



Protective Effects of *Teucrium polium* Leaves Ethanolic Extract against *Eimeria papillata*-Induced Behavioral Changes in Mice

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ABSTRACT

Background: Medicinal plants that are abundant in physiologically active phytochemicals have long been used in traditional medicine. Due to their anti-inflammatory and antioxidant qualities, several of these can lower the chance of developing certain illnesses. Coccidiosis is a disease that affects many animals and results in large financial losses. *Eimeria* spp. strains that are resistant to drugs have emerged as a result of drug addiction and overuse. Therefore, *Teucrium polium* leaves ethanolic extract (TPLE) was assessed on behavioral performance for the animals injected with *Eimeria papillata*.

Methods: *T. polium* leaves ethanolic extract (TPLE) was prepared. Also, the behavior of mice infected with the *E. papillata* parasite and treated with the plant extract was evaluated. In addition, eosin and hematoxylin-stained tissue sections of mice brains were made.

Result: There was an improvement in the number of vertical and horizontal movements in the plant-treated group. In addition, there was an improvement in learning and memory through increased total latency time the number of crossings during the shock. Also declined the number of inertial crossings and several reinforced crossings during the shock. There were no changes in oxidative stress or brain histopathology. Our results showed that TPLE has improved the behavioral performance of mice, which promotes the conduct of numerous researches on the compounds found in plants and their effect on animal behavior and also in investigating the behavior of animals in response to infection and treatment.

Key words: Behavioral performance, *Eimeria papillata*, *Teucrium polium*.

INTRODUCTION

The genus *Eimeria* is a protozoan parasite that belongs to the phylum Apicomplexa. infects many animals, causes intestinal coccidiosis, leading to significant economic losses (Chapman *et al.*, 2013; Khodakaram and Hashemnia, 2017; Tokiwa *et al.*, 2022). This parasite develops in the intestine and has devastating effects on younger animals (Bakunzi *et al.*, 2010; Dakpogan *et al.*, 2019). Subclinical symptoms are frequently linked to poor weight gain, decreased productivity and higher mortality rates (De Macedo *et al.*, 2019). Pneumonia and helminthiasis are two more parasitic disorders that may be made worse by coccidiosis (Etsay *et al.*, 2020). In addition, infection with the *Eimeria* parasite leads to an imbalance in the behavior of the host, which varies according to host sex (Simeonovska and Golemansky, 2015). In one study, *Eimeria vermiformis* was shown to have several behavioral effects on laboratory mice, which could be attributed to changes in neural function (Kavaliers and Colwell, 1992). These impacts include altered predator responses, decreased sensitivity to pain (analgesia) and responses to aversive stimuli (Colwell and Kavaliers, 1993; Kavaliers and Colwell, 1993, 1994).

Anticoccidian medications and live vaccinations are the conventional approaches to prevent and control coccidiosis however, these approaches bring up issues with drug resistance, food security, production costs species cross-protection (Chapman, 1997; Sharman *et al.*, 2010). In addition, overuse and abuse of medications have led to the

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emergence of drug-resistant strains of the *Eimeria* species (Hema *et al.*, 2015). Herbal products have garnered attention globally recently as safe substitutes for conventional treatments for a range of illnesses, with a decreased chance of developing resistance (Abd El-Hack *et al.*, 2020; Abdelnour *et al.*, 2020; Ashour *et al.*, 2020). In addition to being an abundant source of bioactive phytochemicals, many of which have anti-inflammatory and antioxidant properties and have been utilized for ages in traditional medicine (Shahat *et al.*, 2013).

The Lamiaceae family is considered one of the largest and most distinctive families of flowering plants, as it includes

236 genera, is spread throughout the world and has various biological activities and phytochemicals (Naghbi *et al.*, 2005; Raja, 2012). *T. polium* is a perennial shrub that grows to a height of 20 to 50 cm. It is a member of the Lamiaceae family and is found across the arid, rocky areas of hills and deserts in practically all Mediterranean countries, as well as in southern Asia, North Africa and Europe (Bahramikia and Yazdanparast, 2012). The therapeutic benefit of *T. polium* is attributed to its antioxidant properties (Sharififar *et al.*, 2009; Asadi and Farahmandfar, 2020). The biological activities of *T. polium* have been widely reported and it has been shown to possess antibacterial, anti-rheumatoid, anti-inflammatory, antinociceptive, hypolipidemic, hypoglycemic and antihypertensive effects (Hasani *et al.*, 2007), anticoccidial and anthelmintic (Al-Shaebi *et al.*, 2023), anticancer (Vilas-Boas *et al.*, 2020), antiviral and antifungal (Ebadollahi *et al.*, 2019), memory enhancement (Orhan and Aslan, 2009) and hepatotoxicity (Pour *et al.*, 2019).

