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Visceral leishmaniasis infection stimulates anxiety behaviour and social behaviour disorders in rat models

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Abstract

Leishmaniasis, a tropical disease caused by different species of *Leishmania* parasites, spreads through the bite of the female sand fly. Visceral Leishmaniasis (VL) is the most severe manifestation of Leishmaniasis. If untreated, it can lead to a high fatality rate. This research was designed to examine the prospects of alterations in behavior that might arise following an infection of visceral leishmaniasis in rats. This was achieved by evaluating the exploratory or locomotor activity, anxiety levels, and emotional social behaviour of infected rat models. Sixty adult male Wistar rats were divided into four groups: a control group and three infected groups with (10, 50, and 100 million parasites). Behavioural experiments were conducted for two, four, six, eight, and 10 weeks following the injection of visceral leishmaniasis, using an open field test and a social behaviour test. The results indicated a rise in anxiety-related behaviour and a reduction in locomotor activity, as evaluated by the open field test. Furthermore, the results demonstrated an increase in depression, as determined by the social behaviour test. Infection with *Leishmania donovani* increases anxiety-like behaviour, decreases emotion-related social activities and communication, and reduces locomotion. These observations were made using typical behavioural tests like open field and social behaviour assessments.

Keywords: Anxiety behaviour, Locomotor activity, Open field test, Social behaviour test, Visceral leishmaniasis

تحفز الاصابة بداء الليشمانيا الحشوي سلوك القلق واضطرابات السلوك الاجتماعي في نماذج الجرذان

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الخلاصة

داء الليشمانيا هو مرض استوائي تسببه أنواع مختلفة من طفيليات الليشمانيا، ينتشر من خلال لدغة أنثى ذبابة الرمل. داء الليشمانيا الحشوي (VL) هو أشد مظاهر داء الليشمانيا. إذا لم يتم علاجه، يمكن أن يؤدي إلى معدل وفيات مرتفع. تم تصميم هذا البحث لدراسة احتمالات التغيرات في السلوك التي قد تنشأ بعد الإصابة بداء الليشمانيا الحشوي في الجرذان. اذ تم تحقيق ذلك من خلال تقييم النشاط الاستكشافي أو الحركي، ومستويات القلق، والسلوك الاجتماعي العاطفي لنماذج الجرذان المصابة. قسم ستين ذكر من

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الجرذان البالغة إلى أربع مجاميع: مجموعة السيطرة وثلاث مجاميع مصابة باللشمانيا الحشوية (10 و50 و100 مليون طفيلي). أجريت تجارب سلوكية لمدة أسبوعين وأربعة وستة وثمانية وعشرة أسابيع بعد الحقن بداء الليشمانيا الحشوي، باستخدام اختبار المجال المفتوح واختبار السلوك الاجتماعي. أشارت النتائج إلى ارتفاع في السلوك المرتبط بالقلق وانخفاض في النشاط الحركي. وعلاوة على ذلك، أظهرت النتائج زيادة في الاكتئاب، كما تم تحديده من خلال اختبار السلوك الاجتماعي. تؤدي الإصابة بداء الليشمانيا الحشوية إلى زيادة السلوك المشابه للقلق، وتقليل الأنشطة الاجتماعية والتواصل المرتبط بالعاطفة، وتقليل الحركة. تم إجراء هذه المشاهدات باستخدام اختبارات سلوكية نموذجية.

1. Introduction

Leishmaniasis is a tropical disease that affects the poorest communities in over 90 countries worldwide. Leishmaniasis risk factors include poverty, population mobility, malnutrition, inadequate hygiene, and a weakened immune system [1]. The clinical manifestations of leishmaniasis include Visceral Leishmaniasis (VL), Cutaneous Leishmaniasis (CL), and Mucocutaneous Leishmaniasis (MCL) [2]. Visceral Leishmaniasis (VL) caused by *Leishmania donovani* is the most severe manifestation of leishmaniasis, a group of zoonotic and protozoan illnesses. It causes a significant mortality rate if left untreated [3,4].

The disease is transmitted through the bites of sandflies infected with leishmaniasis [5]. This infection is critical in patients with weakened immune systems [6]. For many years, researchers have extensively examined several factors that determine the outcome of leishmaniasis infection in animal models. These factors include the stage of development, the amount of infection, the parasite species or strain, and the method of infection [7]. Previous reports suggest that inflammatory pathways induced by parasitic infection might contribute to developing mood disorders, cognitive impairment, and emotional sensitivity-like behaviors (social behaviour) [8,9]. Interestingly, Alvarez [10] proposed that inflammations serve as a central mechanism via which stress and life adversity can negatively impact health over time. Inflammatory processes have an impact on brain functioning, making individuals with inflammatory diseases - such as those caused by parasitic infection - more susceptible to mood problems and anxiety. This concept has received support in multiple studies that have documented differences in behaviour among individuals, communities, or species with different levels of parasite infestation [11].

Infected organisms either tolerate or fight infection; this phenomenon can also be described as the slope of the correlation between illness and individual behaviour, which are the normal behavioural changes that occur during infection (such as lethargy and anorexia), and the infection itself [12]. A different investigation examined the knowledge, attitudes, and practices in relation to visceral VL among individuals residing in a village in India. Forty-three per cent of the respondents indicated that the sickness had impacted their mental well-being, leading to feelings of tension, aggravation, and melancholy [13]. Behaviour has been identified as the primary means by which hosts protect themselves against parasite infection [14]. The study of animal behaviour is closely connected to the fields of parasite ecology and evolution [15]. In rodents, illness behaviour may be estimated by classical behavioural tests. These are beneficial for estimating anxiety-like behaviors [16]. A behavioural test is specifically designed to validate and reinforce a theory of cognition or emotion. The main emphasis is on tests that can assess anxiety-resembling behaviors (open field tests) and emotional sensitivity-like behaviors (social tests), as they serve as crucial tools in pre-clinical studies [17]. Humans and animals are both known to have a reasonably high comorbidity of anxiety and depression, as stated by Neely [18]. The interaction between the immune and endocrine systems is of paramount importance in the context of leishmaniasis, and understanding the relationship between the immune and endocrine systems in leishmaniasis is

vital for developing targeted treatments and interventions as well as physiological changes that may influence individual behaviors. Managing stress and hormonal imbalance may improve immune function and, consequently, clinical outcomes in patients. [19].
Neuro-inflammation, inflammatory responses, and pain conditions in animals have been observed as being related to illness behaviour. The high generation of pro-inflammatory cytokines can cause injury to tissues and the central nervous system, generating acute behavioural changes such as anxiety and depression [20]. For this reason, the present study focused on evaluating changes in behaviour after *L. donovani* infection by using rat models to estimate the behavioural alterations of rats infected with VL. The motor activities observed during the anxiogenic period were measured, as were emotional relationships by using open-field and a social behaviour test was also used. These suggest that these behavioural changes can provide complementary information on behavioural changes in patients infected with visceral leishmaniasis and find suitable solutions for these behavioural disorders. Understanding the immediate mechanisms underlying alters in host behaviour stimulated by infection with *L. donovani* is important as it will restore our understanding of how these parasites may affect human mental health.

