Global Journal of Pharmacology 9 (1): 01-12, 2015
ISSN 1992-0075

DOI: 10.5829/idosi.gjp.2015.9.1.9232

Protective Effect of Minocycline Against Bacterial Infection-Induced Sickness Behavior in Rats

[1]Maha G. Soliman, [2]A. Hanaa-Mansour, [2]Wedad A. Hasan and [1]Nahla A. Hassaan

[1]Department of Zoology, Faculty of Science, Al-Azhar University, Cairo, Egypt
[2]Department of Pharmacology,
National Organization for Drug Control and Research (NODCAR) Cairo, Egypt

Abstract: Many studies suggest that bacterial infection generate adverse effects on the cognitive, behavioral and emotional status. Inflammation, oxidative stress and altered level of immune-cytokines are involved in the pathogenesis of sickness behavior in rats. Minocycline is a broad spectrum second generation semi synthetic derivative of the bacteriostatic antibiotic tetracycline. The present study aimed to determine effects of minocycline on neurobehavioral and some other related parameters in bacterially infected and non- infected rats. The levels of Interferon gamma (IFNγ) and nitric oxide (NO) were assessed in brain tissue, serum C-reactive protein (CRP) as well as total and differential leukocytic counts (WBCs), also brain histopathological examination was evaluated. Male Sprague-Dawley rats received (90mg/kg) p.o. minocycline for three days. The infected animals were intraperitoneally injected 48 hours before sacrificing with 200 µl of *E. coli* 24 hours bacterial culture in nutrient broth containing approximately 1.8 x 10^8 cfu / ml. Animals were divided into four groups: - (1) Control group, (2) *Escherichia coli* infected group, (3) Minocycline treated group, (4) Minocycline and *Escherichia coli* treated group. The results revealed that minocycline blocked bacterial infection-associated sickness behavior in rats, reduced signs of cognitive impairment, decreased CRP, IFNγ, NO and total leucocytic count (WBCs).

Key words: *Escherichia coli* · Minocycline · Interferon Gamma · C-Reactive Protein

INTRODUCTION

Minocycline is a highly lipophilic molecule easily penetrating the blood–brain barrier. Minocycline is currently receiving attention as a potential new agent for the treatment of major depression. Minocycline reduces transcription of the downstream pro-inflammatory nitric oxide synthase and the subsequent release of interleukin1β (IL-1β), nitric oxide (NO) [1, 2].

The therapeutic mechanisms of minocycline may involve suppression of proinflammatory cytokines [3, 4]. In the central nervous system, it may decrease the occurrence of microglia derived inflammatory mediators [5].

Entrance of gram-negative bacteria into the body causes the liberation of toxic, soluble products of the bacterial cell wall, such as lipopolysaccharide (LPS, also known as endotoxin) which induces activation of the peripheral innate immune system then stimulates the secretion of CNS cytokines that modulate the behavioral symptoms of sickness.

The LPS components of many bacteria are toxic [6]. LPS may cause a marked increase in circulating cytokines, such as Interleukin (IL)-1β, IL-6 and Tumor Necrosis Factor (TNF)-α, as well as a characteristic set of physiological and behavioral responses [7-9]. Specifically, the syndrome elicited by LPS has been termed 'sickness behavior', since it comprises reductions in general activity, exploration, social interest, feeding and body weight [10, 11] as well as decrements in cognitive functioning such as in learning and memory [12]. In addition to these effects, LPS functions as a stressor, by increasing the release of corticotrophin releasing factor (CRF) and adrenocorticotropic hormone (ACTH) from the hypothalamus–pituitary– adrenal (HPA) axis [13].

Corresponding Author: Nahla Ahmed Hassaan, M.Sc Degree in Department of Zoology, Faculty of Science, Al-Azhar University, Cairo, Egypt. E-mail: nahlaahmed329@yahoo.com.

Minocycline is effective against gram-positive and -negative infections [14, 15]. It is a safe, widely-used, inexpensive antibiotic that has minimal side effects [16]. The action of minocycline is assumed to be exerted through the inhibition of cytochrome c release from the mitochondria, the inhibition of caspase expression and the suppression of microglial activation [17, 18]. Furthermore, minocycline attenuated lipopolysaccharide (LPS)-induced expression of pro-inflammatory cytokines and prevented LPS-induced development of depressive-like behaviors in mice [19]. These lines of evidence suggest that minocycline is a potential antidepressant drug.

MATERIALS AND METHODS

Animals: Albino rats weighing 150-200 gm were used in these experiments. Rats were allowed free access to standard diet and tap water at controlled room temperature [25±2°C] and light controlled condition with 12h-light and dark cycles. Animal handling and experimental protocols were approved by the Research Ethical Committee of the National Organization for Drug Control and Research (NODCAR, Cairo, Egypt).

Drugs: Minocycline hydrochloride capsules were brought from (Chemical Industries Development (CID) - Giza- A.R.E Minocycline was used in a dose of 90mg/Kg. for three days according to Shimazawa *et al*. [20] and Homsi *et al*. [21].

Bacteria Strain: *Escherichia coli* (*E. coli*) NCTC 9001 bacterial strain was obtained from the microbiology control lab in NODCAR. 24 hours growth in nutrient broth (Oxid) at 37°C was used in this study, where in the infected animal groups, each animal was injected intraperitoneally with 0.2 ml of 24 hours growth of *Escherichia coli* suspension in nutrient broth containing approximately 1.8 x 10^8 cfu / ml intraperitoneal for once according to Linde [22], Virkamäki and Yki-Järvinen [23] and Sgibnev and Cherkasov [24].

Experimental Design: Animals Were Divided into Four Equal Groups:

Group 1: Animals received 0.2 ml distilled water intraperitoneal and served as control group.

Group 2: Animals received 0.2 ml of 24 hours growth of *Escherichia coli* suspension in nutrient broth containing approximately 1.8 x 10^8 cfu / ml intraperitoneal for once, 72 hours before 3rd day manipulations and served as infected group.

Group 3: Animals received minocycline (90mg/kg) orally for 3 days and served as treated group.

Group 4: Animals received minocycline (90mg/kg) orally for 3 days and *Escherichia coli* 0.2ml of 24 hours growth of *Escherichia coli* suspension in nutrient broth containing approximately 1.8 x 10^8 cfu / ml intraperitoneal for once.

At the end of the experiment (third day) the animals of each group were divided into two sets. The first set was used to undergo behavioral testing namely T-maze, open field, forced swimming test as well as determination of serum C-reactive protein (CRP) titer. The second set was used to determine step-through passive avoidance test and rotarod test as well as collecting EDTA blood samples. Brains were carefully excised from both sets, for determining NO and IFNγ. Other brains were taken to record histological examination.

Behavioral Tasks

Open Field Test (OFT): The apparatus used in this test according to Vorhess [25], Losser [26], Volosin *et al*. [27].

