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ANTI-NECROTIC POTENTIAL OF ETHANOLIC LEAF EXTRACT OF ZIZIPHUS TALANAI AGAINST MONOSODIUM GLUTAMATE-INDUCED CYTOARCHITECTURAL ALTERATIONS IN THE BRAIN OF ALBINO MICE

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Abstract:

Ziziphus talanai (Blanco) Merrill, locally known as the Balakat tree in the Philippines, is an endemic plant reported to have therapeutic properties due to its flavonoid content. Specifically, neuroprotective activities against oxidative stress in mice brains have been previously documented under this plant. Therefore, this study investigated the antinecrotic potential of Z. talanai against MSG-induced cytoarchitectural alterations of the mice's prefrontal cortex. Twenty (20) male albino mice were distributed into four treatment groups: T0 (DW alone at 0.3 mL/20 g b.w.); T- (MSG alone at 180 mg/20 g b.w.); T+ (L-Taurine at 0.2 mL/20 g b.w. and MSG at 180 mg/20 g b.w.); and T1 (Z. talanai at 0.3ml/20g b.w. and MSG at 180 mg/20 g b.w.). Results of the histological assessment of the prefrontal cortex reveal normal histology for T0, marked with an intact nucleus, and prominent and organized cells. Tissue architecture for T- reveals the reduction of cells and necrosis. T+ and T1 groups both maintained intact and well-stained nuclei that are comparable with T0. Interestingly, cellular proliferation has been noted for the Z. talanai group, which indicates possible neuronal differentiation. Overall, Z. talanai extract has been observed to exert antinecrotic capacities in the mouse brain.

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TIỀM NĂNG CHỐNG HOẠI TỬ CỦA CHIẾT XUẤT LÁ ETHANOL CỦA ZIZIPHUS TALANAI CHỐNG LẠI SỰ THAY ĐỔI CẤU TRÚC TẾ BÀO DO BỘT NGỌT GÂY RA TRONG NÃO CỦA CHUỘT BẠCH TẠNG

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Tóm tắt

Ziziphus talanai (Blanco) Merrill, được biết đến với tên địa phương là cây Balakat ở Philippines, là một loài thực vật đặc hữu được báo cáo là có đặc tính chữa bệnh do hàm lượng flavonoit của nó. Cụ thể, các hoạt động bảo vệ thần kinh chống lại căng thẳng và oxy hóa ở não chuột đã được ghi nhận trước đây dưới loại cây này. Do đó, nghiên cứu này đã điều tra tiềm năng chống ung thư của Z. talanai chống lại sự thay đổi cấu trúc tế bào do MSG gây ra ở vỏ não trước trán của chuột. Hai mươi (20) con chuột bạch tạng đực được phân thành bốn nhóm điều trị: T0 (riêng DW ở mức 0,3 mL/20 g bw); T- (chỉ riêng bột ngọt ở mức 180 mg/20 g bw); T+ (L-Taurine ở mức 0,2 mL/20 g thể trọng và MSG ở mức 180 mg/20 g thể trọng); và T1 (Z. talanai ở mức 0,3ml/20 g bw và MSG ở mức 180 mg/20 g bw). Kết quả đánh giá mô học của vỏ não trước trán cho thấy mô học bình thường đối với T0, được đánh dấu bằng một nhân nguyên vẹn và các tế bào nổi bật, có tổ chức. Kiến trúc mô cho T- cho thấy sự giảm tế bào và hoại tử. Các nhóm T+ và T1 đều duy trì nhân nguyên vẹn và được nhuộm màu tốt tương đương với T0. Thú vị là sự tăng sinh tế bào đã được ghi nhận đối với nhóm Z. talanai , điều này cho thấy sự khác biệt hóa tế bào thần kinh có thể xảy ra. Nhìn chung, chiết xuất Z. talanai đã được quan sát thấy có khả năng chống hoại tử trong não chuột.