2. Materials and Method

2.1 Leishmania parasite

An Iraqi isolate (MHOM / IQ /2005 /MRU15) of *L. donovani* was kindly provided by the laboratory of Parasitology Graduate Studies, Department of Biology, College of Science, University of Baghdad. It was previously diagnosed by polymerase chain reaction (PCR). A biphasic culture medium was utilized in this investigation to cultivate and preserve promastigote forms of *L. donovani* at a temperature of 26 °C. Biphasic culture medium (NNN) to produce and maintain promastigote forms of *L. donovani*, this medium comprises two phases: the solid phase and the liquid phase.

2.2 Experimental animals

The study protocols received approval from the Department of Biology, College of Sciences, University of Babylon (Protocol No. 1423/10-9-2023). The experiments were conducted in adherence to the approved guidelines and ethical standards outlined by the National Committee for Research Ethics in Science and Technology (NETNT). Sixty adult male Wistar rats aged 8-10 weeks and weighing 180 g each were purchased from the animal house of the Department of Biology, College of Science, University of Babylon, Iraq. The animals were allowed to adapt for two weeks before the experiment under standard conditions (temperature 24℃ ±1) and with a 12/12 light /dark cycle.

2.3 Study design

Sixty rats were categorized into four classes (each group contained 15 rats):
group 1 was the control group (the rats were not infected with *L. donovani*), group 2 was the rats infected with *L. donovani* by intraperitoneal injections of 10 million promastigotes, group 3 was the rats infected with *L. donovani* by intraperitoneal injections of 50 million promastigotes, group 4was the rats infected with *L. donovani* by intraperitoneal injections of 100 million promastigotes.

2.4 Experimental behaviour

The rodents were confined in enclosures equipped with provisions of sustenance and hydration. All the boxes housing rats were moved to the behaviour testing room 30 minutes before the beginning of the initial test. The rodents were put in enclosures equipped with provisions of sustenance and hydration. The boxes housing rats were moved to the behaviour testing room 30 minutes before the beginning of the initial test. In these experiments, one rat

was designated the control or "unfamiliar" rat, while another rat was used as the test subject. Behavioural experiments were undertaken two, four, six, eight, and ten weeks after the injection of VL promastigotes. All behaviors were recorded using a video camera placed above the experimental box, approximately 50 cm, to record the movements of the rats. Behavioural parameters were counted manually for 5:00 minutes for each test.

2.4.1 The open field test (*OFT*)

The open field assessments were executed to observe the locomotive activities and anxiety-related behaviors. Consequently, the examination took place for two, four, six, eight, and ten weeks consecutively through daily five-minute sessions.

The rats were positioned in a wooden square field (W: 100 cm, L: 80 cm, H: 40 cm), and the box floor was divided into 20 equal squares. A small box was placed in one corner of the big box. On the day of testing, each rat was placed in any corner of the big box, and its motion was recorded. The floor surfaces and walls of the box were cleaned with a 70% alcohol solution after each experiment. Behaviour analysis includes evaluating the number and time of behavioural parameters measured, including risk estimate over the number and time intervals and at different locations within the OFT. The box was designed with some modifications to suit the current study [21].

2.4.2 The social tests

The social tests were performed to observe emotion and depression. Accordingly, the tests took place for two, four, six, eight, and ten weeks in succession, with five-minute sessions. The rats were positioned in a wooden square field (W: 100 cm, L: 80 cm, H: 40 cm). The box consisted of two chambers, each measuring (W: 50cm, L: 40cm), with transparent glass posters containing a central void and identical containers in each chamber. One of the unfamiliar rats was positioned in one container and the test rat was placed in the middle of the two chambers, and its motion was recorded. The floor surfaces and walls of the box were cleaned with a 70% alcohol solution after each experiment. Behavioural emotion parameters measured in this test include the number of times the rat enters into A and B rooms and time spent in A and B rooms. The initial test session enabled an estimation of the social affiliation of the experimental rats. The box was designed with some modifications to suit the present study [22].

2.5 Statistical analysis

One-way analysis of variance (ANOVA) was performed using SPSS version 23, followed by Duncan's multiple range test. Statistical significance was assumed when the p-value was less than or equal to 0.05.

3. Results

3.1 Open field test (OFT)

3.1.1 Locomotor activity and anxiety measure in rats after two weeks of infection by Leishmania donovani. Table (1) demonstrates a significant lowering ($p<0.05$) in the number of times of rearing, the number of central squares they moved, and time spent in the central squares at a dose of 100 million promastigotes, in comparison to the control group. No significant changes were shown in the following measured parameters: latency time, grooming number times instances, peripheral squares number they moved, number of house entries, and time spent in the house. Regarding the length of time spent in the peripheral square for a dosage of 100 million promastigotes, this resulted in a significant increase ($p\leq0.05$) compared to the control group.

3.1.2 Locomotor activity and anxiety measure in rats after four weeks of infection by Leishmania donovani. Table (2) indicates the significant reductions ($p<0.05$) in the number of rearing, grooming, peripheral, and central squares, as well as time spent in the central square, compared to the control group. Additionally, there was a slight but noticeable increase ($p\leq0.05$) in the time spent in the house at a dosage of 50 million promastigotes, and longer latency times were observed at dosages of 100 million promastigotes and 50 million promastigotes compared to the control group. There were no significant changes in any of the studied parameters regarding the number of entries to the house. Regarding the time spent in the peripheral square for doses of 100 million promastigotes and 50 million promastigotes, there were significant increases compared to the control group.

Table 1. Behavioural changes of rats infected with *Leishmania donovani* for two weeks were observed using an open field test for 5 min.