Rotarod Test: Motor coordination was tested by comparing the latency to fall on the very first trial between treatment groups [28]. The rotarod used in this study is a test of sensorimotor coordination and balance [29].

Passive Avoidance Test: The procedure is described by Narayanan *et al*. [30].

T- Maze: T-maze analysis was performed as described by Wenk [31].

Forced Swimming Test: The forced swim test was performed according to the method of Porsolt *et al*. [32] and Cryan *et al*. [33].

Biochemical Parameters

Estimation of Brain Nitric Oxide Content: Determination of brain tissue nitric oxide was carried out using Biodiagnostic kit according to the method of Montgomery and Dymock [34]. Data were expressed as μmol /L.

Determination of Interferon- γ (Elisa): Determination of brain tissue IFN-γ concentration, the enzyme-linked-immunosorbent- assay (Elisa) method was used using Uscn, Inc.

Determination of C- Reactive Protein: Determination of C-reactive protein level was done using CRP- Latex according to the method of Hayashi *et al*. [35].

Statistical Analysis: Data were expressed as means (n=10-12) animals, each subgroup± SEM. A statistical comparison between the different groups was performed using one-way analysis of the variance (ANOVA) followed by Tukey-Kramer multiple comparison test [36, 37]. Statistical analysis was carried out using prism computer program (Graph Pad software Inc. V5, San Diego, CA, USA). Probability values of less than 0.05 were considered statistically significant.

RESULTS

Effects of Minocycline on Neurobehavioral Tasks in *Escherichia coli* Induced Sickness Behavior in Rats

Motor Activity Tests

Open Field Test: *Escherichia coli* infection significantly increased latency time (sec.) and significantly decreased (ambulation, rearing, grooming) when compared with control group. Administration of Minocycline (90mg/kg, p.o.) for 3 days before induction of *Escherichia coli* infection in rats significantly decreased latency time (sec.) but increased ambulation and grooming frequency when compared with *Escherichia coli* infected group (Fig. 1).

Rotarod Test: *Escherichia coli* infection significantly decreased retention time (sec.) when compared with control group. Administration of Minocycline (90mg/kg, p.o.) for 3 days before induction of *Escherichia coli* infection in rats significantly increased rotarod retention time (sec.) when compared with *Escherichia coli* infected group (Fig. 2).

Cognitive and Emotional State

Passive Avoidance Test

Step-through Latency (*Day* 1): In the testing trial (day 1), Minocycline administration (90mg/kg, p.o.) for 3 days before induction of *Escherichia coli* infection in rats significantly increased step-through latency (sec.) when compared with *Escherichia coli* infected rats (Fig. 3).

Step-through Latency (*Day* 2): The results of Passive avoidance testing trial (day 2), Administration of Minocycline (90mg/kg, p.o.) for 3 days before induction of *Escherichia coli* infection in rats significantly increased step-through latency (sec.) when compared with *Escherichia coli* infected group (Fig. 3).

T-Maze: *Escherichia coli* infection significantly increased the T-maze test ratio (Entries into the novel arm/Entries into previous arm) when compared with control group. Administration of Minocycline (90mg/kg, p.o.) for 3 days before induction of *Escherichia coli* infection in rats significantly decreased T-maze test ratio when compared with control group and *Escherichia coli* infected group (Fig. 2).

Forced Swimming Test

Immobility Time: Administration of Minocycline (90mg/kg, p.o.) for 3 days before induction of *Escherichia coli* infection in rats significantly decreased immobility time (sec.) when compared with *Escherichia coli* infected group (Fig. 3).

Struggling Time: Administration of Minocycline (90mg/kg, p.o.) for 3 days before induction of *Escherichia coli* infection in rats significantly increased struggling time (sec.) when compared with *Escherichia coli* infected group (Fig. 3).

Immunological Parameters

Level of IFNγ in Brain Homogenate: Administration of Minocycline (90mg/kg, p.o.) for 3 days before induction of *Escherichia coli* infection in rats significantly decreased the level of IFNγ in brain homogenates (pg/mg) when compared with *Escherichia coli* infected group at $p<0.05$ (Fig. 4).

Level of Nitric Oxide in Brain Homogenate: Administration of Minocycline (90mg/kg, p.o.) for 3 days before induction of *Escherichia coli* infection in rats significantly decreased the level of NO in brain homogenates (μmol/L) when compared with *Escherichia coli* infected group (Fig. 5).

Serum Level of C-Reactive Protein: Administration of Minocycline (90mg/kg, p.o.) for 3 days before induction of *Escherichia coli* infection in rats significantly decreased the level of CRP (mg/L) when compared with *Escherichia coli* infected group (Fig. 6).