Therefore, evaluating behavioral alterations, motor performance and functional deficits in the brains of *E. papillata*-infected mice and treating them with leave extract of *T. polium* (TPLE) was the primary goal of the current investigation.

MATERIALS AND METHODS

This experiment was completed in the zoology department of King Saud University's College of Sciences between August 2023 and February 2024. Twenty-five adult male Swiss albino mice weighing between 22 and 25 g and aged between 8 and 10 weeks were purchased for the current study from the King Saud University College of Science's animal house. Mice were kept in well-ventilated cages with 12 hours of light and 12 hours of darkness and under particular pathogen-free conditions at $23 \pm 5^\circ\text{C}$. The animals were acclimated for seven days before the start of the experiment and were given free access to tap water and regular pellet feeds.

In May 2022, *T. polium* leaves were gathered near Al-Badyah Tabuk, Saudi Arabia. Location: 80 km south of Tabuk at $27^\circ 45' 59.5''\text{N}$ and $36^\circ 31' 48.8''\text{E}$. A scientist from the King Saud University Herbarium (Science College, Botany Department, Riyadh, Saudi Arabia) recognized the plant.

With some modifications, *T. polium* leaves extract was prepared using the technique of Qabaha *et al.*, (2021). The leaves were ground into a powder after being allowed to air dry. The obtained powder was then exposed for a full day to a cold maceration extraction method employing an ethanol solvent solution (50%). The ethanolic extract was collected and stored at -20°C in sealed bottles after being filtered and concentrated in a rotary evaporator operating at 50°C under pressure.

E. papillata parasite, was used as the model in this study. Oocysts of the parasite the *Eimeria* were passaged in laboratory mice (*Mus musculus*). Feces were used to collect unsporulated oocysts, which were then allowed to sporulate in a solution containing 2.5% (w/v) potassium

dichromate ($\text{K}_2\text{Cr}_2\text{O}_7$). To get them ready for the rest of the experiment, they were then washed with a phosphate buffer solution many times. Five groups of seven mice each were used, as follows:

Group 1: Non-infected (negative control).

Group 2: Daily received oral administration (for 5 days) of TPLE (150 mg/kg)

Group 3: Infected (positive control).

Group 4: Infected and treated group with TPLE (150 mg/kg)

Group 5: Infected and treated group with the Amprolium (120 mg/kg body weight).

According to Al-Quraishy *et al.* (2020), weight change was measured on day 0 and 5 of the trial. On the fifth day post-infection (p.i.), fecal pellets from each mouse in the 3rd, 4th and 5th groups were collected and the total number of shed oocysts was calculated according to Schito *et al.* (1996). All of the mice were sacrificed and the brain was harvested and saved for use in the experiment's later stages.

Before being sacrificed, mice were put through cognitive behavioral tests following the experiment's dosage phase. The Shuttle-Box test (Active Avoidance Reflex) by Abu-Taweel *et al.* (2014) and the Activity Cage by Kraeuter *et al.* (2019) were used to assess behavioral changes.

The Ugo Basile 47420-Activity Cage was used to record the spontaneous coordinated activity of mice as well as changes in this activity over time, such as horizontal or vertical movements. The test lasted five minutes for each animal. The infrared beams located above the cage floor on the x and y axes were broken to record the motions in both the vertical and horizontal directions. A digital counter was used to count the electric impulse that was created by this interruption.

This test evaluates the mice's memory and learning capacity using the methodology according to Abu-Taweel *et al.* (2014). using the automatic reflex conditioner in a shuttle box (Ugo Basile, Italy). The device automatically recorded the findings of the investigation into the mice. Every animal underwent 30 trials of the test. Depending on the animal's capacity for learning, the lamp, bell and electricity shocks will be turned on and off thirty times each. To avoid stun electricity, the healthy animals-who pick things up fast-move from room to room as soon as they see the light from the lamp and hear the bell. The animal is first placed in one of the two rooms and allowed to explore the area. After that, the actual test begins and it concludes with the thirty time. The duration of the test may be shorter than the animal's capacity to learn, which allows it to escape when it notices the lamp and hears the bell.

Following ketamine and xylazine injections to induce anesthesia, five mice per group were bled retro orbitally to obtain blood samples. The blood samples were centrifuged for 15 minutes at 3000 rpm after clotting at room temperature. The clear serum was collected and kept at -20°C until biochemical analysis. following blood collection. Samples were homogenized as 0.5 g of tissue in 5 ml of buffered phosphate saline after the brain was dissected.

The homogenate's clear supernatant was removed and kept at -20°C until oxidative stress parameters were measured, following a 10-minute centrifugation at 3000 rpm.

