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Original Research Article

Assessment of Genetic Damage Induced by Toxoplasmosis in Bone Marrow Cells of Female Mice and the Protective Role of Bay Laurel (*Laurus nobilis*) Leaf Extract

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Abstract: This study was designed to assess the capacity of *Toxoplasma gondii* to induce genetic alterations in the somatic bone marrow cells of female mice, as well as to examine the potential therapeutic role of bay leaf (*Laurus nobilis*) extract in reducing or alleviating these alterations. High-Performance Liquid Chromatography (HPLC) was employed to discover the active components in the bay leaf extract that may be advantageous in the treatment of toxoplasmosis. 42 female mice, each weighing an average of 20 to 30 grams, were given the parasite, which was taken from the placentas of aborted females. The mice were categorized into six groups, each consisting of three females. The initial group (negative control) was administered solely oil, whereas the subsequent group (positive control) was infected exclusively with the parasite. The four remaining groups were infected with the parasite and subsequently administered bay leaf extract at varying dosages (1, 5, 10, and 15 mg/kg) for a duration of 14 days. The results showed a significant decrease (P>0.05) in chromosomal abnormalities, including chromatid fragmentation, centromere fragmentation, and circular chromosome fragmentation, in the groups treated with bay leaf extract compared to the control group infected with the parasite. A significant increase in the mitotic index and a decrease in micronucleus frequency were also observed in the treated groups, indicating a protective and therapeutic effect of the bay leaf extract. Our findings confirm that bay leaf extract has the ability to mitigate the genetic damage caused by T, gondii in bone marrow cells.

Keywords: *Toxoplasma Gondii*, Female Mice, Bone Marrow, Somatic Cells, *Laurus Nobilis*.

INTRODUCTION

Toxoplasmosis is a serious zoonotic disease caused by the parasite *Toxoplasma gondii*. *T. gondii* is a protozoan that infects all warm-blooded animals, including humans, with cats being the primary and definitive host and a major source of infection (Nelson and Willims, 2014). The parasite's life cycle is complex, involving both sexual and asexual reproduction in multiple hosts (Szabo and Finney, 2017). Infection usually occurs through the ingestion of meat contaminated with tissue cysts or contaminated eggs, or through direct contact with an environment contaminated with infectious stages of the parasite (Jiang *et al*., 2015). *T. gondii* has three main serotypes (types 1, 2, and 3) that differ in their genetic makeup and virulence Type 1 strains are the most virulent in laboratory settings and associated with ocular toxoplasmosis (Dalimi and Abdoli, 2014). The second variety exhibits lower virulence in mice yet is more prevalent in human infections across Europe and North America. The third type affects animals more often than infected humans (Weiss and Dubey, 2009)

For generations, herbal remedies have served as a fundamental means of prevention and therapy (Alday .and Doggett, 2017). Bay leaves (*Laurus nobilis*) are utilized in the pharmaceutical sector for their antibacterial and antimicrobial qualities, as well as their efficacy as an antibiotic against viruses and fungi (Chmit

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et al., 2014; Merghni *et al*., 2016). The purpose of this study was to evaluate *T. gondii*'s ability to cause genetic changes in female mice's somatic bone marrow cells and investigate the possible therapeutic benefit of bay leaf (*Laurus nobilis*) extract in mitigating or lessening these changes.

MATERIALS AND METHODS

Experimental Design

The placentas of twenty-three women who had undergone abortions were used to isolate the Toxoplasma conidiophore. There were 42 female mice employed, each of which weighed between 20 and 30 grams on average. These mice were then separated into six groups, each consisting of three mice. There was a determination made regarding the median lethal dose (LD50) of a bay leaf extract. These are the groups that participated in the experiment:

Group 1 (negative control): treated with oil only, without parasite infection or bay leaf extract.

Group 2 (positive control): infected with parasite only, without any treatment.

Group 3: infected with parasite and then treated with 1 mg/kg of bay leaf extract.

Group 4: infected with parasite and then treated with 5 mg/kg of bay leaf extract.

Group 5: infected with parasite and then treated with 10 mg/kg of bay leaf extract. • Group 6: Infected with the parasite and then treated with 15 mg/kg of bay leaf plant extract.