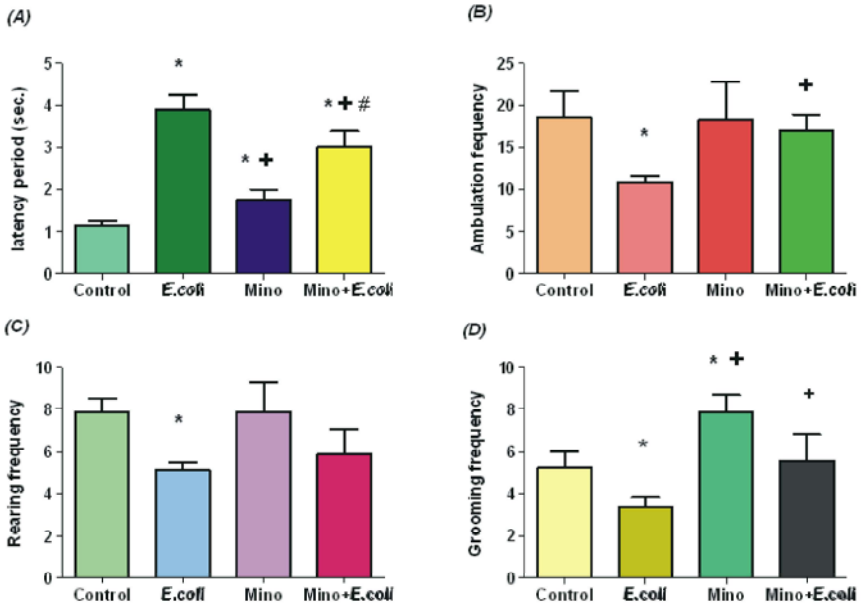

Fig. 1: Open field test

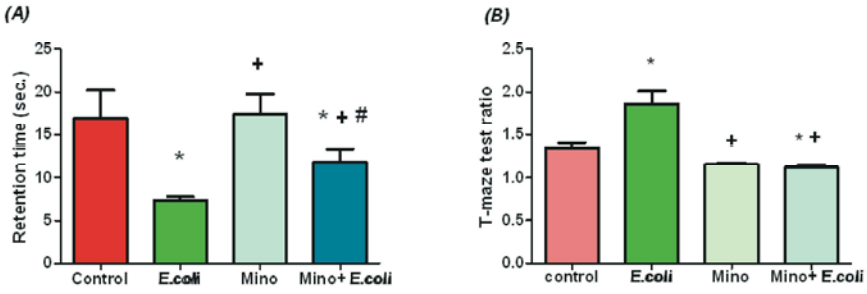

Fig. 2: (A) Rotarod and (B) T-maze tests

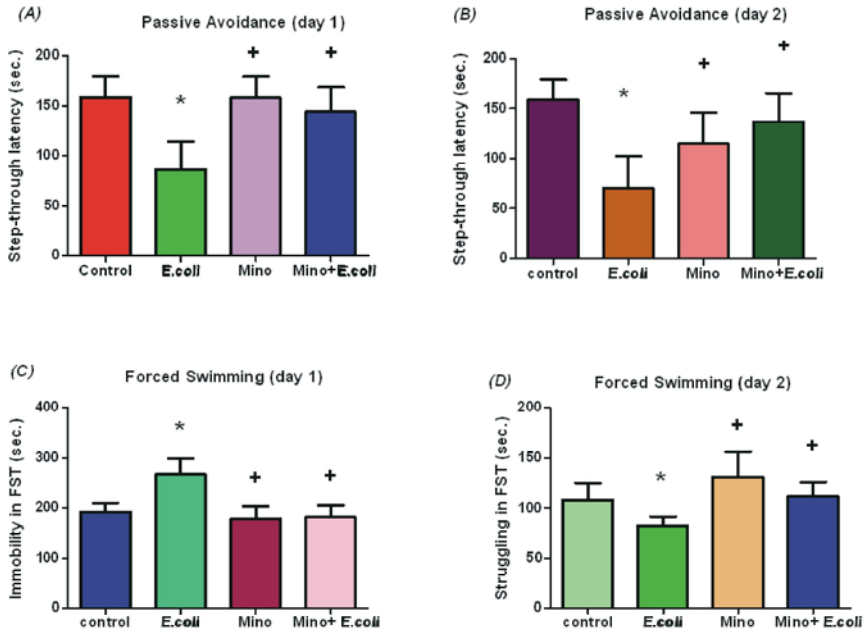

Fig. 3: (A,B) Passive avoidance and(C,D) Forced swimming tests

Table 1: Peripheral blood total leucocytic count and differential leucocytic count in control, *E. coli* and minocycline treated animal groups

Animal group	Total Leukocytic count x10³/Cmm	Neutrophils	Lymphocyte	Monocytes
Normal control	7.938±0.3727	40.50±2.125	52.33±2.603	5.167±0.9804
E. coli	14.68±0.9615 *	53.33±3.040*	41.67±2.871*	6.833±1.515
Minocycline	6.138±0.5109 *+	35.17±4.020+	37.17±1.621*	3.667±0.7601*+
Minocycline + *E. coli*	12.05±1.627 *+#	61.33±2.231*#	28.00±3.38*+#	4.667±1.256

Values are expressed as mean ± S.E. Anova test.

*: Significant at P <0.05 when compared to normal group.

+: Significant at P <0.05 when compared to infected group.

#: Significant at P<0.05 when compared to treated group.

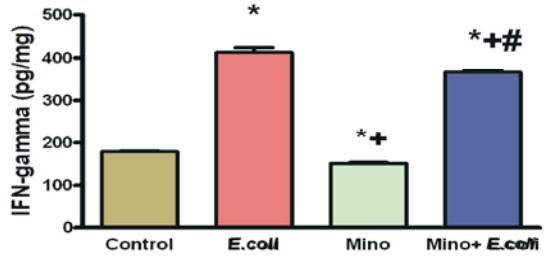

Fig. 4: Effect of Minocycline on the level of IFN-γ in brain homogenate in *Escherichia coli* induced sickness behavior in rats

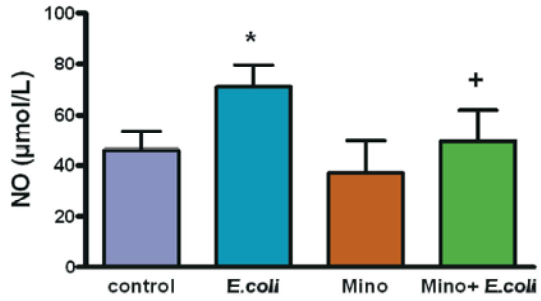

Fig. 5: Effect of Minocycline on Level of Nitric oxide in brain homogenate in *Escherichia coli* induced sickness behavior in rats

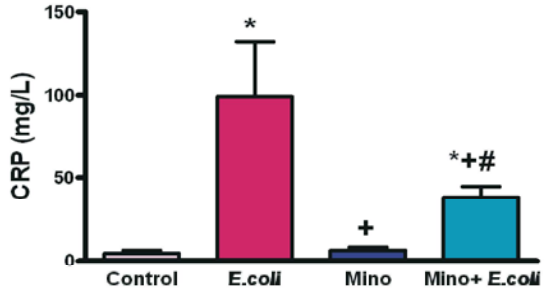

Fig. 6: Effect of Minocycline on Serum Level of C-reactive protein in *Escherichia coli* induced sickness behavior in rats

Hematological Parameters

Total Leucocytes Count:

***Effect of minocycline on total leucocytic count in Escherichia coli induced sickness behavior in rats*:** Administration of Minocycline (90mg/kg, p.o.) for 3 days before induction of *Escherichia coli* infection in rats significantly decreased the total leucocytes count when compared with *Escherichia coli* infected group (Table 1).

Differential Leucocytes Count

Lymphocytes Count:

***Effect of Minocycline on lymphocytes count in Escherichia coli induced sickness behavior in rats*:** *Escherichia coli* infection significantly decreased the peripheral blood lymphocytes count percentage when compared with control group. Administration of Minocycline (90mg/kg, p.o.) for 3 days before induction of *Escherichia coli* infection in rats significantly decreased the lymphocytes percentage when compared with control group, *Escherichia coli* infected group and Minocycline treated group (Table 1).

Neutrophils: *Escherichia coli* infection significantly increased the neutrophils count percentage when compared with control group. Administration of Minocycline (90 mg/kg, p.o.) for 3 days before induction of *Escherichia coli* infection in rats significantly increased the neutrophils percentage when compared with control group and Minocycline treated group (Table 1).

Histological Examination

Effect of Minocycline on Histological Examination in *Escherichia coli Induced Sickness Behavior in Rats*: Histological section of control rat brain showed the typical layered appearance of the cerebral cortex and the underlying structures including the hippocampus and subcortical areas. No histological abnormalities were detected (Fig. 7a).