By measuring the amount of malondialdehyde in brain tissues using Ohkawa *et al.* (1979) method, lipid peroxidation was assessed. Briefly stated, 0.5 ml of the sample and 1 ml of the stock solution-which included 0.375% thiobarbituric acid, 15% trichloroacetic acid and 0.25 mol/L HCl-were mixed and the mixture was heated in a boiling water bath for 30 minutes. The clear supernatant was separated by centrifugation at 3000 rpm for 15 minutes, following cooling. A spectrophotometer was used to measure the pink color's absorption at 532 nm. Tetramethoxypropane (1,1,3,3) was used as a standard.

Using a colorimetric technique, nitric oxide (NO) levels were determined following Green *et al.* (1982) methodology. This involved diazotizing nitrous acid, which coupled a vivid reddish-purple azo dye when combined with sulphanilamide, N-(1-naphthyl) ethylenediamine and an acidic environment in the presence of nitrite. At 540 nm, the resultant dye could be detectable.

The brain homogenate's reduced glutathione (GSH) content was assessed using a modified Beutler *et al.* (1963) technique. In short, 0.5 ml of 10% trichloroacetic acid (TCA) was combined with 0.5 ml of homogenate. The liquid was centrifuged for five minutes at 2000 rpm after being shaken sporadically for ten to fifteen minutes. Elman's reagent (0.1 ml) was added to a separate test tube after 0.2 ml of the clear supernatant from the process was combined with 1.7 ml of phosphate buffer. The color's absorbance was measured at 412 nm after five minutes.

Superoxide dismutase (SOD) activity was measured using the Marklund and Marklund, (1974) suggested methodology. 200 μl of tissue homogenate and distilled water, 500 μl Tris/EDTA buffer and 100 μl of 10 mM pyrogallol made up each 1 ml of the reaction mixture. Pyrogallol

underwent a fast auto-oxidation process that, in the presence of superoxide anions, created a yellow hue. By dissolving the superoxide anions, SOD stopped pyrogallol from auto-oxidizing. Two measurements of the color absorbance were made at 420 nm: one at zero time (after the addition of pyrogallol) and the other after 10 minutes.

Every animal in the group had its cerebellum and cerebral cortex tissues preserved for a period of 24 to 48 hours in newly made 10% neutral buffered formalin. After samples were cleaned and dehydrated in an ethyl alcohol series that was 70% absolute, they were embedded in paraffin wax. Cut, dewaxed, hydrated in a decreasing ethanol series and then stained with hematoxylin and eosin (HandE) sections measuring 4-5 μm . (Adam and Caihak, 1964). Sections stained using a light microscope (Leica, Wetzlar, Germany) were imaged.

The data were presented using the means $\pm$ standard error of the means (SEM). Version 28 of SPSS was used for statistical analysis. Tukey's multiple comparison test was employed after one-way analysis of variance (ANOVA), a statistical technique, to compare various groups. Statistical significance was established using a p-value of less than 0.05 ($p < 0.05$).

RESULTS AND DISCUSSION

Administration of *E. papillata* to mice significantly affected the vertical ($p < 0.01$) and horizontal ($p < 0.05$) motor activity as compared to the control mice (Fig 1,2, respectively). Treatment mice with *T. polium* extract significantly ($P < 0.01$) modulated the vertical (Fig 2) the horizontal motor impairment (Fig 1).

The total latency time and number of crossing during the shock of *E. papillata* -exposed mice were significantly ($p < 0.05$) declined compared to the control mice (Fig 3,6, respectively), On the contrary, the number of inertial crossing and number of reinforced crossing during the shock were

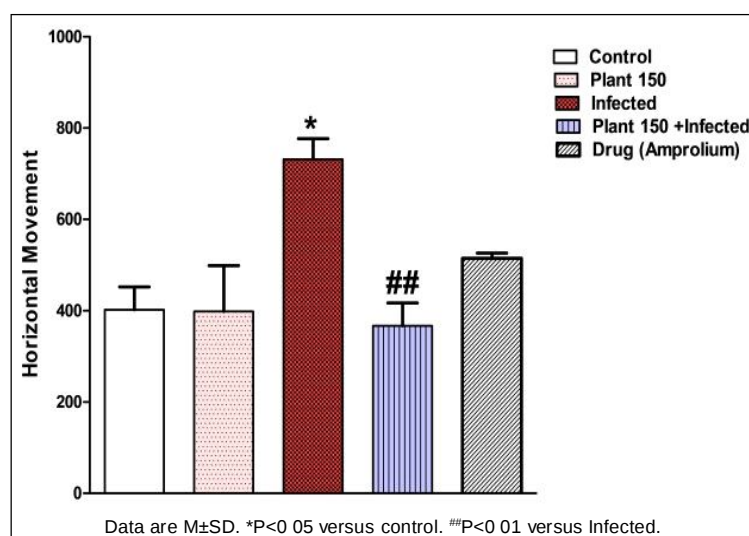


Fig 1: Effect of *E. papillata* exposure and *T. polium* extract on the locomotor activity (horizontal movement) of mice.

significantly ($p < 0.05$) increased in the *E. papillata*-administered group as compared to controls (Fig 4,5), respectively.

