aJava

Asian Journal of Animal and Veterinary Advances

Academic
Journals Inc.

www.academicjournals.com

OPEN ACCESS Asian Journal of Animal and Veterinary Advances

ISSN 1683-9919
DOI: 10.3923/ajava.2024.26.36

Research Article

Ocimum gratissimum L. (Labiateae) Aqueous Extract Prevents Behavioural Impairment, Motor Incoordination and Brain Oxidative Stress Induced by Prenatal Stress in Female Rats

[1]Duplex Youmbie Dchanche, [1]Martin Salah Anchang, [2]Nadège Kouémou Emégam, [1]Mumbi Laurantine Ngenteh, [1]Bih Belta Lilian Fubi, [1]Tangu Patience Neng, [1]Ndifor Rose Nchang, [1]Oumar Mahamat, [3]Edouard Akono Nantia, [4]Faustin Pascal Tsagué Manfo and [1]Antoine Kada Sanda

[1]Department of Zoology, Faculty of Science, University of Bamenda, P.O. Box 39 Bambili, Cameroon
[2]Department of Animal Biology and Conservation, Faculty of Science, University of Buea, P.O. Box 63 Buea, Cameroon
[3]Department of Biochemistry, Faculty of Science, University of Bamenda, P.O. Box 39, Bambili, Cameroon
[4]Department of Biochemistry and Molecular Biology, Faculty of Science, University of Buea, P.O. Box 63, Buea, Cameroon

Abstract

Background and Objective: Prenatal stress is the exposure of an expectant mother to physical or psychological stressors and is a major risk factor for depression anxiety and post-traumatic stress disorders of the offspring. *Ocimum gratissimum* (scent leaf) is an aromatic herbaceous plant which has been used in folk medicine in the treatment of anxiety and depression. The present work was designed to evaluate the protective effect of *Ocimum gratissimum* on behavioural impairment, motor incoordination and oxidative stress induced by prenatal stress in female rats. **Materials and Methods:** Twenty-five pregnant females were at randomly divided into 5 groups of 5 rats each. Group 1 (normal), Groups 2 to 5 were submitted to stress and treated respectively with distilled water (5 mL/kg/day), reference drug (diazepam 1 mg/kg) and the aqueous extract of *O. gratissimum* at the different doses of 100 and 200 mg/kg/day. The pups (3 weeks old) were submitted to behavioral tests including beam walking, open field and forced swimming tests. At the end of the behavioural assessments, the brains of animals were used for the evaluation of oxidative stress parameters and acetylcholinesterase activity. **Results:** Prenatal stress-induced motor dysfunctions, anxiogenic like and depressive-like behaviour in animals. All the behavioural impairments were significantly reversed by the administration of *O. gratissimum* extract at both doses of 100 and 200 mg/kg. Furthermore, the plant extract especially the dose of 100 mg/kg antagonised the reduction of glutathione levels and superoxide dismutase activity induced by the stressors. **Conclusion:** The results of this study give evidence that *O. gratissimum* aqueous extract prevents anxiety-like behaviour, depressive symptoms and motor deficits induced by prenatal stress. This action of *O. gratissimum* could be through the reduction of oxidative stress and acetylcholinesterase activity.

Key words: Acetylcholinesterase, anxiety, depression, neuroprotective, *Ocimum gratissimum*, oxidative stress, prenatal stress

Citation: Dchanche, D.Y., M.S. Anchang, N.K. Emégam, M.L. Ngenteh and B.B.L. Fubi *et al*., 2024. *Ocimum gratissimum* L. (Labiateae) aqueous extract prevents behavioural impairment, motor incoordination and brain oxidative stress induced by prenatal stress in female rats. Asian J. Anim. Vet. Adv., 19: 26-36.