The brain histopathology of rats infected with *Escherichia coli* revealed severe lesions including damage in the cortex, gross disorganization of normal architecture, showing gross disorganization of the typical layered appearance of the cerebral cortex, hippocampus,

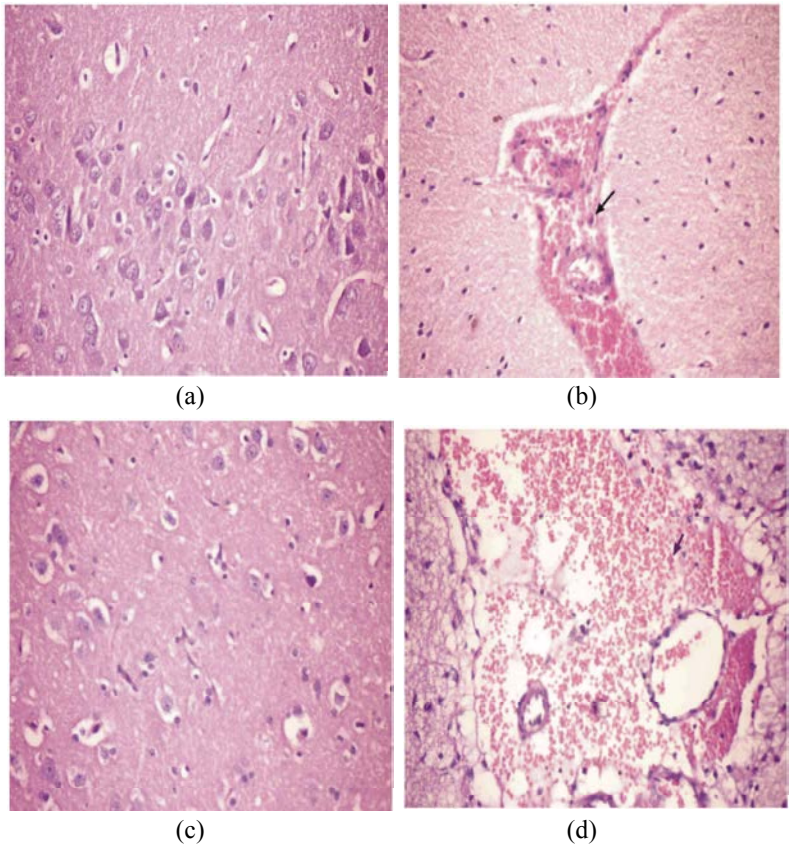

Fig. 7: Shows (a) control (b) infection with *Escherichia coli* showing perivascular hemorrhage (c) treatment with Minocycline showing no histopathological changes with few pyknotic cells and (d) treatment with Minocycline + *E.coli* is showing haemorrhage in Virchow space

as well as the subcortical areas, necrosis, foamy appearance of cytoplasm and severe gliosis, neuronal degeneration, demyelination, separation of nerve fibers, aggregation of glial cells and necrosis. Besides, perivascular edema, dilatation of the vessels and necrosis of neurons were observed.

Escherichia coli infection resulted in brain injury in selective brain areas, including bilateral ventricular enlargement, cell necrosis at the sub- and periventricular areas. Hemorrhage was sometimes observed mainly in the edematous layer. In comparison to control group *Escherichia coli* infection induced endotoxemia that was manifested by inflammatory cellular infiltration and intracellular edema of brain cells. The brain pathology of rats infected with *Escherichia coli* revealed inflammatory infiltrates of polymorphonuclear cells (PMNs) in the meningeal and ventricular spaces. Also, an induced marked recruitment of PMN into brain parenchyma, especially in the white matter was observed. Hippocampus showed severe inflammation and apoptosis of neurons. Acute hemorrhage, most prominent in the white matter of the brain, was observed. Cortex showed increased cellularity attributable to inflammatory exudates (Fig. 7b).

Sections of animals treated with Minocycline (90mg/kg, p.o.) for 3 days showed no lesions in the cortex and no significant damage in underlying structures including the hippocampus or subcortical areas. The brain tissue of the Minocycline treated rats looked almost like the control brain (Fig.7c).

Histopathological sections obtained from animals administered Minocycline (90mg/kg, p.o.) for 3 days before induction of *Escherichia coli* infection revealed mild to moderate degrees of lesions in the cortex without significant damage in other subcortical areas. Changes were comparatively less severe in this group where Minocycline attenuated bacterial infection-induced brain injury in these rat brains. Minocycline treatment reduced histological brain damage. It ameliorated brain injury and abolished the signs of neuroinflammation.

Minocycline could minimize white matter injury, there was less edema in the Minocycline treated group than in *Escherichia coli* infected group. Treatment preserved the histological picture of the brain with decreased microglial cells (Fig. 7d).

DISCUSSION

An animal model with biological and/or clinical relevance in the behavioral neurosciences is a living organism used to study brain-behavior relations under controlled conditions [38].

Neurobehavioral tasks assessment determines both the motor activity, the cognitive and emotional state screening. In this study the motor activity was evaluated by open field test (OFT) and rotarod test. On the other hand, the cognitive and emotional state screening was carried out by passive avoidance test, T-maze test and forced swimming test.

The present study showed that intraperitoneal infection with LD_{10} of *Escherichia coli* was associated with remarkable behavioral changes. This agrees with Penninx *et al*. [39] who mentioned that systemic infection is associated with an increased frequency of behavioral and cognitive complications.

In the present study treatment with minocycline attenuates some of the behavioral and cognitive effects of *Escherichia coli.* Minocycline decreased the latency period in the open field test (OFT) and increased the OFT grooming when compared with *Escherichia coli* infected group. On the other hand, in passive avoidance test (PA) step through latency in 2nd day was significantly increased when compared with bacterial infected group. Administration of minocycline significantly decreased the T-maze test ratio.

We observed that exposure of experimental animals to *Escherichia coli* infection is accompanied by reduced open field activity; minocycline prophylactic treatments improved open field activity and social interaction tests. These agreed with Bauhofer *et al*. [40] who demonstrated that prophylaxis was efficient, especially with antibiotics and G-CSF. Minocycline was able to ameliorate deficits of social interaction and PPI but not hyperlocomotion [41]. The ability of minocycline to modulate inflammatory reactions may be of great importance in the selection of neuroprotective agents, especially in chronic conditions like diabetes and Alzheimer's disease [42].

In a separate study, minocycline ameliorated performance on the Morris Water Maze and Open Field Tasks in a rat model of vascular dementia [43].