1. Introduction

The use of nature as a potential resource for drug development has become an increasing trend in pharmaceuticals this twentieth century. Different civilizations as early as 5,000 BC, have documented the use of plants for medications [1,2]. The therapeutic potentials of these plants are attributed to the various bioactive compounds within them. These include polyphenols, essential oils, alkaloids, flavonoids, polysaccharides, tannins, and saponins [3,4].

Ziziphus talanai (Blanco) Merrill, known for its local name as Balakat Tree in Mabalacat City, Pampanga, Philippines, is an endemic plant under Rhamnaceae that is reported to possess various pharmacological properties. Its phytochemicals including triterpenes, anthraquinones, coumarins, steroids, essential oils,

tannins, phenols, and flavonoids are known to exhibit antioxidant capacities against different reactive oxygen species (ROS) [5,6]. The antioxidative potential of the Balakat tree is also documented to play roles in reproprotection, hepatoprotection [7], cardioprotection [8], nephroprotection [9,10], and neuroprotection [11,12] in mice models.

No existing studies have been performed currently on the investigation of the anti-necrotic potential of *Z. talanai* on the ventromedial prefrontal cortex. Thus, this study investigates its anti-necrotic potential against MSG-induced oxidative stress in the male ICR mouse brain.

2. Materials and Method

2.1 Treatment Groups. Assessment of the antinecrotic potential of *Z. talanai* involves the utilization of its crude ethanolic leaf extract against oxidative stress induced by MSG. Table 1 describes the four treatment groups along with their corresponding agent and dosages.

Table 1: Treatment Groups

Treatment Groups	
T0 (Normal Control)	Male albino mice were treated with distilled water alone; Dosage: 0.3 mL/20 g b.w.
T- (Negative Control)	Male albino mice were treated with MSG alone; Dosage: 180mg/20 g b.w.
T+ (Positive Control)	Male albino mice were treated with LTaurine (0.2 mL/20 g b.w.) and MSG (180mg/20 g b.w.)
T1 (Extract Control)	Male albino mice were treated with ethanolic leaf extract of *Z. talana*i (0.3ml/20g b.w.) and MSG (180mg/20 g b.w.)

2.2 Plant material. Two (2) kilos of matured leaves of *Z. talanai* were collected at Xevera, Tabun, Mabalacat City, Pampanga and were authenticated at the University of the Philippines, Diliman, Quezon City, Philippines [7].

2.3 Animal models. This study used 20 male albino mice to evaluate the anti-necrotic potential of *Z. talanai* against MSG-induced cytoarchitectural alterations in the brain. Animals were acclimatized for two (2) weeks before the administration of chemicals and extracts. Animals were kept at the Biology laboratory of Mabalacat City College and maintained in a normal condition with 12/12 hours light/dark cycle at room temperature. Hygienic conditions include maintenance of proper ventilation with daily removal of accumulated fecal and waste deposits along with changing of water [11,13].

2.4 Preparation of Ethanol Leaf Extract. The collected leaves were washed using tap water, air dried, and kept at room temperature with proper ventilation for seven (7) days without exposure to sunlight. The dried leaves were then cut into pieces and homogenized using an electric food blender. The powdered leaves were measured using a digital weighing scale and soaked using four (4) liters of 95% analytical grade ethanol for seventy-two (72) hours at room temperature. Whatman filtering paper number 2 was utilized to filter and separate the solid materials from liquid portions of the solution, followed by a steam bath to eliminate the solvent [7,13,11,14,9]

2.5 Preparation of Monosodium glutamate. Monosodium glutamate (150g) was utilized in this study and purchased at SM hypermarket Dau, Mabalacat, Pampanga. The MSG was diluted in distilled water with the amount of 9000 mg/kg to 0.2ml/20g mice b.w. [15,16,17] to come with a dosage of 180mg/20g b.w. This MSG solution was administered in mice via the oral gavage technique [18,19] for seven (7) days.