Parameters test	Control	Dose (10) million promastigotes	Dose (50) million promastigotes	Dose (100) million promastigotes
latency time(sec.)	2.833±0.230	3.400±0.755	3.666±1.000	3.1000±1.000
Rearing (number)	30.000 ± 2.000	34.666 ± 2.516[a]	24.000 ± 2.000[ab]	19.666 ± 1.527[abc]
Grooming (number)	9.333 ±1.527	8.000 ± 1.000	9.666 ± 1.527	7.333 ± 1.527
Peripheral squares (number)	4.333 ± 2.516	3.333 ± 0.577	4.333 ± 2.081	1.666 ± 0.577
Central squares (number)	3.000 ± 1.000	2.333 ± 0.577	4.000 ± 1.000 [b]	1.333 ± 0.577 [ac]
Time spent in the Peripheral squares(sec.)	288.533 ± 1.960	286.366 ± 2.103	82.280 ± 1.335[ab]	294.433 ± 2.013[abc]
Time spent in the central squares(sec.)	9.100 ±1.637	6.366 ± 1.101[ac]	4.80 ± 1.42[a]	2.433 ± 0.611[abc]
House enter (number)	1.33 ± 1.55	1.67 ± 0.58	1.33 ± 1.53	0.33 ± 0.58
Time spent in the house (sec.)	1.466 ± 0.208	3.033 ± 0.642[a]	2.033 ± 0.416	2.800 ± 1.352

Values are mean ± S.D. n=3. a: significant difference compared to the control group. b: significant difference compared to the dose (10) million promastigotes. c: significant difference compared to the dose50 million promastigotes.

Table 2: Behavioural changes of rats infected with *Leishmania donovani* for four weeks were observed using an open field test for 5 min.

Parameters test	Control	Dose (10) million promastigotes	Dose (50) million promastigotes	Dose (100) million promastigotes
latency time(sec.)	1.467 ± 0.550	1.967 ± 0.305	3.467 ± 0.750[ab]	3.067 ± 0.450[ab]
Rearing (number)	31.667 ± 2.081	9.667 ± 2.517[abc]	3.333 ± 1.527[ab]	3.333 ± 1.577[ab]
Grooming (number)	7.667 ± 1.527	4.333 ± 1.527[a]	3.333 ± 1.577[a]	3.000 ± 1.000[a]
Peripheral squares (number)	6.667 ± 2.081	3.000 ± 1.000[a]	1.667 ± 1.154[a]	1.000 ± 0.000[a]
Central squares (number)	4.000 ± 1.000	1.667 ± 0.577[a]	1.000 ± 0.000[a]	1.000 ± 0.000[a]
Time spent in the Peripheral squares(sec.)	281.403 ± 0.562	297.466 ± 1.059[ac]	294.366 ± 0.862[ab]	297.000 ± 1.276[ac]
Time spent in the central squares(sec.)	8.467 ± 1.201	2.033 ± 0.305[a]	1.533 ± 0.513[a]	1.300 ± 0.100[a]
House enter (number)	1.000 ± 0.000	1.000 ± 0.000	1.333 ± 0.577	1.000 ± 0.000
Time spent in the house (sec.)	1.867 ± 0.208	1.400 ± 0.200	3.267 ± 1.159[ab]	2.533 ± 0.602

Values are mean ± S.D. n=3. a: significant difference compared to the control group. b: significant difference compared to the dose (10) million promastigotes. c: significant difference compared to the dose50 million promastigotes.

3.1.3 Locomotor activity and anxiety measure in rats after six weeks of infection by Leishmania donovani. Table (3) indicates a significant increase ($p \leq 0.05$) in latency time at a dose of 50 million promastigotes, compared to both the control group and the dose of 10 million promastigotes. There were significant decreases ($p<0.05$) in the rearing number, grooming number, peripheral number, central number, and time spent in the central sector compared to the control group. The duration spent in the peripheral significantly decreased, whereas the time spent in the house significantly increased for all dosages compared to the control group. There were significant increases in the number of entries to the house for dosages of 10 million promastigotes and 100 million promastigotes compared to the control group.

Table 3. Behavioural changes of rats infected with *Leishmania donovani* for six weeks were observed using an open field test for 5 min.

Parameters test	Control	Dose (10) million promastigotes	Dose (50) million promastigotes	Dose (100) million promastigotes
latency time(sec.)	2.400 ± 1.200	2.533 ± 0.737^{c}	19.500 ±1.907^{ab}	2.633 ± 1.150^{c}
Rearing (number)	32.667 ± 2.517	2.000 ± 1.000^{a}	2.000 ± 0.000^{a}	1.667 ± 0.577^{a}
Grooming (number)	5.333 ± 1.528	1.667 ± 0.577^{a}	1.667 ± 1.155^{a}	2.000 ± 1.000^{a}
Peripheral squares (number)	6.667 ± 1.528	4.000 ± 1.000^{a}	2.333 ± 0.577^{a}	3.333 ± 1.155^{a}
Central squares (number)	3.000 ± 1.000	1.000 ± 0.000^{a}	1.000 ± 0.000^{a}	1.000 ± 0.000^{a}
Time spent in the Peripheral square(sec.)	288.600 ± 0.984	264.567 ± 5.200^{ac}	212.733 ± 6.155^{ab}	150.600 ± 4.700^{abc}
Time spent in the central square(sec.)	7.500 ± 1.400	3.933 ± 0.321^{ac}	1.467 ± 0.153^{ab}	1.500 ± 0.200^{ab}
House enter (number)	1.000 ± 0.000	3.000 ± 1.000^{a}	1.667 ± 0.577	3.000 ± 1.000^{a}
Time spent in the house (sec.)	1.833 ± 0.251	35.633 ± 4.652^{ac}	70.500 ± 10.200^{ab}	146.333 ± 3.750^{abc}

Values are mean ± S.D. n=3. a: significant difference compared to the control group. b: significant difference compared to the dose (10) million promastigotes. c: significant difference compared to the dose50 million promastigotes.

3.1.4 Locomotor activity and anxiety measure in rats after eight weeks of infection by Leishmania donovani. Table (4) demonstrates significant reductions ($p<0.05$) in the number of rearing, grooming, central number, peripheral number, and time spent in the central sequence, compared to the control group. There were highly significant increases ($p \leq 0.05$) in latency time for doses 10 million promastigotes and 100 million promastigotes. There were notably significant reductions in time spent in the peripheral square for all dosages, compared to the control group. Additionally, there was a small rise in the number of house entries for dose 10 million promastigotes. Regarding the time spent in the house, noteworthy increases were recorded at all doses compared to the control group.

Table 4: Behavioural changes of rats infected with *Leishmania donovani* for eight weeks were observed using an open field test for 5 min.