Minocycline thereby reduces transcription of the downstream pro-inflammatory nitric oxide synthase and cyclooxygenase-2 and the subsequent release of interleukin1β (IL-1β), nitric oxide (NO) and prostaglandin E2. Minocycline may be particularly helpful in patients with depression and co morbid cognitive impairment, as well as depression associated with organic brain disease. The antinociceptive effect of minocycline and proposed role for minocycline in the treatment of patients with major depression and prominent somatic discomfort and somatoform spectrum disorders [1, 2]. In addition, our results observed that treatment with minocycline attenuated LPS- induced motor behavioral deficits in rotarod test. This observation agreed with Kobayashi *et al*. [44] who mentioned that minocycline significantly increased rotarod performance. Their study demonstrated those microglia were stimulated by lipopolysaccharide (LPS). LPS stimulates the NF-κB pathway, To investigate the mechanism of action of minocycline, they examined the LPS–NF-κB axis. The NF-κB expression was upregulated by LPS and inhibited by the co-treatment of minocycline.

Minocycline therapy provided an enhanced beneficial effect, ameliorating behavioral and neuropathological alterations. Minocycline treatment significantly extended survival and improved rotarod performance to a great degree [45].

Our results revealed that minocycline treated rats improves cognitive impairment in passive avoidance test when compared with bacterial infected group.

This result is also consistent with previous studies, which reported that minocycline reduces signs of cognitive impairment in preclinical and preliminary clinical trials. For example, reducing microglial activation with minocycline prevents an exaggerated hippocampal IL-1β response and prevents memory impairment on the Contextual Fear Condition task in adult rats infected with *Escherichia coli* as neonates. This effect was also evident when minocycline was administered prior to fear conditioning but after the LPS challenge Williamson *et al*. [46].

In a rat model of traumatic brain injury, minocycline improved active place avoidance learning that required memory lasting less than 2 h. Minocycline prevented cognitive deficits by improving learning and memory spanning at least 24 h in the active place avoidance task [47]. Improvements in spatial learning have also been demonstrated in a mouse model of Alzheimer's disease following 4 weeks of minocycline therapy [48]. Similarly, amyloid precursor protein transgenic mice fed minocycline-containing food exhibited better performance on the Morris Water Maze task as compared to untreated animals [49].

Campbell *et al*. [50] mentioned that minocycline is a tetracycline antibiotic that has been proposed as a potential conjunctive therapy for cognitive disorder. In accordance with our findings Borre *et al*. [51] showed that treatment with minocycline attenuates some of the behavioral and cognitive effects of olfactory bulbectomy. Minocycline partially rescued cognitive decline as seen in the T-maze paradigm, a hippocampus-dependent spatial memory task. In contrast, minocycline was not effective in restoring fear memory impairment following bulbectomy (as measured in the passive avoidance paradigm).

Minocycline monotherapy and in combination treatment, minocycline significantly reduced immobility by increasing climbing behavior on the FST test as compared to vehicle treated rats [52]. Reduced immobility by increased climbing suggests that minocycline may produce antidepressant action via modification of noradrenergic mechanisms [53].

Our results demonstrated that minocycline reduced floating time and increased climbing in FST when compared to bacterial infected group (prevented LPS-induced development of depressive-like behaviors).

This data is in line with the findings of O'Connor *et al*. [19] who reported that minocycline attenuated lipopolysaccharide (LPS)- induced expression of pro-inflammatory cytokines and prevented LPS-induced development of depressive-like behaviors in mice.

Mello *et al*. [54] preliminary evidence indicates that minocycline has antidepressant properties. LPS-treated animals presented an increase in immobility time in the forced swimming test (FST) when compared to controls 24 h after endotoxin administration. Minocycline prevented and reversed LPS-induced alterations in the FST. Taken together, their results demonstrate that Minocycline effectively ameliorate LPS-induced depressive-like behavior.

However, a separate study with minocycline monotherapy prior to the FST on day 1 and day 2, did not find any significant effects on immobility; Therefore, behavioral responses during the FST are not affected by acute systemic injection of LPS [55]. Macrolides, tetracyclines are known to have immunomodulatory activities. Tetracyclines suppress the antibody responses of mice to T-cell-dependent and T-cell-independent antigens [56].

Pro-inflammatory cytokines and other molecules traditionally associated with immune function have been implicated in mediating behavioral and physiological consequences of stressor exposure. There is also evidence that cytokines are aberrantly expressed in depressive populations, suggesting they may play an etiological role in the development of depression/despair-related processes [55].

It was observed that minocycline decrease NO level. Several studies showed that minocycline reduces the expression of inducible nitric oxide synthase and subsequent nitric oxide production [57].

Tetracyclines induced oxidative stress and immunosuppression, Glutathione-S-transferase activity was significantly increased in the blood, liver, kidney and spleen samples of the group that received Tetracycline; It also appeared to suppress specific and nonspecific immune system parameters, such as the haematocrit, leucocyte count, oxidative radical production (nitroblue tetrazolium activity), total plasma protein and immunoglobulin levels and phagocytic activity [58].

Intraperitoneal injection of tetracyclines protects mice from lethal endotoxemia downregulating inducible nitric oxide synthase in various organs and cytokine and nitrate secretion in blood. Tetracyclines are able to protect mice from lipopolysaccharide (LPS)-induced shock, a cytokine-mediated inflammatory reaction. Altogether, these results indicate that tetracyclines are advantageous candidates for the prophylaxis and treatment of septic shock in mice, having both antimicrobial activity and the ability to inhibit endogenous TNF-alpha, IL-1 alpha and iNOS, hence, exerting, potent anti-inflammatory effects. Tetracyclines were found to inhibit NO synthesis by peritoneal macrophages stimulated in vitro with LPS [59].

According to Deak *et al*. [55] it was concluded that endogenous inflammatory mediators do not appear to be involved in the normal progression of behavioral responses during the FST.

The present study showed a decrease in IFN-γ level in minocycline treated rats when compared with bacterial infected group.

This results agreement with previous studies, in which minocycline reduces the expression of pro-inflammatory cytokines (e.g. IL-1β, IL-6, IL-2, TNF-α, IFN-γ) [60, 61].

Houri-Haddad *et al*. [62] recognized that following bacterial challenge, the antibacterial agent's minocycline HCl attenuated the inflammatory process, in its own fashion concluding that antibacterial agents have the ability to induce an anti-inflammatory response. They also modify the inflammatory response to bacterial infection.

Minocycline treatment increased the counts and/or percentages of splenic B-cells. The drug-alone increased the levels of cytokines, including interleukin-1α (IL-1α) and IL-6. In addition, minocycline suppressed the production of interferon-γ that can prevent hematopoiesis [63].

Minocycline prevents microglia activation and rescue behavioral deficits induced by neonatal intrahippocampal injection of lipopolysaccharide LPS in rats [41]. Intrahippocampal injection of LPS results in persistent elevation in cytokines in several brain regions [64]. They speculated that LPS injection in the ventral hippocampus induces significantly increased microglia activation in the ventral hippocampus first and the activated microglia could produce cytokines and free radicals and these factors, in turn, affect Iba1 expression in the brain regions outside hippocampus.