Treatment of *E. papillata*-administered mice with *T. polium* extract improved learning and memory in the treatment group compared to the infected group.

Regarding antioxidant and oxidative stress, there were no changes between the different study groups (Fig 7,8, 9,10), respectively.

HandE-stained sections from different brain regions of mice exposed to *E. papillata* reflected the normal cells of the cerebral cortex and cerebellum, where there are no obvious changes in these areas (Fig 11,12, respectively).

All living things in the natural world are constantly exposed to a complex environment and run the risk of being attacked by pathogenic bacteria. So, organisms need to be able to identify diseases and put up a strong defense against

them in order to survive. Also, animals that are ill have changes in their behavior and physiological responses, which can be caused by either host- or pathogen-dependent mechanisms (Lopes *et al.*, 2016). Since the brain controls behavior, alterations in brain physiology brought on by an illness may result in behavioral changes. According to Kent *et al.* (1992), infections cause the immune system to release pro-inflammatory cytokines, which disrupt brain function and result in illness-related behavior.

In biomedical research, the evaluation of behavioral changes during infection is essential, especially when assessing the pharmacological and toxic effects of new anti-parasitic drugs. Where changes in locomotor activity should be assessed before doing other behavioral characterizations since it is necessary for a number of distinct behavioral characterizations (Karl *et al.*, 2003). Here, *E. papillata* led to an increase in locomotor activity when compared with

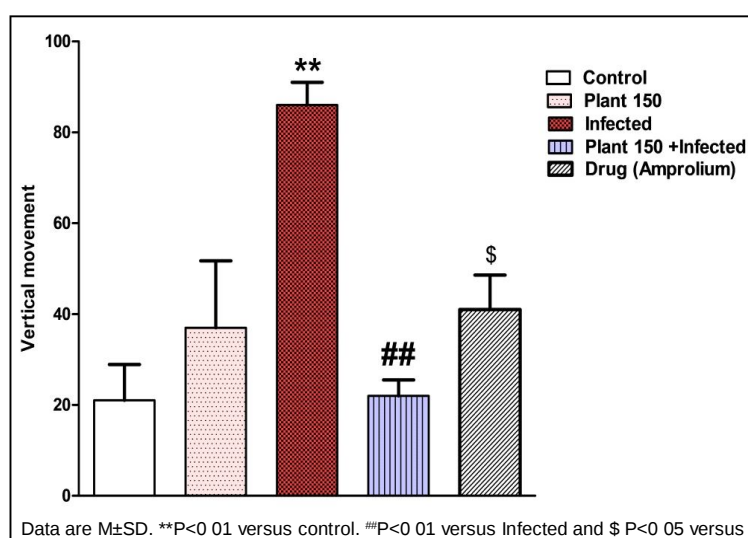


Fig 2: Effect of *E. papillata* exposure and *T. polium* extract on the locomotor activity (Vertical movement) of mice.

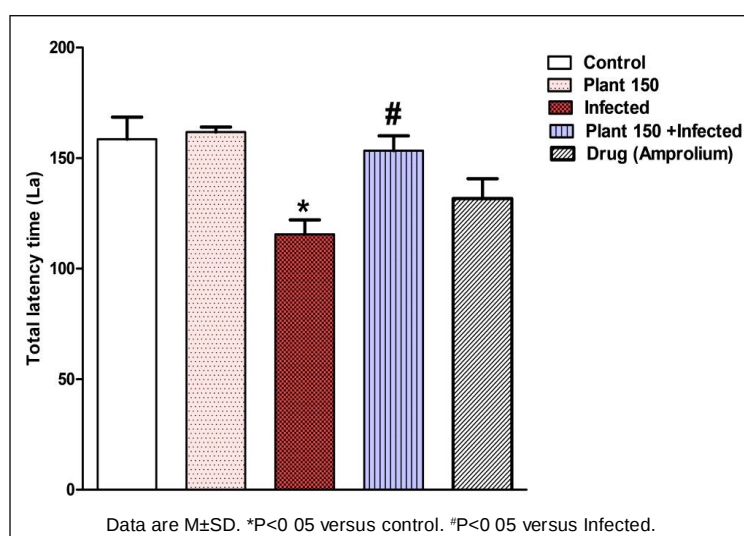


Fig 3: Effect of *E. papillata* exposure and *T. polium* extract on Total latency time of mice.

the control group. In contrast, the treatment with *T. polium* improved the locomotor activity.