Corresponding Author: Antoine Kada Sanda, Department of Zoology, Faculty of Science, University of Bamenda, P.O. Box 39, Bambili, Cameroon
Tel: (+237) 677738662/(+237) 655258429

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Liaudat *et al.*[1], defined stress as a real or supposed threat to physical or psychological integrity of an individual, resulting in a physiological and/or behavioural response. Stress is a feeling of emotional or physical tension resulting in learning and memory disabilities, hence causing diseases such as depression, insomnia, anxiety and post-traumatic stress disorder[2]. Stress alters hippocampal neuronal activity and synaptic plasticity, which leads to an increase in hippocampal glucocorticoid receptor activation and a decrease in neuronal cell survival and neurogenesis[3]. This can lead to excessive production of free radicals that cause oxidative damage to the brain[4].

The frequent psychiatric conditions identified as anxiety and depression are the most common stress-related mood disorders causing disability and premature death. More than 20% of the adult population suffer from these conditions at some time during their life[5]. The brain is the central organ of stress and adaptation to social and physical stressors because it determines what is threatening, stores memories and regulates the physiological as well as behavioural responses to stressors that may be damaging or protective[6].

In human's prenatal stress may rise to behavioural changes including hyperactivity, anxiety, aggression, attention deficits disorders and cognitive alterations in adolescence and adulthood[4]. Animals exposed to stress prenatally subsequently exhibit alterations in reproductive functions and sexual behaviour as well as altered function of the Hypothalamic-Pituitary-Adrenal (HPA) axis[7]. In prenatally stressed rats, the female offspring had increased potential for the development of male sexual behaviour, disrupted oestrous cycles and increased intrauterine mortality and induced spontaneous abortions[8].

Prenatal maternal stress has been associated with physiological, neurological and psychological consequences in the offspring[9]. Therefore, it is important to prevent prenatal stress or counteract their negative effects on humans and other organisms. Medicinal plants have for long been used as a source of relief either in the form of traditionally prepared concoctions or in the form of pure active principles to prevent and alleviate a wide variety of diseases empirically, including dysfunctions induced by prenatal stress[10]. *Ocimum gratissimum* L. (Labiateae) is a medicinal plant from Cameroon commonly called scent leaf. It is an aromatic herbaceous plant from the Labiatae family and is the most abundant of the genus *Ocimum* which is a shrub reaching up to 1.9 m in height with branched stems. The leaves measure up to 10×5 cm and are ovate to ovate-lanceolate, sub-acuminate to acuminate at apex, cuneate and decurrent at base with a coarsely crenate, serrate margin, pubescent and dotted on both sides[11]. *Ocimum gratissimum* is used for abdominal pains, sore eyes, ear infections and like in treatment for blocked nostrils where the leaves are rubbed between the palms and sniffed, regulation of menstruation and as a cure for prolapse of the rectum, for coughs, barrenness, fever, convulsions and tooth gargle[12]. Previous studies have reported potent anti-oxidant and free radical scavenging capacity of *O. gratissimum*[13,14], which may present a potential interest to counteract prenatal stress-induced neurotoxicity. Based on the above, this research focuses on assessing the effects of a leaf aqueous extract of *O. gratissimum* on prenatal stress-induced neurodevelopment and behavioural impairments in female rats.

MATERIALS AND METHODS

Study area: This study was carried out at the University of Bamenda, Department of Zoology in the Laboratory of Phytopharmacology from March to June 2022.

Plant collection and extraction: The fresh leaves of *Ocimum gratissimum* were collected in Babungo Village in the North West region of Cameroon, on November 2021. The plant was identified and compared with the material; Letouzey R. 7905 of the National Herbarium of Cameroon, where a voucher specimen is registered under the number No: 17298 SRFCam. *Ocimum gratissimum* leaves were washed and shade dried under for a week. The dried leaves were ground using an electric blender (Binatone model) and sieved to obtain a fine powder of 300 g. The 300 g which was then macerated in 3 L of distilled water for 72 hrs. After filtration using Whatman No. 1 filter paper the water was evaporated in an oven at 40°C. Then a brown extract of 24.56 g was obtained giving an extraction yield of 8.19%. The plant extract was administered at the doses of 100 and 200 mg/kg/day the dosage used was selected based in our pre-screening tests.