Cvh.	Control	Dose (10) million promastigotes	Dose (50) million promastigotes	Dose(100)million promastigotes
latenkcy time(sec.)	1.500 ± 0.200	7.733 ± 2.150^{ac}	1.233 ± 0.153^{b}	4.367 ±1.700^{abc}
Rearing (number)	30.000 ± 5.000	1.667 ± 0.577^{a}	1.333 ± 0.577^{a}	1.000 ± 0.000^{a}
Grooming (number)	6.000 ± 3.000	1.667 ± 0.577^{a}	1.000 ± 0.000^{a}	1.000 ± 0.000^{a}
Peripheral squares (number)	5.333 ± 2.517	3.667 ±0.577	2.000 ± 1.000^{a}	1.000 ± 0.000^{ab}
Central squares (number)	4.000 ± 2.000	1.000 ± 0.000^{a}	1.000 ± 0.000^{a}	1.000 ± 0.000^{a}
Time spent in the Peripheral squares(sec.)	290.567 ±4.709	236. 467 ± 1.650^{ac}	65.200 ± 1.179^{ab}	15.467 ± 4.650^{abc}
Time spent in the central squares(sec.)	6.767 ± 1.172	1.333 ± 0.153^{a}	1.300 ± 0.200^{a}	1.267 ± 0.153^{a}
House enter (number)	1.333 ± 0.577	3.000 ± 1.000^{a}	2.000 ± 1.000	1.000 ± 0.000^{b}
Time spent in the house (sec.)	1.800 ± 0.300	61.467 ± 3.600^{ac}	234.53 ± 1.750^{ab}	280.40± 4.651^{abc}

Values are mean ± S.D. n=3. a: significant difference compared to the control group. b: significant difference compared to the dose (10) million promastigotes. c: significant difference compared to the dose50 million promastigotes.

3.1.5 Locomotor activity and anxiety measure in rats after ten weeks of infection by Leishmania donovani. Table (5) demonstrates the noteworthy decreases ($p<0.05$) in the frequency of rearing, grooming, peripheral, central, and time spent in the peripheral, in comparison to the control group. There were significant increases ($p\leq0.05$) in latency time for doses 10 million promastigotes and 100 million promastigote compared to the control group, as well as for dosage 50 million promastigotes compared to dose 10 million promastigotes. There were notably significant reductions in time spent in the central square for all dosages compared to the control group. In addition, there were minor increases in the number of house entries for doses 10 million promastigotes and 50 million promastigotes compared to the control group, as well as a higher number of entries for dosage 50 million promastigotes compared to dose 100 million promastigotes. In terms of time spent in the house, significant increases were observed at all dosage levels when compared to the control group.

Table 5: Behavioural changes of rats infected with *leishmania donovani* for ten weeks were observed using an open field test for 5 min.

Parameters test	Control	Dose(10)million promastigotes	Dose (50) million promastigotes	Dose (100) million promastigotes
latency time(sec.)	1.467 ± 0.251	7.500 ± 1.600^{ac}	3.433 ± 0.650^{b}	6.667 ± 1.856^{ac}
Rearing (number)	26.000 ± 3.000	2.333 ± 0.577^{ac}	1.667 ± 0.577^{ab}	1.333 ± 0577^{abc}
Grooming (number)	10.000 ± 1.000	1.667 ± 0.577^{ac}	1.667 ± 0.577^{ab}	1.333 ±0.577^{abc}
Peripheral squares (number)	6.000 ± 1.000	3.000 ± 1.000^{ac}	3.000 ± 1.000^{ab}	2.000 ± 1.000^{abc}
Central squares (number)	4.000 ± 1.000	1.667 ± 1.155^{ac}	1.333 ± 0.577^{ab}	1.333 ± 0.577^{abc}
Time spent in the Peripheral squares(sec.)	293.467 ± 2.815	196.30 ± 1.200^{ac}	102.633 ±1.059^{ab}	41.63 ± 1.007^{abc}
Time spent in the central squares(sec.)	6.500 ± 1.868	1.533 ± 0.153^{a}	2.167 ± 0.902^{a}	1.833 ± 0.666^{a}
House enter (number)	1.000 ±0.000	2.667 ± 0.577^{a}	3.333 ± 1.528^{a}	1.333 ± 0.577^{c}
Time spent in the house (sec.)	1.900 ± 0.200	96.677 ± 2.917^{ac}	190.567 ±1.106^{ab}	250.367 ± 2.084^{abc}

Values are mean ± S.D. n=3. a: significant difference compared to the control group. b: significant difference compared to the dose (10) million promastigotes. c: significant difference compared to the dose50 million promastigotes.

The open-field test can be used to estimate locomotor activity and measure anxiety. Normal rodents typically acclimate to the box area and eventually explore the center area. More anxious rodents spend less time in the center area of the box and more time in the peripheral closer to the walls and inside the house Figure 1 C, D and E.

The effect of the infection with *L. donovani* for ten weeks using the open field test provided concurrent assessments of locomotor activity, exploration, and anxiety. The number of times entries to the house and the time spent at the house is often used as a measure of anxiety behaviour. The frequent occurrence of this behaviour suggests that a significant increase ($p \leq 0.05$) in mean anxiety levels was observed in the group receiving a dose of 100 million promastigote at week 8 after the infection when compared to the control group, as shown in Figure 1 A. There were no significant changes in the studied parameters regarding the tests at two and four weeks. The effect of the *L. donovani* infection for ten weeks in the open field test quantified the anxiety behaviour by assessing the total number of entries into the central square and the amount of time spent in the central square. The mean frequency and length of these behaviour significantly reduced ($p<0.05$) in activity, indicating that high levels of anxiety were observed in the fourth, sixth, eighth, and 10th weeks, compared to the second week and the control group, as shown in Figure 1 B.