Cytogenetic Testing

Animals were injected with 0.5 mL of colchicine solution intraperitoneally. Two and a half to three hours after injection, the animals were sacrificed. Mice were dissected, and the femur bone was removed, cleaned of tissue remnants, and washed with 0.9% physiological saline until it turned white. The bones were placed in test tubes and centrifuged at 2000 rpm for 10 minutes. The supernatant was removed, and potassium chloride (KCl) solution was added to the precipitate on the tube walls. The tubes were immersed in a water bath at 37 degrees Celsius for approximately twenty to twenty-five minutes. They were then centrifuged again. Glass slides were prepared by sterilizing and cleaning them, and drops of the cell-containing solution were placed on them at an angle to ensure the dispersion of chromosomes and nuclei. The slides were refrigerated for examination under a light microscope using an oil lens. One thousand bone marrow cells from all females were examined to identify dividing cells. The mitotic index of somatic cells was calculated using the following equation:

Mitotic Index (%) = (Number of dividing cells / Total number of cells (dividing and non-dividing)) × 100

This assay was performed in several steps, beginning with the removal of the skin and muscle surrounding the femur of a mouse. The bone contents were washed with a human (Ab) syringe. The solution was then centrifuged at 1000 rpm for 10 minutes. The precipitate was collected and smeared onto a glass slide, which was then allowed to dry at room temperature. The cells were fixed with methyl alcohol for 1 minute and then stained with Giemsa stain until dry. The slides were then examined under a light microscope at 100x magnification. The percentage of micronuclei was calculated in 500 polymorphochromatic red blood cells (P.C.E.) per slide, with 100 P.C.E. cells per slide. The percentage of micronuclei was calculated using the following equation:

Micronucleus Percentage (%) = (Number of micronuclei / Total number of cells) × 100

Statistical Analysis: The SAS (2012) statistical analysis program was used to detect significant differences between the study groups.

RESULT

Forty-two female mice, averaging 20–30 grams, were utilized and allocated into six groups of three mice each. The initial group (negative control) received treatment solely with oil. The second group, serving as the positive control, was solely infected with the parasite. The third, fourth, fifth, and sixth groups were infected with the parasite and subsequently administered doses of 1, 5, 10, and 15 mg/kg of bay leaf extract, respectively.

The results indicated chromosomal abnormalities, including centromeric fractures, chromatid breaks, and circular chromosomes, in female mice infected with *T. gondii* for 14 days. The alterations were (9.33 ± 1.33, 44.00 ± 2.30, and 14.66 ± 1.33), respectively, at (P>0.05) when juxtaposed with the oil-treated control group were 2.66 ± 1.33, 9.33 ± 1.33, and 6.66 ± 1.33, respectively, at (P>0.05). Concerning chromatid fragmentation, all mixed treatments administered with bay leaf extract at varying dosages (1, 5, 10, and 15 mg/kg body weight alongside the parasite) shown a notable decrease. The values were (21.33 ± 1.33, 13.33 ± 1.33, 9.33 ± 1.33, and 22.66 ± 1.33), respectively, in contrast to the parasite-infected control group, where chromatid fragmentation was (44.00 ± 2.30). The values were (5.33 ± 1.33, 6.66 ± 1.33, 2.66 ± 1.33, 2.66 ± 1.33), respectively, in comparison to the parasite-infected control group, which exhibited centromere fractures of (9.33 ± 1.33). The concentrations of 10 mg/kg and 15 mg/kg were the most efficacious in diminishing centromere fractures. Concerning ring chromosomes, all cross-treatments administered with bay leaf extract and the parasite at various dosages exhibited a considerable reduction. The values were (18.66 ± 1.33, 6.66 ± 2.66, 8.00 ± 0.00, 6.66 ± 1.33), respectively, in contrast to the parasite-infected control group, which exhibited ring chromosomes measuring (14.66 ± 1.33). The 15 mg/kg concentration proved to be the most efficacious in diminishing ring chromosomes. As shown in Table (1).

Table 1: Presents the mean values of the chromosomal abnormalities for all study groups

Treatments	Normal Chromosomes	chromosomal abnormalities		
		chromatid breaks	centromeric fractures	circular chromosomes
	Mean ± SE			
Oil control	81.33±2.66 a	6.66±1.33 b	2.66±1.33 b	9.33±1.33 d
Parasite control	32.00±2.30 d	14.66±1.33 a	9.33±1.33 a	44.00±2.30 a
Extract concentration 1 mg/kg + parasite	53.33±1.33 c	18.66±1.33 a	5.33±1.33 ab	22.66±1.33 b
Extract concentration 5 mg/kg + parasite	64.00±2.30 b	8.00±0.00 b	6.66±1.33 ab	21.33±1.33 b
Extract concentration 10 mg/kg + parasite	77.33±2.66 a	6.66±2.66 b	2.66±1.33 b	13.33±1.33 c
Extract concentration 15 mg/kg + parasite	81.33±1.33 a	6.66±1.33 b	2.66±1.33 b	9.33±1.33 d

Different letters indicate significant differences at the P-value< 0.05.

The results in Table (2) show a decrease in the mitosis index for all groups infected with *T. gondii* for 14 days at a level of (P>0.05). These values reached (323.00 ± 2.64) compared to the oil-treated control group, which reached (319.67 ± 13.69). In contrast, all crossover treatments injected with bay leaf extract along with the gondii at all concentrations (1, 5, 10, and 15 mg/kg body weight) showed a significant increase in the mitosis index. These values reached (381.00 ± 4.93, 452.00 ± 12.48, 472.00 ± 5.29, and 584.33 ± 8.42), respectively. The 15 mg/kg concentration had the greatest effect in raising the mitosis index.