Experimental animals: Thirty Wistar rats *Rattus norvegicus* (twenty-five females and five males) with an average weight of 160-180 g were obtained from the animal house of the University of Yaoundé 1, Cameroon. Rats were housed in plastic cages under ambient conditions and had free access to rodent pellets and water *ad libitum*.

All the experiments were done in agreement with the guide for the care and use of Laboratory Animals published by the National Institute of Health (NIH publication no. 85-23, revised 1996) and the guidelines and the authorization of the Cameroon National Ethical Committee

on the use of laboratory animals for scientific research. Rats were acclimatized for two weeks in the laboratory before the beginning of the experiment.

Animal treatment: Adult female rats were allowed to mate with adult male rats and the vaginal smear was done every morning to monitor for the presence of sperms which the presence in the vaginal smear was considered as day 1 of pregnancy. On the first day of pregnancy, females were randomly assigned to unstressed (group 1 or normal) and stress groups (groups 2-5). Stressed pregnant female rats were divided into 4 groups of 5 rats each and treated orally respectively with distilled water (5 mL/kg/day) (group 2), reference drug diazepam (1 mg/kg)[5] (group 3), the aqueous extract of *O. gratissimum* at the doses of 100 and 200 mg/kg/day (group 4 and 5), respectively. Groups 1, 2, 4 and 5 were orally administered their treatments while group 3 was intramuscularly administered the drug diazepam for a period of 7 days. Pregnant female rats were treated and followed until the delivery day (28-30 days).

Stress induction procedure: Prenatal stress was conducted as previously described by Braastad[15]. Briefly, from day 14 of pregnancy until delivery, female rats were subjected every day to one stress session at 9 O'clock during which they were placed in smaller bowls and exposed to a bright light for 45 min. During the stressing period, effectiveness of the stressor was observed when rats fur and tails were raised couple with sweating. Control pregnant females were left undisturbed in their home cages. Stress during gestational period has been proved to alter the regulation of the hypothalamic adrenal axis (HPA) and to disturbs the development of both hypothalamic and hippocampal brain areas[16].

Evaluation of the effect of prenatal stress and *O. gratissimum* on motor function, exploratory activity and depression: Post-delivery, offspring were allowed to stay with their mother till weaning (for three weeks). The pups were then subjected to behavioural assessment of the prenatal effect of stress on neurodevelopment using: Beam walking, open field and forced swimming tests.

Beam walking test (BWT): Motor coordination was tested using the beam walking test in a wood strip (20-mm) as described by Sanda *et al.*[17] . In this procedure, 3 week old rats had to cross a 1 m long wooden stick located at 1 m above the ground and observers counted the total distance travelled, the number of turns of the animal and the number of slips committed by the rats. The number of foot faults (slips) served as a measure motor of incoordination.

Open-field test (OFT): The open-field test used for exploration of anxiety was conducted on a square arena (24×24×23 cm) on day 21 post-delivery. The floor of the arena was divided into sixteen squares (6×6 cm). Each rat naïve to the arena was placed individually at one corner of the arena and its behavior was recorded for over 5 min period[18]. The following parameters were recorded: Arena centre crossing frequency, distance travelled, stretch attend posture, horizontal activity (total squares crossed), vertical activity (rearing frequency), the total time of freezing (s), the grooming total time (s), grooming frequency and mean grooming duration (s). Grooming behaviour is a displacement response and it is expected to be displayed in a novel environment. The arena was illuminated with direct light of 140 lux and was cleaned after the passage of each rat with alcohol 70%.