2.6 Preparation of L-taurine. L-Taurine was procured at Bambang Enterprise, Manila, and used to make a stock solution. 1000g of L-taurine was diluted in 20ml distilled water, which was then administered to the mice models for seven (7) days at 0.2ml/20g b.w. (20mg/20g b.w. dosage) [20,15].

2.7 Administration of Treatments. Male albino mice were acclimatized and distributed into four treatment groups. Each treatment group contains three (3) replications. The administration of distilled water, L-taurine, the leaf extract of *Z. talanai*, and MSG for all treatment groups was done every 7 a.m. to noon for 7 days before refilling food [7,21,18,15,20,11]. The weights of mice were assessed weekly using a digital weighing scale, to have accurate dosages of the treatment chemicals [7]. The administration route used was oral gavage for all the animal groups. Specifically, the male albino mice in T0 were treated with distilled water alone with a dosage of 0.3mL/20g b.w. The

male albino mice of T- were treated with MSG alone, following the dosage of 180mg/20g b.w. The male albino mice of T+ were treated with 180mg/20g b.w. MSG and L-taurine at 20mg/20g b.w. The male albino mice of T1 were treated with 180mg/20g MSG and 180mg/20g b.w. leaf ethanolic extract of *Z. talanai.*

2.8 Cytoarchitectural Assessment. On the 8th day, extraction of the prefrontal cortex was conducted at the Biology laboratory of Mabalacat City College and performed in accordance with the protocols [22,23,24]. The fixatives were purchased at Pudjed Enterprises, Bambang St., Sta. Cruz, Manila. The specimens were fixed using formalin at 10% concentration overnight to prevent freezing artifacts and loss of cytoarchitecture of the tissues [11, 25, 16]. The prepared slides were then observed at the Mabalacat City College Laboratory using a light microscope with magnifications of 100x, 400x, and 1000x. The specimens were then examined for pathological findings of oxidative stress in mice's prefrontal lobes. The variations among the tissues of different treatments were analyzed and compared using the protocols used by the researchers [16,26, 27, 28, 29, 30].

2.9 Statistical Analysis. The comparison between the groups was evaluated using One-Way Analysis Variation (ANOVA) followed by Tukey's Multiple Comparison Test for Post Hoc Test to verify the statistical significance among the groups. The level of significance was set up at $p < 0.05$ level. The software pack used was Graph Pad Prism Version 6 [13,11,7].

3. Results and Discussion

Figure 1a showed the normal histology of the water-alone group with an intact nucleus, prominent cells, and no histological alterations. Also, organized arrangements of cells were seen in this specimen. These results are normal in the treated group under T0 (water alone) as reported in various studies involving rats. Particularly, rats treated with distilled water didn't exhibit histological alterations in the cerebral part of the brain [31,26]. As shown in figure 1b, reductions in cells were observed with few stained nuclei. These results were also observed in the research involving rodents, wherein the MSG-treated group underwent neural degeneration. Also, shrunken nuclei in the cerebrum cells were observed in these experiments [27, 31]. These cytoarchitectural alterations occurred due to MSG intoxication which is related to oxidative stress [32, 33].