Parasites are known to alter a wide range of characteristics of their hosts, including behavior (Barber *et al.*, 2000; Gegear *et al.*, 2006; Barber and Dingemans, 2010). Behavior changes in infected hosts may be non-adaptive and the consequence of the host's response to the infection or its harmful effects. Numerous behaviors, including those related to memory and motor activity, have been demonstrated to be impacted by cytokines (Dantzer and Kelley, 2007). In contrast to animals that were not infected, we found that *E. papillata* infection reduced latency time in the inhibitory avoidance task, which may indicate memory impairment. This study supports previous research that links depressed people to cognitive deficits, such as memory problems (Bearden *et al.*, 2006; Vasic *et al.*, 2008).

Eimeria protozoan parasites are the source of coccidiosis, one of the most serious diseases threatening the commercial chicken industry. Currently, anticoccidial medicines derived from plants are added to chicken and animal feed (Muthamilselvan *et al.*, 2016). Whereas, numerous parasites cause specific behavioral alterations in their hosts that promote the spread of their infectious stages from one host to another (Stepanka *et al.*, 2000).

Because certain intestinal illnesses could send a signal to the neurological system that controls behavior, the behavior was studied (Singh and Aballay 2019a, 2019b), where they found a brain pathway that sets off the host's defensive behavior against infection.

In this study, only locomotor activity, learning and memory were changed after infection and treatment with TP. The present findings imply that the behavioral alterations

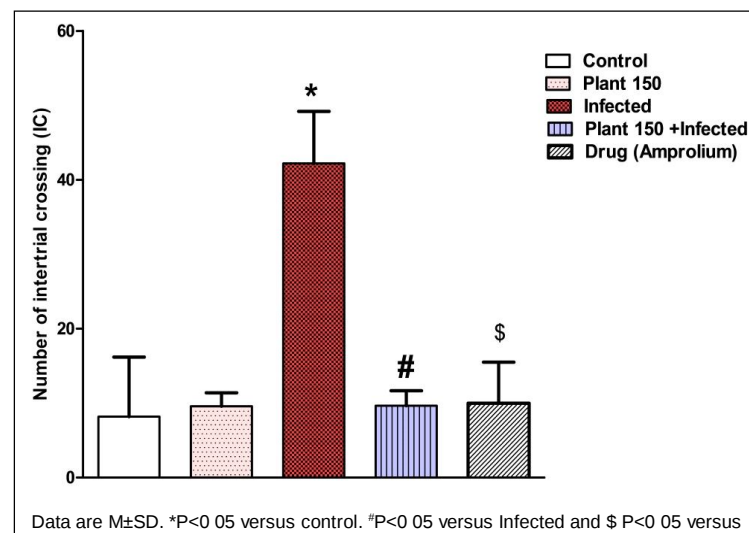


Fig 4: Effect of *E. papillata* exposure and *T. polium* extract on number of intertrial crossing of mice.

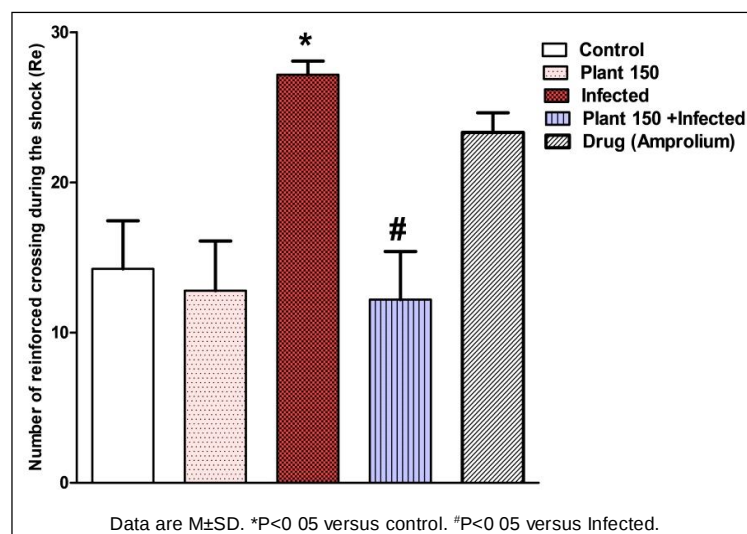


Fig 5: Effect of *E. papillata* exposure and *T. polium* extract on number of reinforced crossing during the shock (Re) of mice.

in infected mice that have been reported by numerous authors and seen in our experiments.

The results of our current study are consistent with the findings of Martin *et al.* (1995), who found that infection with the *Eimeria* parasite led to memory impairment. Where there was no obvious motor, visual, or motivational deficits, nor were there any signs of illness or malaise to explain this reduction in spatial learning. These impairments in spatial learning brought on by parasitic infection are probably the result of the host's immunological and neuromodulator responses and they could be viewed as a fitness cost of the infection response (Martin *et al.*, 1995).

