# Genetic Study of the Effect of the *Toxoplasma gondii* on Somatic Cells of the Bone Marrow in Female Mice and a treatment Attempt by *Laurus Nobilis* Leaf Extract

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## Summary

This study aimed to find out the ability of T. gondii parasite to induce genetic changes in the bone marrow cells of female mice and to try therapeutically for it using bay leaf extract. The parasite was extracted from (23) placentas for aborted women and injected into the 42 mice with an average weight of (20-30) gram. It was divided into six groups at a rate of (3 females) per group. The first group doses the oil only as a negative control, the second group was infected with the parasite as a positive control, while the four study groups dose the plant extract of the bay leaves for (14) days and at doses of (15, 10, 5, 1) mg. for Kg after a period of infestation with the parasite for 14 days. The results of the chromosomal changes showed the type of chromatid fracture among the females injected with the parasite + the extract and with all its concentrations significantly reduce the level at ($P < 0.05$). The concentration was 15 mg/kg, the lowest as the results reached ($9.33 \pm 1.33$) compared to the control of the parasite ($44.00 \pm 2.30$), while the centromere fracture showed All concentrations showed a significant decrease at the level of ($p<0.05$) and showed concentrations 15 and 10 more decreases with a result of $2.66 \pm 1.33$ for both concentrations compared to the control of the parasite, As for the annular chromosome, all concentrations showed a significant decrease at the level of ($p<0.05$) for females injected with the parasite extract, and the concentration was 15 mg/kg, the lowest was ($6.66 \pm 1.33$) compared to the control of the parasite.

***Keyword****: Toxoplasma gondii parasite, somatic cells, mice, bone marrow, Laurus nobilis leaves.*

## Introduction

Toxoplasmosis is a serious disease common to humans and animals. The main cause of it is a type of parasitic protozoan. *Toxoplasma gondii* infects all warm-blooded animals and humans those are intermediate Hostesses. The final host and the main source of infection are cats [(1).] A complex life cycle of the parasite is one of the main reasons for its success, as it depends on sexual and non-sexual reproduction, which includes multiple hosts [(2) (3).] Infection occurs through food contaminated with egg sacs or meat contaminated with tissue sacs or by contact with cats or the environment contaminated with its infectious phases [(4).] There are three types (Type I, II and III) depending on the genotype of the parasite, which differs in virulence and epidemiology, where strains of type I that grow rapidly in the laboratory are more virulent in mice and are often associated with visual toxoplasmosis, As for type II, it is not virulent in mice, but it is more common in humans in Europe and North America, while type III infect animals more unlike the types, I and II where humans are mainly infected [(5)] and since centuries ago. Herbal medicine has been considered as a major source of prevention and treatment [(6).] Laurus nobilis bay leaves have been used in the pharmaceutical industry, as it is entered in the composition of some medicines, as it has anti-microbial and pathogenic activity and is an antibiotic against fungi and viruses [(7)(8).] The current study aimed to know the genetic changes caused by the parasite in the bone marrow cells of mice and the use of the baby plant as a therapeutic attempt to reduce the damage caused by the toxoplasma parasite.

## Material and Methods

**Experimental design**:

Initially, the parasite was extracted from (23) placentas from aborted women and used to inject and infect mice. (42) mice were used with an average weight of (20-30) gram, as they were divided into six groups at a rate of (3 females) in each group. Dosage identified (LD50) about the plant extract of bay leaves and giving a dose of (0.5, 1.0) milliliter of the parasitic suspension of *Toxoplasma gondii*. the groups were dosed as follows: The first group (oil control): This group was treated with oil only without the plant extract and without being infected with the parasite. The second group (parasite control): This group was infected with the parasite only and this group was not treated with oil or plant extract. The third group included infection with the parasite and treated with the plant extract at a concentration of 1 mg/kg of BW. Fourth group: This group was infected with the parasite and then treated with the plant extract at a concentration of (5) mg/kg of animal weight. Fifth group: This group was infected with the parasite and then treated with the plant extract with a concentration of (10) mg/kg of body weight of the animal. The sixth group: This group was infected with the parasite and then treated with the plant extract with a concentration of (15) mg/kg of body weight for the animal.

