can lead to premature breakdown of the basement membrane, detachment of Sertoli cells, and apoptotic loss of germ cells (4). Similarly, in the ovary, while MMPs facilitate normal follicular rupture and luteal regression, their overexpression has been associated with pathological remodelling, increased fibrosis, and failure of follicular maturation. These processes have been well-characterized in the literature, with studies showing that deviations in MMP activity can lead to ovulatory dysfunction and compromised fertility.

In conclusion, our findings provide direct evidence that atrazine exposure leads to increased MMP expression in gonadal tissues, accompanied by clear structural disruptions that indicate aberrant ECM degradation. While multiple pathways may contribute to this dysregulation, the involvement of oxidative signalling appears particularly relevant. These results not only align with established mechanisms but also offer a molecular explanation for the reproductive dysfunctions observed in both male and female rats following atrazine exposure.

# Conclusion

Our present study showed that both male and female rats on being exposed to atrazine showed considerable reproductive disturbances. Signs of gonadal damage were distinctively noticed. Females showed absence of Corpus lutea, disorganised stroma, increased follicular atresia leading to disturbed folliculogenesis and ovulatory failure. The males on other hand showed vacuolisation, degradation of seminiferous tubules and damaged leydig cells which indicated reduced spermatogenesis and impaired production of testosterone hormone.

A strong upegulation of MMPs , specially MMP-2 was noticed on PAGE gel analysis. This increased band suggests enhanced degradation of extra-cellular matrix which matches tissue disintegration. Oxidative stress and activation of NF-KB and MAPK pathways are linked to the over expression.
The findings are supported by hormonal analysis too. Decrease in release of FSH,LH, estradiol and testosterone were significantly noticed in treated group in comparison to control groups.

Blood smear analysis showed increase in neutrophils and mild phagocytic activity showing inflammatory response for oxidative stress. Increase in protein concentration were noticed in testes whereas ovaries showed increase,which indicates biochemical alternations.
Thus, atrazine disrupts the hormone levels, tissue damage,MMP upegulation,gonadal damage and ECM remodeling in both female and male albino rats confirming it's gonadotoxic nature.

# References

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