It has been suggested that parasites have an impact on the decision-making and behavioral responses of their hosts. These effects may originate indirectly from or reflect the physiological and energetic restrictions that the parasite imposes on the host (Holmes and Zohar, 1990; Milinski,

1990). Thus, changes in an animal's spatial performance caused by parasites may have a direct impact on a range of distinct behavioral responses. Therefore, it is unlikely that the parasite uses the declines in spatial learning and memory shown in this study as a means of aiding in transmission. Rather, it suggests that the host's reaction to the parasite infection could be the cause of the changes in spatial learning.

It is unknown if the parasite can change the behavior of its natural host. Among other impacts, a number of studies have revealed that neurotransmitter levels in the brain are altered by parasite brain infections (Prandovszky *et al.*, 2011; Skallová *et al.*, 2006). Nevertheless, the precise mechanism responsible for altering host behavior is still unclear.

The results of our current experiment are similar to the results of Tayyeb *et al.* (2019), which mentioned that *T. polium* includes active chemical components that can improve

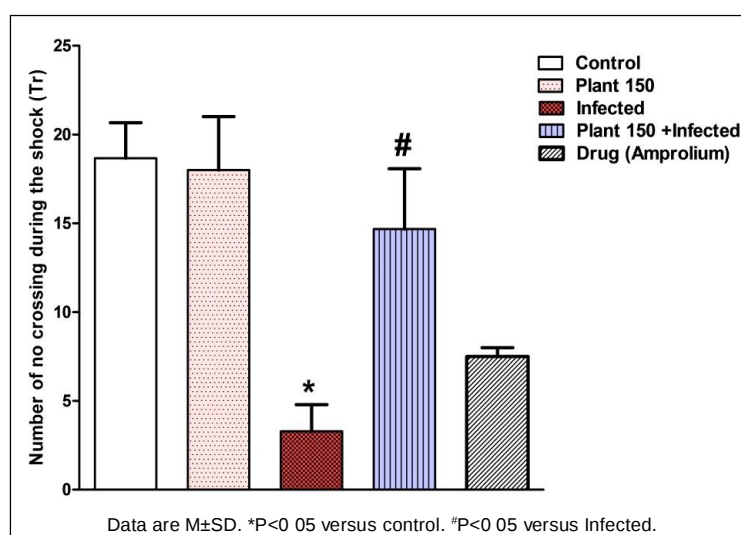


Fig 6: Effect of *E. papillata* exposure and *T. polium* extract on number of no crossing during the shock (Tr) of mice.

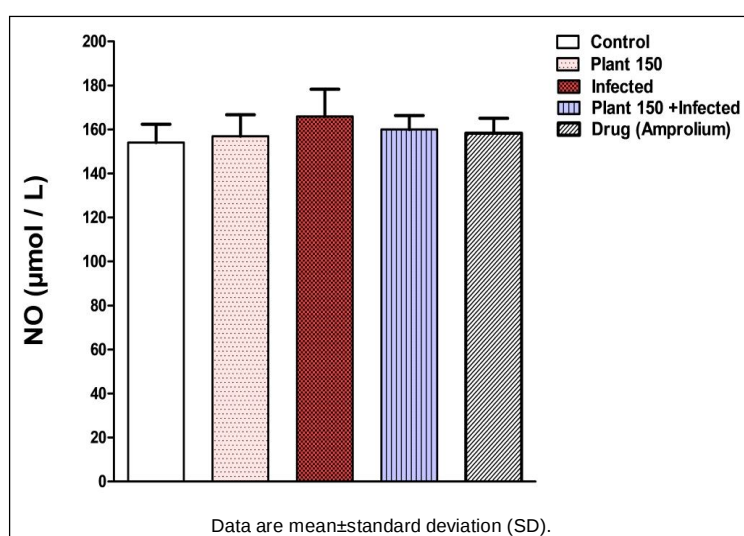


Fig 7: *T. polium* decreased nitric oxide (NO) in brain of *E. papillata* -exposed in mice.

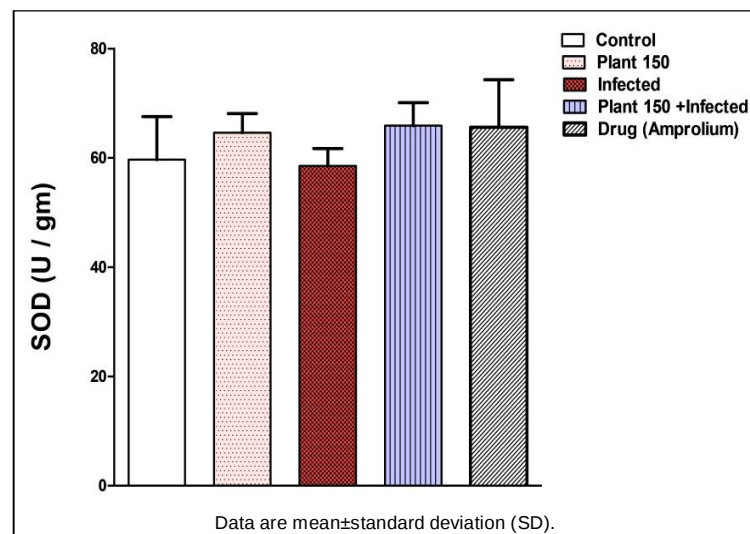


Fig 8: *T. polium* increased superoxide dismutase (SOD) in brain of *E. papillata* -exposed in mice.