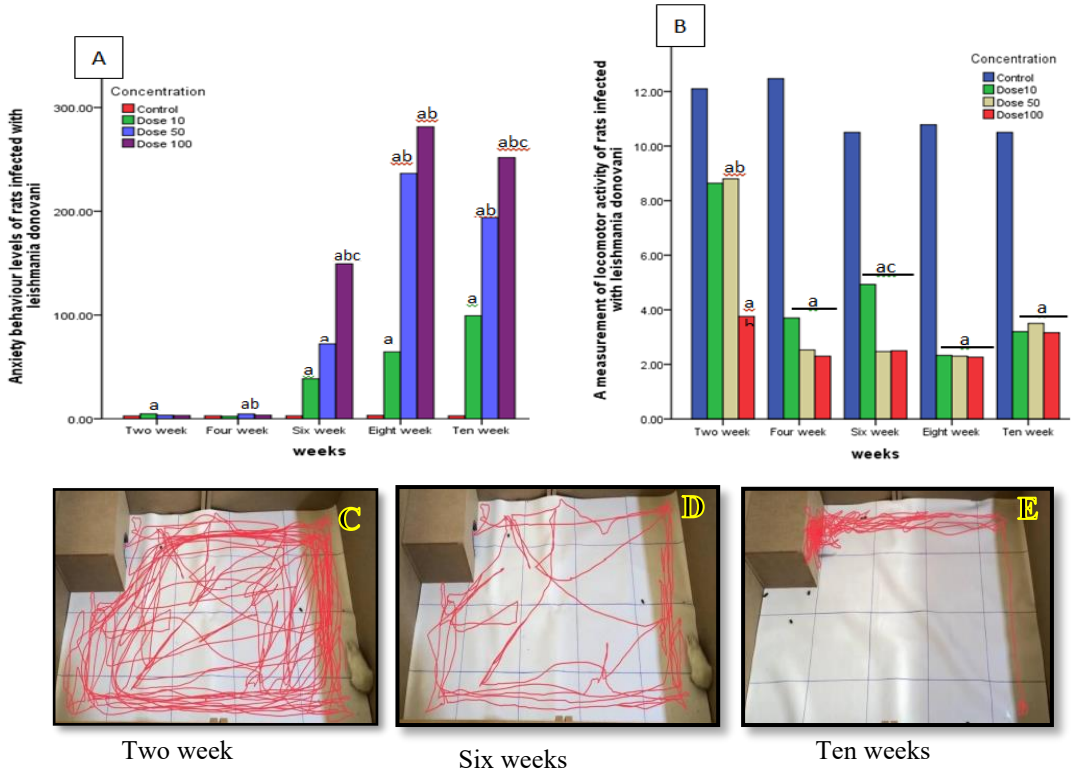

Figure 1 : A- Anxiety behavior measured by the sum of the house enters (number) and time spent in the house in the open field test. B- Locomotors activity measured by the sum of the number of central squares entries and the duration of time spent in the central squares in the open field test. C- After being acclimated to the open field box, rats normally explored the entire box area (peripheral and central squares) two weeks after infection. D- After six weeks from infection, rats showed a decreased exploration of the entire box area with increased movement in the peripheral squares, but E- rats' model of anxiety, rats stayed in the house for

a long time or near the perimeter of the box after ten weeks from infection. a: significant difference compared to the control group. b: significant difference compared to the dose (10) million promastigotes. c: significant difference compared to the dose50 million promastigotes.

3.2 Social behaviour test

3.2.1 Sociability behaviour changes of rats after two weeks of infection by Leishmania donovani

Table (6) displays the significant slight decrease ($p<0.05$) in the duration rats spent in a chamber (B) for the dose 100 group compared to the dose 10 million promastigotes, dose 50 million promastigotes, and control groups. No significant changes were detected in the parameters examined, including the number of visits to chambers (A) and (B). The results showed a significant increase in the time rats spent in the chamber (A) in the dose 100 million promastigotes group compared to the dose 10 million promastigotes, dose 50 million promastigotes, and control groups.

Table 6: Effect the infection with *Leishmania donovani* for two weeks on the social behaviour of rats by using a social test for 5 min

Parameters test	Control	Dose(10)million promastigotes	Dose(50)million promastigotes	Dose(100)million promastigotes
Number of times the rat entered chamber A	1.000 ± 0.000	1.000 ± 0.000	1.000 ± 0.000	1.333 ±0.577
Number of times the rat entered chamber B	1.000 ± 0.000	1.000 ± 0.000	1.000 ± 0.000	1.333 ±0.577
Time spent in chamber A	2.400 ± 0.755	2.367 ± 0.755	2.233 ± 0.907	45.567 ± 4.801[abc]
Time spent in chamber B	298.200 ± 0.954	297.267 ± 0.907	298.233 ± 2.013	255.567 ± 4.806[abc]

Values are mean ± S.D. n=3. a: significant difference compared to the control group. b: significant difference compared to the dose (10) million promastigotes. c: significant difference compared to the dose50 million promastigotes.

3.2.2 Sociability behaviour changes of rats after four weeks of infection by Leishmania donovani

Table (7) indicates that no notable alterations were observed in any of the variables analyzed, including the numerical number of visits to the chambers (A) and (B). There was a significant reduction ($p<0.05$) in the length of time spent in the chamber (B) for the group receiving a dose of 100 million promastigotes, compared to the groups receiving doses of 10 million promastigotes and 50 million promastigotes, as well as the control group. The duration spent in the chamber (A) showed a significant increase ($p\leq0.05$) in a dose of 10 million promastigote compared to the control group, as well as in a dose of 50 million promastigotes and 100 million promastigotes when compared to the other groups.

3.2.3 Sociability behaviour changes of rats after six weeks of infection by L. donovani

Table (8) demonstrates a significant increase ($p\leq0.05$) in the visits of the chambers (A) and (B) at a dose of 100 million promastigotes compared to the dose of 10 million promastigotes, a dose of 50 million promastigotes, and control groups. The analysis of time spent in a chamber (A) revealed a significant increase in the dose of 10 million promastigotes compared to the control group, as well as in the dose of 50 million promastigotes compared to both the control group and dose of 10 million promastigotes, in addition, dose of 100 million

promastigotes showed a significant increase compared to all the other groups. There was a significant reduction ($p<0.05$) in the time spent in chamber (B) for the dose of 10 million promastigotes compared to the control group and the dose the 50 million promastigotes. Similarly, there was a substantial decrease in the time spent in chamber (B) for the dose of 50 million promastigotes compared to the control group and the dose of 10 million promastigotes. In addition, the dose of 100 million promastigotes resulted in a decrease in the time spent in chamber (B) for all groups.

Table 7: Effect the infection with *Leishmania donovani* for four weeks on the social behaviour of rats by using a social test for 5 min.

Parameters test	Control	Dose(10)million promastigotes	Dose(50)million promastigotes	Dose(100)million promastigotes
Number of times the rat entered chamber A	1.000 ± 0.000	1.000 ± 0.000	1.000 ± 0.000	1.000 ± 0.000
Number of times the rat entered chamber B	1.000 ± 0.000	1.000 ± 0.000	1.000 ± 0.000	1.000 ± 0.000
Time spent in chamber A	3.667 ± 0.907	10.567 ± 4.601^{ac}	5.000 ± 2.000	50.433 ± 5.150^{abc}
Time spent in chamber B	296.533 ± 0.874	290.567 ± 5.105	294.533 ± 1.850	250.467 ± 5.150^{abc}

Values are mean ± S.D. n=3. a: significant difference compared to the control group. b: significant difference compared to the dose (10) million promastigotes. c: significant difference compared to the dose50 million promastigotes.