Test of chromosomal changes in somatic cells of bone marrow:

The animals were injected into the cell membrane (0.5) ml of the solution of the colsagen, and after an hour and a half to two hours after the injection of the colsagen, the animals were sacrificed after Anatomize the mouse, holding the femur with forceps, cleaning the muscle residue, and then washing the bone marrow with a 0.9% normal slain until it became white, After being placed in test tubes and in a centrifuge at a speed of 2000 rpm, the suspended was removed and the precipitate was left and KCL solution was added to it on the tube walls with continuous shaking and put in a water bath at 37 ° C for 20-25 minutes and taken out and placed again in the centrifuge then After that the glass slides were prepared and after sterilization and cleaning it was placed as sloping Drops of it were dropped from the solution containing the cells vertically in order to allow the nuclei and chromosomes to spread, and then the glass slides were kept in the refrigerator for the purpose of examining them with the oil lens of the optical microscope.

## Result and Discussion

The results showed the presence of chromosomal changes, that included (chromatid fractures, centromere fractures and annular chromosomes) in females of mice injected with toxoplasma parasites for a period of (14) days which reached (44.00 ± 2.30, 9.33 ± 1.33 and 14.66 ± 1.33) respectively. In addition, a significant difference at the level of $P<0.05$ compared with the oil control group which amounted to (9.33 ± 1.33, 2.66 ± 1.33, 6.66 ± 1.33) respectively. It also showed a significant decrease at $P <0.05$ in the structural chromosomal changes that included (chromatid fractures, centromere fractions, and annular chromosomes) in the interference groups injected with the bay leaf extract and with all its concentrations with the parasite compared with the group injected with the parasite alone as the table (1).

**Table (1) showing the mean Values of differences for female chromosomal changes for the study groups**

| changes chromosomal | | | Natural chromosome Mean ± SE | Group |
|---|---|---|---|---|
| annular chromosomes Mean ± SE | centromere fractures Mean ± SE | chromatid fractures Mean ± SE | | |
| 6.66±1.33 B | 2.66±1.33 B | 9.33±1.33 DC | 81.33±2.66 A | oil control |
| 14.66±1.33 A | 9.33±1.33 A | 44.00±2.30 A | 32.00±2.30 D | parasite control |
| 18.66±1.33 A | 5.33±1.33 BA | 22.66±1.33 B | 53.33±1.33 C | 1 mg / kg |
| 8.00±0.00 B | 6.66±1.33 BA | 21.33±1.33 B | 64.00±2.30 B | 5 mg / kg |
| 6.66±2.66 B | 2.66±1.33 B | 13.33±1.33 C | 77.33±2.66 A | 10 mg / kg |
| 6.66±1.33 B | 2.66±1.33 B | 9.33±1.33 DC | 81.33±1.33 A | 15 mg / kg |

As for the chromatid fractures, it also showed that all the interference treatments injected with the extract of the bay leaves of different concentrations are 15, 10, 5, 1 mg/kg of body weight with the parasite. And there was a significant decrease in it (22.66 ± 1.33, 21.33 ± 1.33, 13.33 ± 1.33C, 9.33 ± 1.33) respectively compared to the control of the parasite, as the chromatid fractures in it reached (44.00 ± 2.30) and the concentration was 15 mg/kg which is the largest Reducing chromatid fractures. As for the centromere fracture, the results showed that all the interference treatments injected with the extract of the bay leave with the parasite had a significant decrease, as they reached in different concentrations, which are 15, 10, 5, 1 mg/kg of body weight (5.33 ± 1.33, 6.66 ± 1.33, 2.66 ± 1.33, 2.66 ± 1.33) respectively. Comparing to the parasite control group as the centromere fraction reached (9.33 ± 1.33), and the 10 and 15 mg/kg concentrations were the most influential in reducing the centromere fracture.

Concerning the annular chromosome, it showed that all the interference treatments injected with the extract of the bay leaves with the parasite had a significant decrease. as it reached in different concentrations, which are 15, 10, 5, 1 mg/kg of body weight (18.66 ± 1.33, 8.00 ± 0.00,6.66 ± 2.66, 6.66 ± 1.33), respectively. Compared to the control group of the parasite, as its annular chromosome reached (14.66 ± 1.33), and the concentration was 15 mg/kg, which is the largest reduction in the annular chromosome.