Forced swimming test (FST): The forced swimming test was conducted based on the method of Sanda *et al.*[17]. The apparatus was consisting of a plastic cylinder (25 cm diameter×50 cm height) filled with 30 cm deep water at 24±2°C. A pre-test was carried out for 10 min the day before the test. During this phase, the rats individually were allowed to swim for 10 min in the swimming tank. The water was changed after a passage with each animal. The probe test was conducted 24 hrs later for a total duration of 5 min. All the behavior of the animal was video recorded. The recorded videos were analysed by an observer blind to the treatment. The duration of immobility was calculated using a stopwatch. After each passage in the swimming tank, the animals were dried with a towel and placed under a heat lamp for 10 min to avoid hypothermia and returned to their respective home cages.

Evaluation of the effect of prenatal stress and *O. gratissimum* on brain oxidative stress parameters and acetylcholinesterase activity, cortisol concentration and histology

Brain collection: After behavioural assessments, pups of the different groups were anesthetized with 50 mg/kg ketamine then sacrifice by cervical decapitation. Skull was dissected and the brain collected and weighed. Half of the brain was kept in formalin solution 10% (V/V) for histological analysis and the other half (0.5 g) were used to prepare brain homogenate with phosphate buffer (pH 7.4, 50 mM).

Oxidative stress parameters evaluation: These analyses included reduced Glutathione (GSH) levels, superoxide dismutase (SOD), Thiobarbituric Acid Reactive Substances (TBARS), total oxidative stress (TOS) and total antioxidant defense (TAD) activities which were estimated as described by Ellman[19], Misra and Fridovich[20], Sanda *et al.*[21] and Saha *et al.*[22], respectively. The acetylcholinesterase activity and cortisol levels were also determined as reported by Ellman[19] and using a cortisol ELISA kit from Elabscience Biotechnology Inc. (EBI) according to the manufacturer's instructions.

Histological examination: Histological examination was done according to the protocol previously described by Sanda *et al.*[23].

Statistical analysis: The software origin pro version 2018 was used for data analyses. Data was presented as Mean±Standard Error of Mean (SEM) values. One-way Analysis of Variance (ANOVA), followed by Turkey's multiple comparison test was used to calculate differences between groups. Differences were considered statistically significant at $p<0.05$.

RESULTS

Beam walking parameters: As shown in Table 1, prenatal stress led to a significant reduction of the distance travelled by rats when compare to the normal control. The distance travelled that was 94.17cm in the normal group dropped to a value of 37.5 in the distilled water group. *Ocimum gratissimum* (100 and 200 mg/kg) likewise diazepam significantly increased ($p<0.05$) the distance travelled. In the other hand, we observed an increase in the number of slips in the distilled water group when compared to the normal control. The number of slips significantly ($p<0.05$) raised from 3.83±0.75 in the normal control to 6±0.73 in the distilled water group. Diazepam and *O. gratissimum* (100 and 200 mg/kg) reverted the impact of prenatal stress and significantly reduced the number of slip. The same previous trend was observed with the number of turns (Table 1).

Open field parameters: The behaviours of the rats in the open field (Table 2) showed, that prenatally stressed rats that received distilled water had a significant decrease ($p<0.05$) of the time in center square, total distance travelled and lines crossed compared to the normal group. However, the grooming time of the animals was not affected by prenatal stress and treatment with *O. gratissimum.* Both doses of *O. gratissimum* induced a significant decrease in the stretch attend posture and duration of grooming when compared to the normal animals. Distilled water (vehicle) group significantly increased the stretch attend posture when compared to normal group. However, the grooming time of the animals was not affected by prenatal stress and treatment with *O. gratissimum*.

Force swim parameters: The immobility time of rats exposed to prenatal stress (distilled water group) significantly increased ($p<0.05$) as compared to the normal group. The immobility time increased from 17. 33±1.22 sec in the normal control to 59.17±5.94 sec in the vehicle group that increase in the immobility time induced by prenatal stress was counteracted by diazepam (1 mg/kg) and both doses of *O. gratissimum* bringing the values close to that of the normal group (Fig. 1).