Table 8: Effect the infection with *Leishmania donovani* for six weeks on the social behaviour of rats by using a social test for 5 min.

Parameters test	Control	Dose(10)million promastigotes	Dose(50)million promastigotes	Dose(100)million promastigotes
Number of times the rat entered chamber A	1.000 ± 0.000	1.000 ± 0.000	1.000 ± 0.000	2.333 ± 0.577^{abc}
Number of times the rat entered chamber B	1.000 ± 0.000	1.000 ± 0.000	1.000 ± 0.000	1.667 ± 0.577^{abc}
Time spent in chamber A	2.333 ± 0.751	15.667 ± 1.069^{ac}	86.900 ± 1.300^{ab}	114.667 ± 1.904^{abc}
Time spent in chamber B	298.367 ± 1.124	284.567 ± 0.961^{ac}	213.500 ± 1.952^{ab}	186. 467 ±1.986^{abc}

Values are mean ± S.D. n=3. a: significant difference compared to the control group. b: significant difference compared to the dose (10) million promastigotes. c: significant difference compared to the dose50 million promastigotes.

3.2.4 Sociability behaviour changes of rats after eight weeks of infection by Leishmania donovani

Table (9) shows a slight significant increase ($p\leq0.05$) in the number of visits to the chamber (A) in the dose of 10 million promastigotes and 50 million promastigotes compared to the control group, as well as in the dose of 100 million promastigotes compared to the dose of 50 million promastigotes. The time spent in the chamber (A) resulted in a significant increase in the dose of 10 million promastigotes compared to the control group and the dose of 50 million promastigotes. Additionally, the dose of 50 million promastigotes showed a significant increase in time spent in chamber (A) compared to the control group and the dose of 10 million promastigotes, while the dose of 100 million promastigotes showed a

significant increase with all groups. There was a significant increase in the number of visits to the chamber (B) in the doses of 10 million promastigotes and 50 million promastigotes compared to the control group, as well as in the dose of 100 million promastigotes compared to the doses of 10 and 50 million promastigotes. The duration of time spent in the chamber (B) exhibited a significant reduction ($p<0.05$) in the dose of 10 million promastigotes when compared to the control group and the dose of 50 million promastigotes. Additionally, the dose of 50 million promastigotes showed a substantial drop in the time spent in room (B) compared to both the control group and the dose of 10 million promastigotes. Furthermore, the dose of 100 million promastigotes showed a significant decrease in the time spent in room (B) when compared to all the other groups.

Table 9: Effect the infection with *Leishmania donovani* for eight weeks on the social behaviour of rats by using a social test for 5 min.

Parameters test	Control	Dose(10)million promastigotes	Dose(50)million promastigotes	Dose(100)million promastigotes
Number of times the rat entered chamber A	1.000 ± 0.000	2.667 ± 0.577[a]	3.000 ± 1.000[a]	1.667 ± 0.577[c]
Number of times the rat entered chamber B	1.000 ± 0.000	3.000 ± 1.000[a]	3.000 ± 1.000[a]	1.333 ± 0.577[bc]
Time spent in chamber A	1.833 ± 0.416	97.300 ± 1.800[ac]	55.067 ± 1.761[ab]	220.667 ± 5.101[abc]
Time spent in chamber B	299.200 ± 0.954	202.600 ± 2.052[ac]	245.500 ± 2.152[ab]	80.600 ± 5.112[abc]

Values are mean ± S.D. n=3. a: significant difference compared to the control group. b: significant difference compared to the dose (10) million promastigotes. c: significant difference compared to the dose50 million promastigotes.

3.2.5 Sociability behaviour changes of rats after ten weeks of infection by L. donovani

Table (10) shows that upon observation, no notable alterations were found in any of the parameters that were analyzed, including the numerical of visits to chambers (A) or (B). The duration spent in chamber A exhibited a significant increase ($p\leq0.05$) in the dose of 10 million promastigotes when compared to the control group and the dose of 50 million promastigotes, as well as in the dose of 50 million promastigotes when compared to the control group and the dose of 10 million promastigotes, and in the dose of 100 million promastigotes when compared to the control group and the dose of 10 million promastigotes. The time spent in room (B) exhibited a significant reduction ($p<0.05$) in the dose of 10 million promastigotes compared to both the control group and the dosage of 50 million promastigotes. Additionally, the dose of 50 million promastigotes showed a significant decrease in the time spent in chamber B in comparison to the control group and the dose of 10 million promastigotes, while the dose of 100 million promastigotes showed a significant decrease in comparison to both the control group and the dose of 10 million promastigotes.

Table 10. Effect the infection with *Leishmania donovani* for eight weeks on the social behaviour of rats by using a social test for 5 min**.**

Parameters test	Control	Dose(10)million Promastigotes	Dose(50)million promastigotes	Dose(100)million promastigotes
Number of times the rat entered chamber A	1.000 ± 0.000	1.333 ± 0.577	1.667 ± 0.577	1.333 ± 0.577
Number of times the rat entered chamber B	1.333 ± 0.577	1.000 ± 0.000	1.000 ± 0.000	1.000 ± 0.000
Time spent in chamber A	2.500 ± 0.917	293.433 ± 2.914^{ac}	298.433 ± 1.701^{ab}	299.400 ± 0.755^{ab}
Time spent in chamber B	297.400 ± 0.917	6.400 ± 3.200^{ac}	2.700 ± 1.114^{ab}	1.833 ± 0.757^{ab}

Values are mean ± S.D. n=3. a: significant difference compared to the control group. b: significant difference compared to the dose (10) million promastigotes. c: significant difference compared to the dose50 million promastigotes.

3.2.6 Measurement of emotion and social communication of rats infected with L. donovani

The sociability test involves measuring the interaction of a rat with an unfamiliar rat. This test is used to assess sociability. A rat is considered more social if it spends more time with an unfamiliar rat than an empty cup. Like these social tests are useful for estimating rat abnormality. Rats infected with parasites are noticeably less social. The effect of the infection with *L. donovani* for ten weeks using the social test provided concurrent assessments of emotion and communication. The number of rats entering the chamber (A) and the time spent in this chamber showed that there was a significant increase ($p \leq 0.05$) in the mean for the groups that received a dose of 100 million promastigotes at all weeks after infection, and there was a significant increase in the number of rats entering chamber A of doses 10, 50 million promastigotes at six, eight and ten weeks as compared to the control group. Chamber (B), which contained the stranger, showed a significant decrease ($p<0.05$) in the mean for all doses at eight and ten weeks after infection and in dose 10 for week eight and in doses 50, 100 million promastigotes for week six, as shown in Figure (2).