Tetracycline antibiotics have anti-inflammatory and antioxidant properties. Minocycline prevented and reversed LPS-induced increase in IL-1β. It prevented and reversed LPS-induced alterations in nitrite content and oxidative stress parameters (lipid peroxidation and reduced glutathione levels). Taken together, their results demonstrated that minocycline effectively ameliorates LPS-induced depressive-like behavior.

Furthermore, treatment with minocycline reduced C-reactive protein level in serum and total leucocytic count when compared to bacterial endotoxin group.

Campbell *et al*. [50] demonstrated that minocycline treatment *in vitro* reduced IL-6 production by monocytes following LPS stimulation concluding that neuroprotective effects of minocycline are due in part to reduction of activated monocytes, monocyte traffic.

The present study revealed minocycline significant decrease of total leucocytic count, relative spleen and thymus weights and non significant decrease in neutrophils percentage. Al-Ankari and Homeida [65] mentioned that tetracyclines significantly decreased the total number of leukocytes, lymphocytes and thymus but not spleen or body weight. On the contrary, Maximova *et al.* [66] reported neutropenia that was suspected to be secondary to tetracyclines exposure which suggested tetracyclines influence on myeloid cells survival.

Minocycline increased the counts and/or percentages of splenic macrophages, granulocytes, natural killer, while suppressing cytokines that could prevent hematopoiesis, e.g. macrophage inflammatory protein-1α, tumor necrosis factor-α and interferon-γ [67].

Brain histological sections obtained from *Escherichia coli* infected animals showed congestion, perivascular cellular infiltration and damaged neurons. These findings agreed with Liu *et al.* [68] who recognized endotoxin induced neuronal loss in substantia nigra with more intense immuno-staining of alpha-synuclein and inflammatory markers detected in brain sections exposed to LPS. According to Mittal *et al.* [69] the brain pathology of wild-type mice infected with *E. coli* revealed inflammatory infiltrates of PMNs in the leptomeningeal and ventricular spaces. Hippocampus showed severe inflammation and apoptosis of neurons in the Ammon horn. Acute hemorrhage, most prominent in the white matter of the brain, was observed. Cortex and molecular layer showed increased cellularity attributable to inflammatory exudates.

Histopathological examination in tetracycline antibiotic treated animals revealed no obvious specific changes in any tissue analysed including the brain tissue.

Minocycline is a second-generation, semi-synthetic tetracycline analog which is a highly lipophilic molecule easily penetrating the blood–brain barrier. It is effective against gram-positive and -negative infections and has powerful anti-inflammatory and neuroprotective effects.

The current results showed that minocycline thereby reduces release of nitric oxide (NO) in brain tissue and also decreased level of serum CRP. Furthermore, minocycline decreased immobility time by increasing struggling behavior. Also, minocycline decreased the level of IFNγ in brain homogenates.

In Conclusions, The present study suggests that Minocycline has powerful anti-inflammatory and exerts neuroprotective, antidepressant, anti-oxidant and immunomodulatory effects via improving cognitive impairment, blocking bacterial infection-induced sickness behavior in rats and decreasing pro-inflammatory cytokines.

REFERENCES

1. Pae, C.U., D.M. Marks, C. Han and A.A. Patkar, 2008. Does minocycline have antidepressant effect? Biomed. Pharmacotherapy, 62: 308 -11.
2. Hashimoto, K., 2009. Emerging role of glutamate in the pathophysiology of major depressive disorder. Brain Res. Rev., 61: 105-23.
3. Cata, J.P., H.R. Weng and P.M. Dougherty, 2008. The effects of thalidomide and minocycline on taxol-induced hyperalgesia in rats. Brain Res., 1229: 100-110.

4. Sriram, K., D.B. Miller and J.P. O'Callaghan, 2006. Minocycline attenuates microglial activation but fails to mitigate striatal dopaminergic neurotoxicity: role of tumor necrosis factor-alpha. J. Neurochem., 96: 706-718.
5. Henry, C.J., Y. Huang, A. Wynne, M. Hanke, J. Himler, M.T. Bailey, J.F. Sheridan and J.P. Godbout, 2008. Minocycline attenuates lipopolysaccharide (LPS)-induced neuroinflammation, sickness behavior and anhedonia. J. Neuroinflammation, 5: 15.
6. Beutler, B. and E.T. Rietschel, 2003. Innate immune sensing and its roots: the story of endotoxin. Nat. Rev. Immunol., 3: 169-176.
7. Yirmiya, R., 1996. Endotoxin produces a depressive like episode in rats. Brain Res., 711: 163-174.
8. Larson, S.J. and A.J. Dunn, 2001. Behavioral effects of cytokines. Brain Behav. Immun., 15: 371-387.
9. Anisman, H., Z. Merali, M.O. Poulter and S. Hayley, 2005. Cytokines as a precipitant of depressive illness: animal and human studies. Curr. Pharm. Des., 11: 963-972.
10. Shen, Y., T.J. Connor, Y. Nolan, J.P. Kelly and B.E. Leonard, 1999. Differential effect of chronic antidepressant treatments on lipopolysaccharide-induced depressive-like behavioural symptoms in the rat. Life Sci., 65: 1773-1786.
11. Kelley, K.W., R.M. Bluthe, R. Dantzer, J.H. Zhou, W.H. Shen, R.W. Johnson and B. A. Broussard, 2003. Cytokine induced sickness behavior. Brain Behav. Immun., 17: 112-8.
12. Sparkman, N.L., L.A. Martin, W.S. Calvert and G.W. Boehm, 2005. Effects of intraperitoneal lipopolysaccharide on Morris maze performance in year-old and 2-month-old female C57BL/6J mice. Behavioural Brain Research, 159: 145-151.
13. Turnbull, A.V. and C.L. Rivier, 1999. Regulation of the hypothalamic-pituitary-adrenal axis by cytokines: actions and mechanisms of action. Physiol. Rev., 79: 1-71.
14. Yong, V.W., J. Wells, F. Giuliani, S. Casha, C. Power and L.M. Metz, 2004. The promise of minocycline in neurology. Lancet Neurol., 3: 744-51.
15. Luccarini, H., C. Ballerini, T. Biagioli, F. Biamonte, A. Bellucci, M.C. Rosi, C. Grossi, L. Massacesi and F. Casamenti, 2008. Combined treatment with atorvastatin and minocycline suppresses severity of EAE. Exp. Neurol., 211: 214-26.
16. Sapadin, A. and R. Fleischmajer, 2006. Tetracyclines: nonantibiotic properties and their clinical implications. J. Am. Acad. Dermatol., 54: 258-65.
17. Domercq, M. and C. Matute, 2004. Neuroprotection by tetracyclines. Trends in Pharmacological Science, 25: 609-612.
18. Kim, H.S. and Y.H. Suh, 2009. Minocycline and neurodegenerative diseases. Behav. Brain Res., 196: 168-79.
19. O'Connor, J.C., M.A. Lawson, C. Andr´e, M. Moreau, J. Lestage, N. Castanon, K.W. Kelley and R. Dantzer, 2009. Lipopolysaccharide- induced depressive-like behavior is mediated by indoleamine 2,3-dioxygenase activation in mice. Molecular Psychiatry, 14: 511-522.
20. Shimazawa, M., T. Yamashima, N. Agarwal and H. Hara, 2005. Neuroprotective effects of minocycline against in vitro and in vivo retinal ganglion cell damage. Brain Res., 1053: 185-94.
21. Homsi, S., F. Federico, N. Croci, B. Palmier, M. Plotkine, C. Marchand-Leroux and M. Jafarian-Tehrani, 2009. Minocycline effects on cerebral edema: relations with inflammatory and oxidative stress markers following traumatic brain injury in mice. Brain Res., 1291: 122-32.
22. Linde, K., 1978. [Use of attenuated mutants of Pasteurella multocida strain, pathogenic to calves, in experimental mouse model studies. 4. Immunogenicity of selected revertants of temperature-sensitive, streptomycin-dependent mutants as potential vaccine strains]. Arch. Exp. Veterinarmed, 32: 943-9.
23. Virkamäki, A. and H. Yki-Järvinen, 1994. Mechanisms of insulin resistance during acute endotoxemia. Endocrinology Ay., 134: 2072-8.
24. Sgibnev, A.V. and S.V. Cherkasov, 2009. [Influence of active forms of oxygen on antilysozyme activity of bacteria]. Zh. Mikrobiol. Epidemiol. Immunobiol., 4: 59-62.
25. Vorhess, C.V., 1974. Some behavioral effects of maternal hypervitaminosis A in rats. Teratolo., 10: 269-274.
26. Looser, R., 1982. In: Wirkung Von Halluzinogene "Cyclazocin und Psilocybin" auf das Verhalten von Ratternin in open-field und Wohn-Labyrinth, Dipl. Biol. Eth. Zurich Zrich., pp: 28.
27. Volosin, M., L. Cancela and V. Molina, 1988. Influence of adrenocorticotrophic hormone on the behavior in the swim test of rats treated chronically with desipramine. J. Pharm. Pharmacol., 40: 74-76.
28. Buitrago, M.M., J.B. Schulz, J. Dichgans and A.R. Luft, 2004. Short and long-term motor skill learning in an accelerated rotarod training paradigm. Neurobiol. Learn. Mem., 81: 211-216