Acetylcholinesterase activity: The effect of the aqueous extract of *O. gratissimum* and stress on the activity of acetylcholinesterase was shown in Fig. 2. The stress significantly reduced acetylcholinesterase activity in prenatally stressed exposed rats as compared to the normal group. The stress-induced effects on acetylcholinesterase activity was however alleviated by the plant extract of *O. gratissimum* with the dose of 100 mg/kg showing comparable effects with the normal group.

Brain oxidative stress parameters: As shown in Table 3, stress significantly reduced glutathione level, superoxide dismutase activity and total antioxidant defense while increasing TBARS and total oxidative stress levels in rats interestingly, the negative effects of the prenatal stress on oxidative stress parameters (glutathione, superoxide dismutase and total antioxidant defense) were reversed by the plant extract. The TBARS and total oxidative stress was normalized by both doses of *O. gratissimum* extract with effect comparable with the normal group.

Serum cortisol levels: There was a significant increase in the serum level of cortisol ($p<0.05$) in prenatally stressed rats as compared to the normal group (Fig. 3). Treatment pregnant rats with either diazepam (1 mg/kg) group or the aqueos extract of *O. gratissimum* prevented the rise of cortisol levels in their pups.

Histological findings of (H&E) stained sections in the cerebellar cortex: In Fig. 4a, the (H&E) stained in the cerebral tissue of the normal group showed the characteristic pattern of the cerebral cortex formed of three layers: The molecular cell layer (M), purkinje cell layer (P), granular cell

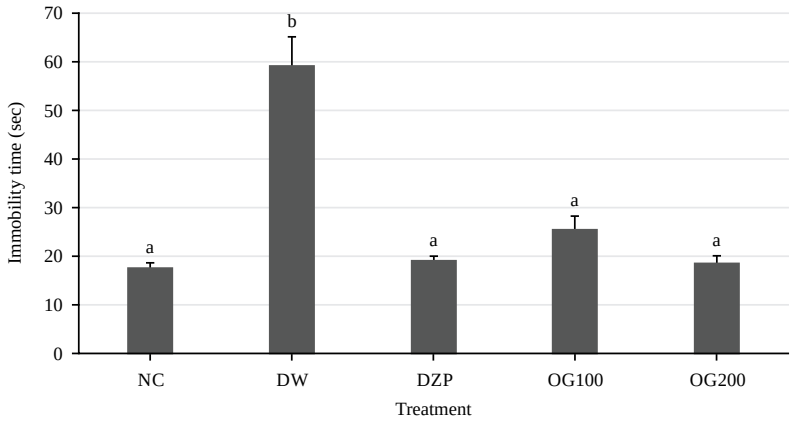

Fig. 1: Effect of the aqueous extract of *Ocimum gratissimum* and stress on the immobility time of force swim test

Each bar in the histogram represent Means±Standard Error of Means (SEM), n = 6 rats per group, data were analyzed using origin pro 2018, DZP: Diazepam (1mg/kg), DW: Distilled water, NC: Normal control, OG100: *O. gratissimum* 100 mg/kg and OG200: *O. gratissimum* 200 mg/kg