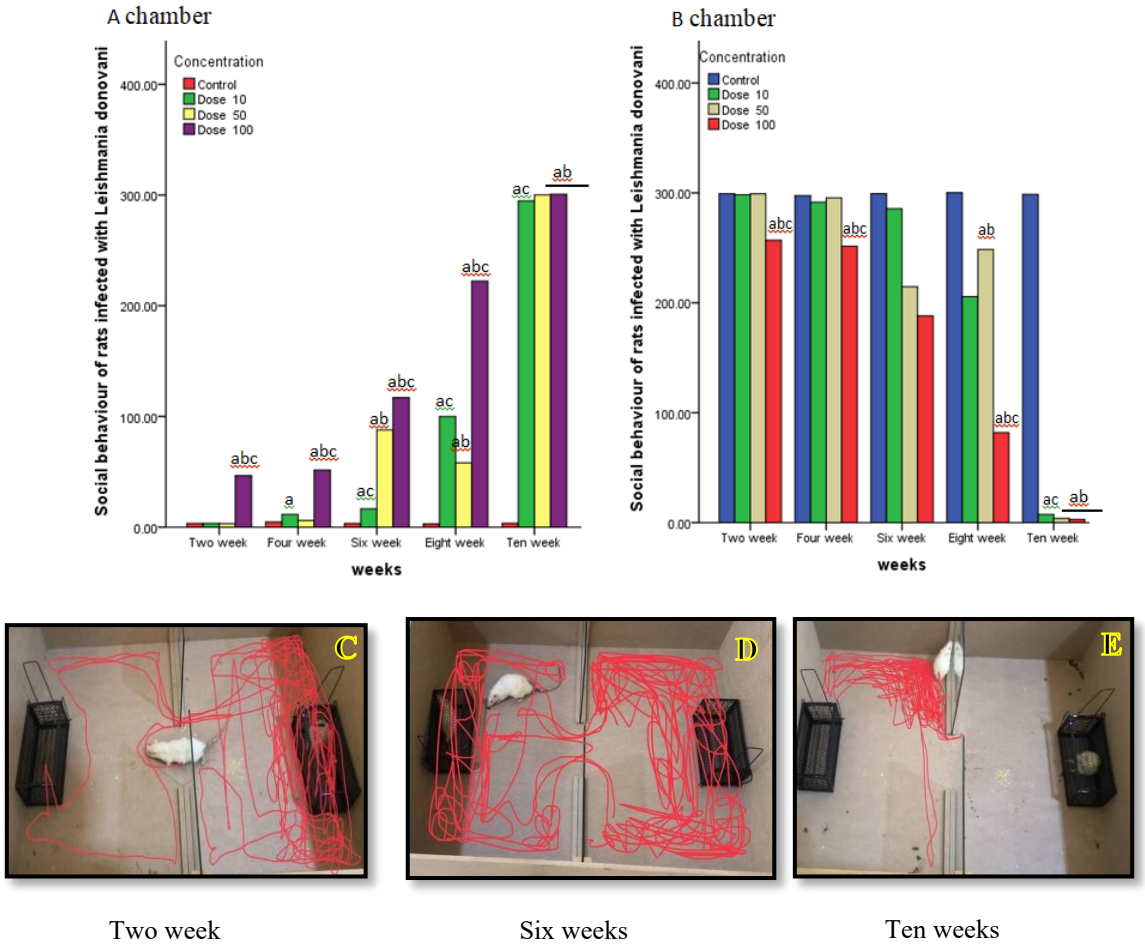

Figure 2: Effect the infection with *L. donovani* for ten weeks on the social interaction behaviour of rats by using the social behaviour test for 5 min. **A chamber** is measured by the time rats spent in the chamber (A) plus the number of the times rats visited this chamber. **B chamber** measured by time rats spent in chamber (B) plus the number of times rats visited this chamber. Picture C shows rats spent more time in the B chamber with an unfamiliar rat compared to the time spent in the A chamber during two weeks of infection. Picture D shows rats spent time in the B chamber with an unfamiliar rat approximately equal to the time spent in the A chamber during six weeks of infection. Picture E shows that rats spent significantly less time in the B chamber with an unfamiliar rat than in the A chamber during ten weeks of infection. a: significant difference compared to the control group. b: significant difference compared to the dose (10) million promastigotes. c: significant difference compared to the dose of 50 million promastigotes.

4. Discussion:

Scientific evidence indicates a correlation between infection, inflammation, and illness behaviour in people and particularly in animals. Such behaviour in rodents can impact performance in tests designed to evaluate anxiety, locomotor activity, and emotional responses. In the present work, a conventional behaviour approach was used to assess the social behaviour and open field (OF) behaviour of rats following infection with *L. donovani*. The present study's findings demonstrate the relationship between parasitic infection and the behavioural changes observed in visceral leishmaniasis infection. The present study is in agreement with a previous study [23], which examined the consequences and impacts of a

high rate of VL relapse, as well as the effects of stress, depression, anxiety, and fear. It demonstrated a direct correlation between VL and a decline in patients' quality of life [24]. All 96 (100%) individuals with VL exhibited symptoms of mental depression.
Tests indicated a substantial reduction in social behaviour in the infected groups following VL infection as the infection progressed until the tenth week, whereby the number of times entering the empty room and the time spent in it increased, which was possibly related to emotional sensitivity and depression-like behaviour. Furthermore, during the OF test, we noticed a noteworthy reduction in the duration spent in the central area and an increase in the average duration spent in the home (dark room) in the infected groups at the highest dosage until the final week of the experiment. This behaviour is typically linked to anxiety, suggesting a decline in general activity and locomotor behaviour. A previous study showed that women affected with leishmaniasis, along with depression and any other psychiatric diseases that were experienced, tended to have quite high rates of depression [25]. Furthermore, reducing some cytokines inside the central nervous system (CNS) during leishmaniasis infection can contribute to the worried behaviour observed in infected mice. Changes in BDNF (brain-derived neurotrophic factor) levels have also been linked to the development of anxiety symptoms, whereas behavioural inhibition temperament has been linked to anxiety disorders [26].