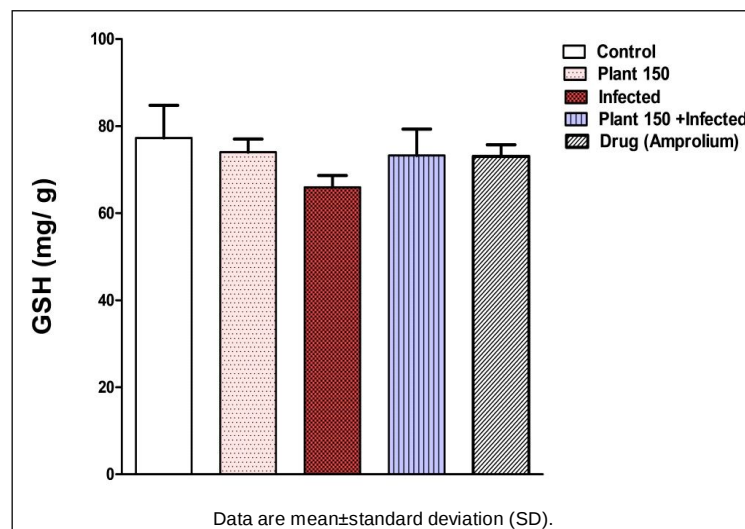


Fig 9: *T. polium* increased glutathione (GSH) in brain of *E. papillata* -exposed in mice.

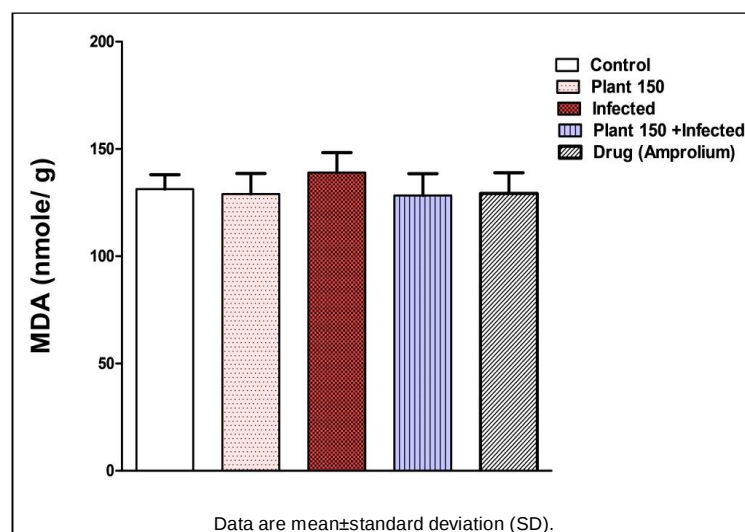


Fig 10: *T. polium* decreased malondialdehyde (MDA) in brain of *E. papillata* -exposed in mice.

memory in a Morris water maze (MWM) setting and may have neuronal survival properties in hippocampus tissue. The effects of this plant extract have been attributed by several researchers to one or more of its active components, including tannin, saponin, sterol, β -caryophyllene, diterpenoids (Niazmand *et al.*, 2008; Niazmand *et al.*, 2007)

flavonoid, terpenoid, iridoid and phenylpropanoid glycosides (Galstyan *et al.*, 1992).

Phytoestrogens are estrogen-like compounds derived from plants that mimic the neuroprotective effects of endogenous estrogen with the fewest negative side effects. These compounds act through their alpha and beta