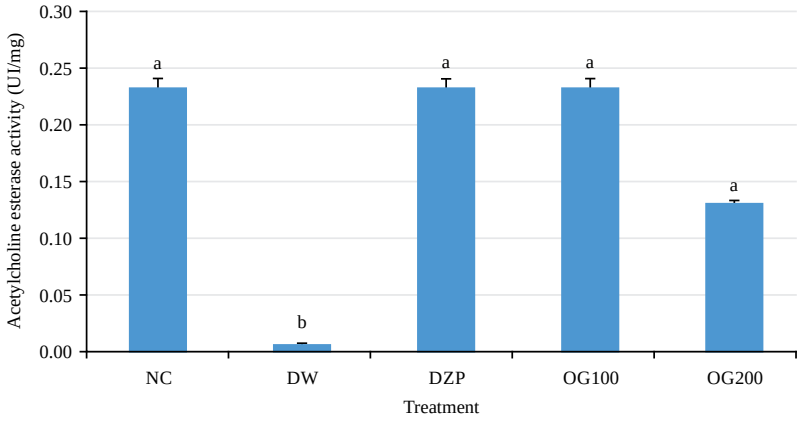

Fig. 2: Effect of the aqueous extract of *Ocimum gratissimum* and stress on the acetylcholine esterase activity

Each bar in the histogram represent Means±Standard Error of Means (SEM), n = 6 rats per group, data were analyzed using origin pro 2018, DZP: Diazepam (1mg/kg), DW: Distilled water, NC: Normal control, OG100: *O. gratissimum* 100 mg/kg and OG200: *O. gratissimum* 200 mg/kg

Table 1: Effect of the aqueous extract of *Ocimum gratissimum* on the beam walking test parameters

Parameters/treatments	Distance travelled (cm)	Number of slips	Number of turns	Number of foot slips	Pauses
Normal	94.17±3.75^{a}	3.83±0.75^{a}	4.17±0.31^{a}	2.33±0.49^{a}	4.67±0.61^{a}
Distilled water (5 mL/kg)	37.5±8.92^{b}	6±0.73^{b}	7.17±0.47^{b}	4.00±0.58^{a}	2.83±0.48^{a}
Diazepam (1 mg/kg)	96.5±2.43^{a}	1±0.63^{c}	2.83±0.40^{a}	2.33±0.42^{a}	2.33±0.61^{a}
Ocimum gratissimum (100 mg/kg)	81.67±4.22^{a}	0.00±0.0^{d}	2.67±0.33^{a}	2.33±0.49^{a}	2.17±0.65^{a}
Ocimum gratissimum (200 mg/kg)	94.17±4.17^{a}	0.17±0.17^{d}	4.33±0.56^{a}	1.97±0.49^{a}	2.17±0.91^{a}

Values in the table represent Means±Standard Error of Means (SEM), n = 6 rats per group and values not sharing a common letter differ significantly with each other ($p<0.05$, origin pro 2018)

Table 2: Effect of the aqueous extract of *Ocimum gratissimum* in the open field test parameters

Parameters/treatments	Distance (cm)	Line crossed (cm)	Grooming	Duration of grooming (s)	Time in center square (s)	Stretch attend posture
Normal	1066.83±191.16^{a}	35.33±3.96^{a}	2.33±0.33^{a}	38±3.17^{a}	4.17±0.60^{b}	37.83±4.13^{a}
Distilled water (5 mL/kg)	433.5±104.22^{b}	20.50±2.57^{b}	3.5±0.22^{a}	28.83±8.98^{a}	2.17±0.87^{a}	42.17±6.38^{a}
Diazepam (1 mg/kg)	1014.33±90.35^{a}	37.00±2.93^{a}	2±0.58^{a}	21.17±3.96^{a}	7.33±1.26^{c}	37.17±1.89^{a}
Ocimum gratissimum (100 mg/kg)	891.5±41.03^{a}	32.67±3.66^{a}	1.67±0.33^{b}	8.33±2.80^{b}	5.17±0.40^{b}	16±3.12^{b}
Ocimum gratissimum (200 mg/kg)	1234±126.35^{a}	46.00±3.09^{a}	1.67±0.42^{b}	8.50±2.64^{b}	6.67±0.88^{c}	14.50±3.55^{b}

Values represent Means±Standard Error of Means (SEM), n = 6 rats per group and values not sharing a common letter differ significantly with each other ($p<0.05$, origin pro 2018)