Table 2: Shows the average values of cell division differences for female groups across all study groups

Treatments	Mitotic Index in Females Mean ± SE
Oil control	319.67 ± 13.69 d
Parasite control	323.00 ± 2.64 d
Extract concentration 1 mg/kg + parasite	381.00 ± 4.93 c
Extract concentration 5 mg/kg + parasite	452.00 ± 12.48 b
Extract concentration 10 mg/kg + parasite	472.00 ± 5.29 b
Extract concentration 15 mg/kg + parasite	81.33±1.33 a

Different letters indicate significant differences at the P-value< 0.05.

The results showed an increase in the micronuclear count (P>0.05) in all groups of female mice infected with *T. gondii* for 14 days. This value was 168.00 ± 2.31 compared to the oil-treated control group (48.33 ± 1.76). In contrast, all crossover treatments injected with bay leaf extract along with the parasite at all concentrations (1, 5, 10, and 15 mg/kg body weight) showed a significant decrease in the micronuclear count. These values were 155.00 ± 17.32, 136.67 ± 4.91, 121.33 ± 2.33, and 115.00 ± 1.15, respectively. The 15 mg/kg concentration was the most effective in reducing the micronuclear count. As shown in Table (3).

Table 3: Shows the mean values of micronuclear count differences for female groups across all study groups.

Treatments	Micronuclear count Mean ± SE
Oil control	48.33 ± 1.76 e
Parasite control	168.00 ± 2.31 a
Extract concentration 1 mg/kg + parasite	155.00 ± 17.32 ab
Extract concentration 5 mg/kg + parasite	136.67 ± 4.91 b
Extract concentration 10 mg/kg + parasite	121.33 ± 2.33c
Extract concentration 15 mg/kg + parasite	115.00 ± 1.15 d

Different letters indicate significant differences at the P-value< 0.05.

DISCUSSION

The results relating to chromosomal changes in the parasite-infected control group revealed a decrease in normal chromosomes and an increase in chromosomal abnormalities, indicating a direct relationship between increased toxoplasmosis infection and chromatid exchange (Shubber, 1987). These chromosomal abnormalities arise from DNA damage, coupled with

inadequate repair. The involvement of bay leaf extract in mitigating chromosomal changes across all concentrations indicates the plant's antiparasitic properties. This can be ascribed to its active chemical constituents, including flavonoids, quercetin, luteolin, thymol, and carvacrol, as evidenced by HPLC in the findings of the present study (Stammati *et al*,.1999: Chang *et al*., 2000).

Infection with the tachyzoite stage of the Toxoplasma conidiophore indicates that it accelerates the G1 phase, the stage of cytoplasmic cell growth and synthesis. The parasite also slows down the G2 phase, the second stage of cell growth and synthesis during which DNA integrity is confirmed. Furthermore, there is a decrease in M-mitosis, the actual division phase that produces two new cells, as confirmed by our current study, which showed a reduced mitotic rate in the infected control group (Luo *et al*., 2014). Infection with the parasite also leads to host cell necrosis or programmed cell death, as the parasite has the ability to interfere with the host cell cycle and affect the G2/M phases, effectively halting the cell cycle at the mitotic stage (Kishimoto, 2015). Studies have shown the parasite's ability to influence the cell cycle by affecting the expression of Cyclin B1, a crucial component of the M-mitosis phase of mitosis. The parasite interacts with Cdc2 and plays a key role in cell regulation during mitosis (Yokoyama *et al*., 2015). Cellular genetic damage, often accompanied by damage to other genes, frequently leads to mutations (Doherty *et al*., 2012).

Micronuclei originate in the cytoplasm of interphase cells and are chromosomal fragments lacking a centromere or complete chromosomes that failed to migrate to the spindle poles during anaphase of cell division (Aiassa, 2018). A study by (Bonassi *et al*., 2003) have confirmed a link between chromosomal abnormalities and micronucleus formation. These micronuclei arise in dividing cells from deviations in chromosome structure and from impaired function of the mitotic spindle fibers (Fenech, 2003). Micronucleus formation may be attributed to abnormalities in the cleavage of chromosomal centrioles or to DNA damage that renders centrioles ineffective (Hayashi, 2016). Micronucleus assays can be effective in assessing biological and chemical effects, a test used in in vivo mutation studies that compares to molecular mechanisms demonstrating the relationship between DNA damage and mutation (Georgakilas, 2008). Bay leaf extract reduces micronucleus replication due to its borneol content, which acts as an antioxidant (Zhang *et al*., 1997). On the other hand, the chemical compound eugenol, found in bay leaf extract, reduces the rate of micronuclear replication in mice (Ellahuene *et al*., 1994).

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