An investigation demonstrated a notable reduction of the activity in the infected group following infection with *L. amazonensis*. This was correlated with anxiety-like behavioural patterns and led to a reduction in overall physical activity. Notably, the infected group exhibited a significant decrease in the time allocated to the center and across the line during observation in an OF test. This showed that the infected group had markedly elevated concentrations of IL-1β, IL-6, and produced TNF-α 10 weeks post-infection [27]. Sustained and excessive synthesis of pro-inflammatory cytokines can lead to tissue and central nervous system damage, resulting in significant changes in behaviour, such as depression and anxiety. These mechanisms are precisely controlled by cytokines, particularly the main inflammatory interleukin-1 beta (IL-1β) and tumour necrosis factor-alpha (TNF-α) [28]. These compounds have the ability to both stimulate and heighten the sensitivity of the neurons of the spinal cord responsible for transmitting peripheral nociceptive data to the brain. Scientific evidence has shown a correlation between sickness behaviour and inflammatory response, neuro-inflammation, and pain problems in animals [29,30].

In addition, the absence of IFN-γ in mice led to heightened levels of anxiety-like and depressive-like behaviors. IFN-γ is crucial in regulating the central nervous system processes that influence the modulation of anxiety and depressed states, which were linked to a decrease in the expression of nerve growth factor (NGF) in the prefrontal cortex of the mouse brain submitted for testing [31]. Neurological manifestations have been associated with *Leishmania* infection, the assessment of parasitic load, and alterations in behaviour in mice infected with *L. amazonensis*. *L. amazonensis* is commonly associated with various clinical manifestations of cutaneous leishmaniasis. Nonetheless, it can also disseminate to organs and induce the visceral form of the disease. Infected mice exhibited reduced levels of cytokines in the prefrontal cortex, which correlated with an increase in anxiety-related behaviour at two and four months after injection [32].

According to this study, a large proportion of the observed phenomena may be illustrated by hopelessness, social isolation, and a deficiency of interpersonal communication. Consistent with a prior study, the results elucidated the psychosocial approach for analysis individuals by considering their personal experiences and the combined influence of their surroundings by evaluating how these factors affect their mental and emotional state. Culture

and society have considerable influences on the impact of psychosocial illness. It might be perceived as a psychiatric condition or emotional distress and defined by the emotions of anxiety, sadness, and despair, which are frequently impacted by societal discrimination [33]. Some studies provide valuable insights into the correlation between stigma and leishmaniasis. There is a strong correlation between leishmaniasis, and the strength of this stigma is determined by the severity and frequency of the leishmaniasis lesions. A study found that individuals experienced social, physical, and emotional isolation due to exclusion from social groups. Furthermore, they reported experiencing psychological effects in the form of despair and anxiety [34, 35, 36].

Animal models represent complex cognitive and emotional processes relevant to human behavior. They are important methodological tools in pre-clinical studies. Behavioural testing is primarily and specifically developed to validate and support a theory of cognition or emotion. Open field and social testing are especially important behavioural test batteries. Individual, sex, breed, stock, early life stress, temperature, pH, immunological response, biomarkers, and others are animal models [17].

The present results align with previous research that demonstrates both direct and indirect links between parasite infection and mental disorders. For instance, *Toxoplazma gondii* has been demonstrated to have an impact on various psychiatric disorders, including schizophrenia, bipolar disorder, and major affective disorder (MAD) [37]. Neuromodulation is believed to potentially have an impact. Several studies have linked the development of psychiatric problems to the indirect consequences of toxoplasmosis, which involve the release of cytokines and other inflammatory molecules [38, 39]. An investigation carried out on various age groups infected with *T. gondii* revealed a significant incidence of serious depressive illness, which was associated with elevated IgG levels in comparison to the control group [40].

Alterations in behaviour during infection may arise from immunological dysregulation or the host genetics, perhaps responsible for the regulation of depression. Parasitic infection and the absence of important immune cells such as T lymphocytes have been observed to negatively affect emotional well-being and cognition by modifying the immune system [41]. Parasite infections are widely recognized for their ability to stimulate the immune system, leading to heightened immunological activation. This immune response is linked to specific genetic variations that enhance the likelihood of developing psychopathological conditions. Specific genetic differences in the genes responsible for interleukin-1beta (IL-1β), tumour necrosis factor-alpha (TNF-α), and C-reactive protein (CRP), as well as genetic changes impacting T-cell function, could elevate the likelihood of developing depression [42].
Chronic inflammation in the gastrointestinal tract leads to anxiety-like behaviour and changes the biochemistry of the central nervous system. *Trichuris muris* causes mild to moderate inflammation in the colon and promotes anxiety-like behaviour. The levels of circulating tumour necrosis factor-α and interferon-γ, along with the kynurenine and kynurenine/tryptophan ratio, were shown to rise. The proteomic analysis revealed significant changes in the amounts of many proteins associated with inflammation and brain function [43]. A complex network of neurotransmitters regulates the physiological pathways connected to behavioural and neurocognitive function, which might modify behaviour. Serotonin (5-HT) production requires tryptophan. On the other hand, the kynurenine pathway requires IDO overexpression, which is linked to depression. TNF and IFNγ, pro-inflammatory cytokines, stimulate tryptophan degradation via IDO-mediated degradation, leading to TRYCAT synthesis. These catabolites can cause neurodegeneration since

tryptophan is an initial form of the essential serotonin in the brain. CNS IDO levels can influence serotonin levels and may play a role in depression [44].

The present study provides evidence in favor of network theory, which asserts that an initial mental difficulty can stimulate alternative processes and function as a shared element, therefore facilitating a trajectory of progressive changes, as observed in anxiety and depressive illnesses [45]. Social and psychological stress can produce *Trypanosoma cruzi* infection-related pathology, anxiety, cognitive impairment chronic, and Chagas disease. Persistent infection in mice causes behavioural changes, increased GABA, glutamate, and lipid peroxidation byproducts, as well as reduced BDNF synthesis. Prior stimulation of IFNγ and TNF enhances the impact of serotonin on parasite uptake. Thus, cytokines may increase astrocyte Toxoplasma parasite uptake by increasing serotonin absorption. This increases brain parasitism, serotonin deficiency, neurotransmitter and network disruption, and neurotoxicity. This may cause behavioural and cognitive issues [46, 47].

5. Conclusion

This study established a correlation between behavioural modifications that could be influenced by visceral leishmaniasis. The findings indicate that infection with *L. donovani* causes an elevation in anxiety-like behaviour, along with a reduction in social behaviour associated with emotion and social communication, as well as lowering locomotor activity. These observations were made using traditional behavioural tests, including an open field test and a social behaviour assessment.

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Conflict of interest

The authors announced that they have no conflict of interest.

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