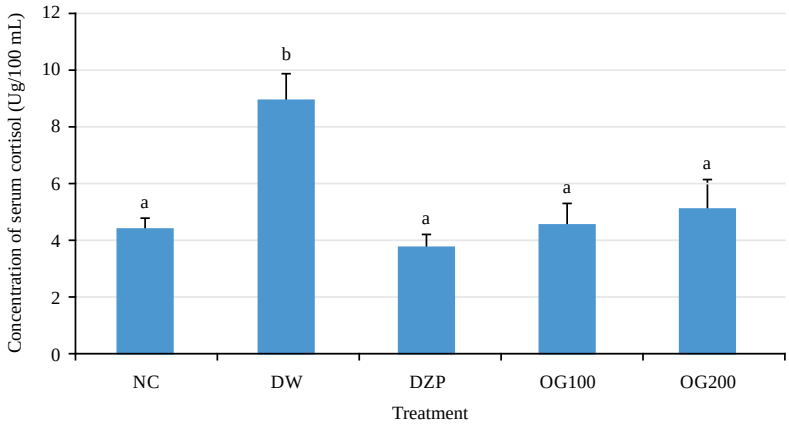

Fig. 3: Effect of the aqueous extract of *Ocimum gratissimum* and stress on the concentration of serum cortisol

Each bar in the histogram represent Means±Standard Error of Means (SEM), n = 6 rats per group, data were analyzed using origin pro 2018, DZP: Diazepam (1mg/kg), DW: Distilled water, NC: Normal control, OG100: *O. gratissimum* 100 mg/kg and OG200: *O. gratissimum* 200 mg/kg

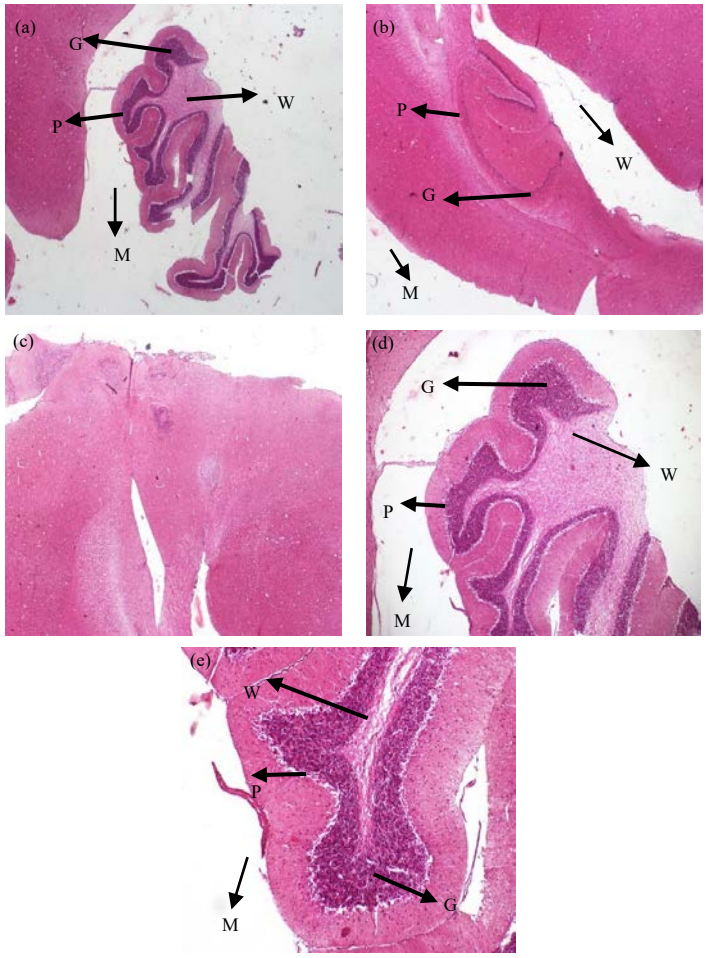

Fig. 4(a-e): Effect of the aqueous extract of *Ocimum gratissimum* and stress on the litter brain histological examination, (a) Normal control, (b) Distilled water, (c) *O. gratissimum* (100 mg/kg), (d) *O. gratissimum* (200 mg/kg) and (e) Diazepam (1 mg/kg)