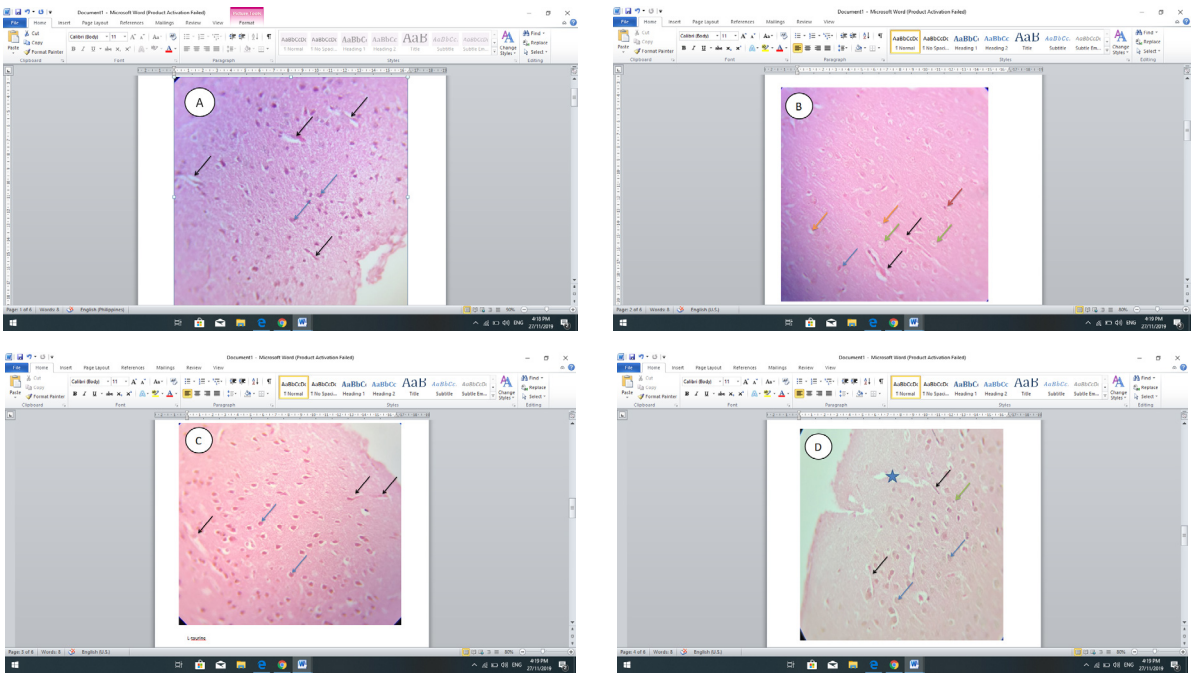

Figure 1: Histopathological observation in the ventromedial prefrontal cortex of mice using LPO. (A) Representative mice treated with water alone showed the normal appearance of brain tissue with the prominent nucleus of the cells. (B) In mice treated with MSG alone, prominently, cells in this treatment lost their structural integrity and almost all the nuclei of the cells were not visible/stained. (C) Representative mice treated with MSG + L-taurine showed normal brain tissue and conspicuous nucleus. (D) Group treated with MSG + Z. talanai extract showed alleviation of the brain tissue in terms of the gross features and cytoarchitecture parameters. Black arrow = blood vessels; Blue arrow = normal cells; orange arrow = karyolitic cell; Green arrow = karryorhectic cell; Red arrow = pynotic cell; Star shape = artefacts. H&E; HPO magnification.

In figure 1c, mice treated with the L-taurine group had normal results with alleviated tissue samples. This is due to the neuroprotective of L-taurine [34,35] It has been reported previously that this antioxidant agent has anti-apoptotic potential in mice and rats, leading to undamaged cells in the L-taurine group [36]. Moreover, L-taurine has antioxidant properties that neutralize the oxidative damage of the MSG-treated group [37, 38]. On the other hand, mice treated with *Z. talanai* extract have proliferated cells with intact and well-stained nuclei. This result is almost the same in the study conducted on *Z. jujube* a relative plant of *Z. talanai* which has proliferative potential in cultured PC12 cells [39,40]. In addition, it is speculated that flavonoids present in *Z. jujube* possess biological activities that promote neuronal differentiation. Interestingly, these secondary metabolites are also present in *Z. talanai,* thus providing the potential of the ethanol leaf extract against cytoarchitectural aberrations such as necrosis.

4. Conclusion

The results of this study suggest the potential of *Z. talana*i ethanol leaf extract as an agent to fight oxidative stress brought about by MSG. Accordingly, the crude extract has exhibited protection in the ventromedial prefrontal cortex against neuronal necrosis. This is attributed to the flavonoid constituents of *Z. talanai*. Further studies are encouraged to be conducted to elucidate the neuroprotective potential of *Z. talanai* against MSG-induced toxicity in the mouse brain.

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