The results of chromosomal changes about the parasite control group for females infected with the parasite and the lack of natural chromosomes and the increase in chromosomal changes as in Figure 1, show that increased toxoplasma infection is directly related to chromatid exchanges[9]. and these chromosomal changes occur as a result of the damage that occurs in the DNA and its attendant From incorrectly re-repairing it [10]. As for the role of bay leaf extract, which reduced chromosomal changes and all the concentrations used, this indicates that the plant has an effect on the parasite. and may be due to what it contains Effective chemical compounds such as flavonoids, quercetin, and luteolin, that the plant contains thymol with carracrol shows the plant can repair DNA because it contains these two compounds [11] [12].

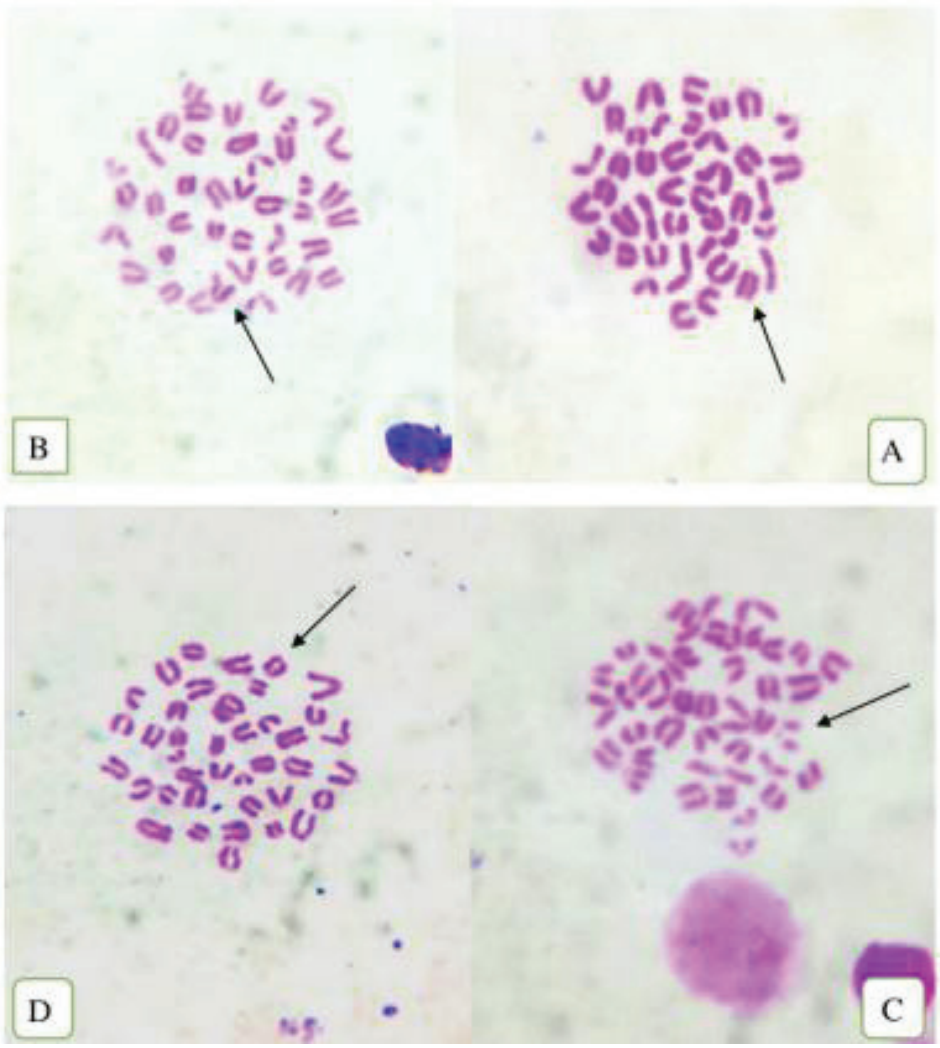


**Figure 1: Natural chromosome B: chromatid fractures C: centromere fractures D: annular chromosomes (Giemsa stain with a strength of 100 X).**

## Conclusion

Ours study concluded there are many chromosomal changes in females mice which injected with *toxoplasma spp* parasites. Significant decrease in the structural chromosomal changes is included of the chromosome. Our study suggest treatment by *Laurus nobilis* leaf extract in females mice have positive effect on bone marrow in females mice that infected by the *Toxoplasma gondii*.

**Ethical Clearance**: The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

**Conflict of Interest:** Non

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