29. Dulcich, M.S. and R.E. Hartman, 2013. Pomegranate supplementation improves affective and motor behavior in mice after radiation exposure. Evidence-based Complementary and Alternative Medicine, In Press.
30. Narayanan, S.N., R.S. Kumar, B. K. Potu, S. Nayak, P.G. Bhat and M. Mailankot, 2010. Effect of radio-frequency electromagnetic radiations (RF-EMR) on passive avoidance behaviour and hippocampal morphology in Wistar rats. Ups. J. Med. Sci., 115: 91-96.
31. Wenk G.L., 2001. Assessment of spatial memory using the T- maze. Current protocols in neuroscience, Supplement 4:5B.1-8.5B.7
32. Porsolt, R.D., G. Anton, N. Blavet and M. Jalfre, 1978. Behavioural despair in rats: a new model sensitive to antidepressant treatments. European Journal of Pharmacology, 47: 379-391.
33. Cryan, J.F., A. Markou and I. Lucki, 2002. Assessing antidepressant activity in rodents: recent developments and future needs. Trends in Pharmacological Sciences, 23: 238-245.
34. Montgomery, H.A. and J.F. Dymock, 1961. The determination of nitrite in water. Analyst, 86: 414.
35. Hayashi, H., G.A. LoGrippo and M. Perry, 1970. Quantitative determination of C-reactive protein (CRP) by micro-double diffusion technic. Henry Ford. Hosp. Med. J. Fall., 18: 155-64.
36. Kirkwood, B.R., 1998. Comparison of several means analysis of variance In. Essentials of Medical Statistics. 1st edition. London, pp: 46-49. Blachwell Scientific Publications.
37. Gennaro, A.R., 2000. The Science and Practice of Pharmacy. 20th ed., Icppincott Williams. London, pp: 158.
38. Fisch, G.S., 2007. Animal models and human neuropsychiatric disorders. Behav. Genet., 37: 1-10.
39. Penninx, B.W., S.B. Kritchevsky, K. Yaffe, A.B. Newman, E.M. Simonsick, S. Rubin, L. Ferrucci, T. Harris and M. Pahor, 2003. Inflammatory markers and depressed mood in older persons: results from the health, aging and body composition study. Biol. Psychiatry, 54: 566-572.
40. Bauhofer, A., R.K. Schwarting, M. Köster, A. Schmitt, W. Lorenz and C.R. Pawlak, 2004. Sickness behavior of rats with abdominal sepsis can be improved by antibiotic and G-CSF prophylaxis in clinic modeling randomized trials. Inflamm. Res., 53: 697-705.
41. Zhu, F., Y. Zheng, Y.Q. Ding, Y. Liu, X. Zhang, R. Wu, X. Guo and J. Zhao, 2014b. Minocycline and risperidone prevent microglia activation and rescue behavioral deficits induced by neonatal intrahippocampal injection of lipopolysaccharide in rats. PloS One, 9:e93966.
42. Cai, Z., Y. Yan and Y. Wang, 2013. Minocycline alleviates beta-amyloid protein and tau pathology via restraining neuroinflammation induced by diabetic metabolic disorder. Clin. Interv. Aging, 8: 1089-95.
43. Cai, Z.Y., Y. Yan, S.Q. Sun, J. Zhang, L.G. Huang, N. Yan, F. Wu and J.Y. Li, 2008. Minocycline attenuates cognitive impairment and restrains oxidative stress in the hippocampus of rats with chronic cerebral hypoperfusion. Neuroscience Bulletin, 24: 305-13.
44. Kobayashi, K., S. Imagama, T. Ohgomori, K. Hirano, K. Uchimura, K. Sakamoto, A. Hirakawa, H. Takeuchi, A. Suzumura, N. Ishiguro and K. Kadomatsu, 2013. Minocycline selectively inhibits M1 polarization of microglia. Cell Death Dis., 4: e525.
45. Stack, E.C., K.M. Smith, H. Ryu, K. Cormier, M. Chen, S.W. Hagerty, S.J. Del Signore, M.E. Cudkowicz, R.M. Friedlander and R.J. Ferrante, 2006. Combination therapy using minocycline and coenzyme Q10 in R6/2 transgenic Huntington's disease mice. R. J. Biochim. Biophys. Acta, 1762: 373-380.
46. Williamson, L.L., P.W. Sholar, R.S. Mistry, S.H. Smith and S.D. Bilbo, 2011. Microglia and Memory: modulation by early-life infection. Journal of Neuroscience, 31: 15511-21.
47. Abdel Baki, S.G., B. Schwab, M. Haber, A.A. Fenton and P.J. Bergold, 2010. Minocycline synergizes with N-acetylcysteine and improves cognition and memory following traumatic brain injury in rats. PLoS One, 5: e12490.
48. Fan, R., F. Xu, M.L. Previti, J. Davis, A.M. Grande, J.K. Robinson and W.E. Van Nostrand, 2007. Minocycline reduces microglial activation and improves behavioral deficits in a transgenic model of cerebral microvascular amyloid. Journal of Neuroscience, 27: 3057-63.
49. Seabrook, T.J., L. Jiang, M. Maier and C.A. Lemere, 2006. Minocycline affects microglia activation, Abeta deposition and behavior in APP-tg mice. Glia, 53: 776-82.