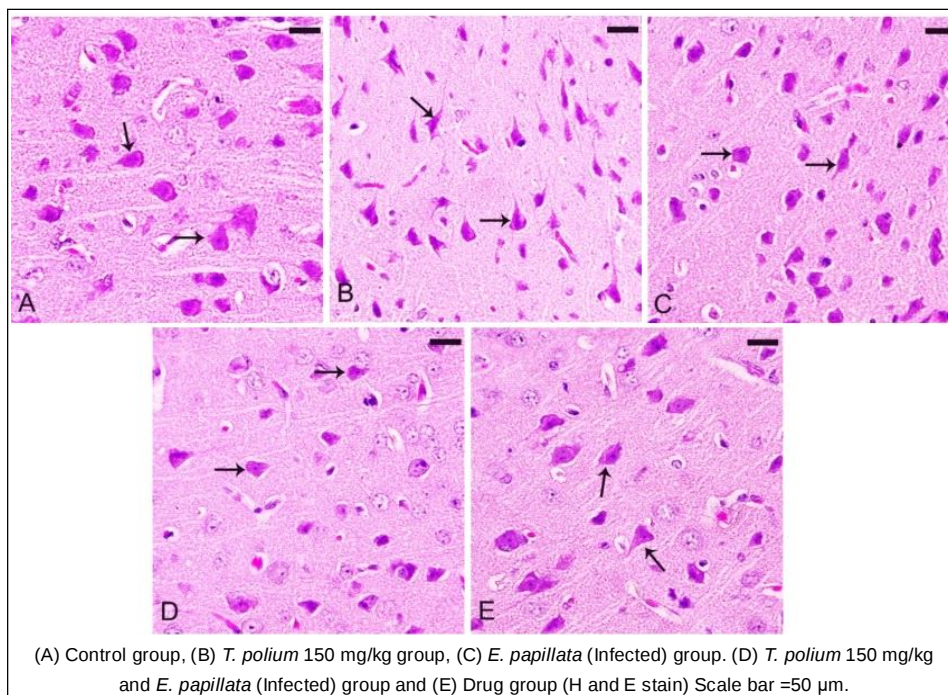


Fig 11: Sagittal sections in mice cerebral cortex showing pyramidal cells distribution (PYC) (Thin arrow).

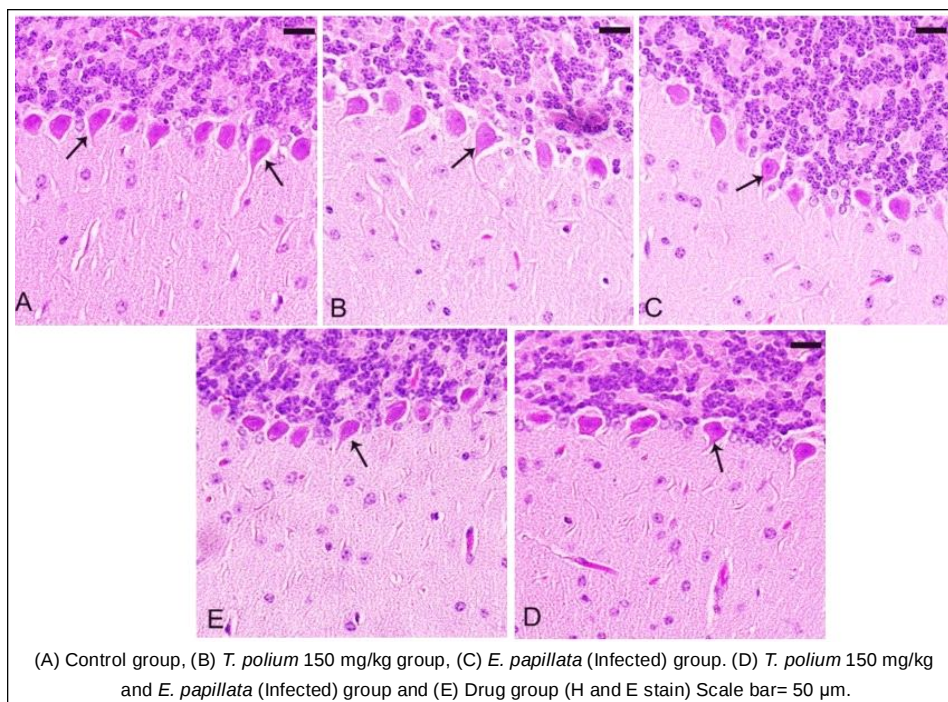


Fig 12: Sagittal sections in mice cerebellum showing Purkinje cell layer (PCL) (Thin arrow).

receptors, which are specifically distributed in the CA3 intrahippocampal region. It is possible that these receptors will mediate cell survival signaling and memory-enhancing responses (Simonyan and Chavushya, 2016).

CONCLUSION

It could be concluded that TPLE has improved the behavioral performance of mice. So, more research should be done to determine the in vitro effectiveness of TPLE. This will inform ongoing studies geared toward the development of TPLE as a novel drug. Additionally, future studies that further elucidate these conserved pathways will contribute to a better understanding of the behavioral and physiological alterations induced by intestinal infection and gut dysbiosis.

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Institutional review board statement

The animal study protocol was approved by the Institutional Review Board (or Ethics Committee) of Kingdom of Saudi Arabia (Ethics Committee, King Saud University, Ethics Agreement ID: KSU-SE- 23-56).

Conflict of interest

The authors declare that there are no conflicts of interest.

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