50. Campbell, J.H., T.H. Burdo, P. Autissier, J.P. Bombardier, S.V. Westmoreland, C. Soulas, R.G. González, E.M. Ratai and K.C. Williams, 2011. Minocycline suppresses interleukine-6, its receptor system and signaling pathways and impairs migration, invasion and adhesion capacity of ovarian cancer cells: *In vitro* and *In vivo* studies. PLoS One, 6: e18688.
51. Borre, Y., V. Sir, S. De Kivit, K.G. Westphal, B. Olivier and R.S. Oosting, 2012. Minocycline restores spatial but not fear memory in olfactory bulbectomized rats. European Journal of Pharmacology, 697: 59-64.
52. Molina-Hernandez, M., N.P. Tellez-Alcantara, J. Perez Garcia, J.I. Olivera-Lopez and M.T. Jaramillo-Jaimes, 2008. Antidepressant-like actions of minocycline combined with several glutamate antagonists. Prog. Neuropsychopharmacol. Biol. Psychiatry, 32: 380-386.
53. Detke, M.J., M. Rickels and I. Lucki, 1995. Active behaviors in the rat forced swimming test differentially produced by serotonergic and noradrenergic antidepressants. Psychopharmacol., 121: 66-72.
54. Mello, B.S., A.S. Monte, R.S. McIntyre, J.K. Soczynska, C.S. Custódio, R.C. Cordeiro, J.H. Chaves, S.M. Vasconcelos, H.V. Nobre, F.C. Florenço De Sousa, T.N. Hyphantis, A.F. Carvalho and D.S. Macêdo, 2013. Effects of doxycycline on depressive-like behavior in mice after lipopolysaccharide (LPS) administration. Journal of Psychiatric Research, 47: 1521-1529.
55. Deak, T., C. Bellamy, L.G. D'Agostino, M. Rosanoff, N. K. McElderry and K.A. Bordner, 2005. Behavioral responses during the forced swim test are not affected by anti-inflammatory agents or acute illness induced by lipopolysaccharide. Behavioural Brain Research, 160: 125-134.
56. Woo, P.C., H.W. Tsoi, L.P. Wong, H.C. Leung and K.Y. Yuen, 1999. Antibiotics modulate vaccine-induced humoral immune response. Clin. Diagn. Lab. Immunol., 6: 832-7.
57. Amin, A.R., M.G. Attur, G.D. Thakker, P.D. Patel, P.R. Vyas, R.N. Patel, I.R. Patel and S.B. Abramson, 1996. A novel mechanism of action of tetracyclines: effects on nitric oxide synthases. Proc. Natl. Acad. Sci., U.S.A., 93: 14014-14019.
58. Yonar, M.E., 2012. The effect of lycopene on oxytetracycline-induced oxidative stress and immunosuppression in rainbow trout (Oncorhynchus mykiss, W.).Fish & Shellfish Immunol., 32: 994-1001.
59. Milano, S., F. Arcoleo, P. D'Agostino and E. Cillari, 1997. Intraperitoneal injection of tetracyclines protects mice from lethal endotoxemia downregulating inducible nitric oxide synthase in various organs and cytokine and nitrate secretion in blood. Antimicrob. Agents Chemother., 41: 117-21.
60. Ryu, J.K., H.B. Choi and J.G. McLarnon, 2006. Combined minocycline plus pyruvate treatment enhances effects of each agent to inhibit inflammation, oxidative damage and neuronal loss in an excitotoxic animal model of Huntington's disease. Neuroscience, 141: 1835-48.
61. Noble, W., C.J. Garwood and D.P. Hanger, 2009b. Minocycline as a potential therapeutic agent in neurodegenerative disorders characterised by protein misfolding. Prion, 3: 78-83.
62. Houri-Haddad, Y., A. Halabi and W.A. Soskolne, 2008. Inflammatory response to chlorhexidine, minocycline HCl and doxycycline HCl in an in vivo mouse model. J. Clin. Periodontol., 35: 783-788.
63. Mehrotra, S., M.J. Pecaut and D.S. Gridley, 2013. Effects of minocycline on hematopoietic recovery after whole-body irradiation. *In vivo*, 27: 11-28.
64. Feleder, C., K.Y. Tseng, G.G. Calhoon and P. O'Donnell, 2010. Neonatal intrahippocampal immune challenge alters dopamine modulation of prefrontal cortical interneurons in adult rats. Biological Psychiatry, 67: 386-392.
65. Al-Ankari, A.S. and A.M. Homeida, 1996. Effect of antibacterial growth promoters on the immune system of broiler chicks. Vet. Immunol. Immunopathol., 53: 277-83.
66. Maximova, N., D. Zanon, F. Verzegnassi and M. Granzotto, 2013. Neutrophils engraftment delay during tigecycline treatment in 2 bone marrow-transplanted patients. J. Pediatr. Hematol. Oncol., 35: e33-7.
67. Mehrotra, S., M.J. Pecaut and D.S. Gridley, 2012. Analysis of minocycline as a countermeasure against acute radiation syndrome. *In vivo*, 26: 743-58.
68. Liu, Y., L. Qin, B. Wilson, X. Wu, L. Qian, A.C. Granholm, F.T. Crews and J.S. Hong, 2008. Endotoxin induces a delayed loss of TH-IR neurons in substantia nigra and motor behavioral deficits. Neurotoxicology, 29: 864-870.
69. Mittal, R., I. Gonzalez-Gomez, K.A. Goth and N.V. Prasadarao, 2010. Inhibition of inducible nitric oxide controls pathogen load and brain damage by enhancing phagocytosis of Escherichia coli K1 in neonatal meningitis. Am. J. Pathol., 176: 1292-305.