W: White cell, M: Molecular cell, P: Purkinje cell and G: Granular cell

Table 3: Effects of the aqueous extract of *Ocimum gratissimum* and stress oxidative stress

Parameters/treatments	Glutathione (µmole/mL)	Superoxide dismutase (IU/mg of protein)	TBARS (µmol/mL)	Total oxidative stress H_2O_2 (mmol/L)	Total antioxidant defense Trolox (mmol/L)
Normal	0.807±0.132[a]	375±44.72[a]	0.176±0.014[a]	0.200±0.034[a]	0.342±0.056[a]
Distilled water (5 mL/kg)	0.541±0.137[b]	100±30.62[b]	0.620±0.051[b]	0.649±0.106[b]	0.009±0.020[b]
Diazepam (1 mg/kg)	0.847±0.024[a]	355±9.34[a]	0.157±0.002[a]	0.282±0.041[a]	0.474±0.022[a]
Ocimum gratissimum (100 mg/kg)	0.868±0.034[a]	350±44.02[a]	0.144±0.009[a]	0.272±0.095[a]	0.386±0.086[a]
Ocimum gratissimum (200 mg/kg)	1.100±0.153[a]	350±28.50[a]	0.114±0.005[a]	0.345±0.055[a]	0.670±0.065[a]

Values represent Means±Standard Error of Means (SEM), n = 6 rats per group and values not sharing a common letter differ significantly with each other ($p<0.05$, origin pro 2018)

layer (G) and also the medulla formed of white matter fibers (W) was observed. The prenatal stressed animals exhibited a marked reduction in the density of neurons in the three cortical layers (Fig. 4b). The histological sections of the animals treated with *Ocimum gratissimum* extract and diazepam respectively was comparable to that of the normal group and demonstrating restoration of the cellular density in the three cortical layers (Fig. 4c-e).

DISCUSSION

This study was aimed at evaluating the protective effect of the aqueous of extract of *O. gratissimum* on prenatal stress-induced neurodevelopment and behavioural impairments in rats.

Beam walking tests help in assessing the status of locomotion and motor deficits in litter rats exposed to prenatal stress[24,25]. Exposure of rats to prenatal stress resulted in decreased distance travelled and number of turns compared to the normal group. These results suggest that prenatal stress alters motor coordination and balance in rats. Similar results were previously reported by Sanda *et al.*[17]. Interestingly, the motor incoordination induced by prenatal stress was prevented by *O. gratissimum* extract, suggesting that the extract contains bioactive compounds with protective potentials vis-à-vis of stress-induced neurodevelopment. The same results were obtained by Mainul Haque and Abubakar[26] who reported the protective effect of ethanolic extract of grape pomace against the adverse effects of Cypermethrin pesticide on weanling female rats. The current report was evident of increasing reports stating the protective effect of some medicinal plants on neurodevelopment[27,28].

The open field test (OFT) has been appropriate for the evaluation of the basal activity in rodents as well as its evolution in response to novelty or an anxiety-inducing environment, pharmacological treatment, lesions, or genetic modification[17]. The number of center square entries, number of lines crossed and distance travelled were all dropped in prenatal stress-exposed animals (distilled water). These parameters measure exploratory behaviour and anxiety and their low frequency indicates low exploratory and high anxiety induced by the stress which was similar to the work of Taïr *et al.*[27]. Animals exposed to prenatal stress which received distilled water also showed a significant increase in other anxiety-related parameters such as stretch attend posture. Administration of the aqueous extract of *O. gratissimum* effectively prevented the changes induced by prenatal stress in animals. This result suggests that the aqueous extract of *O. gratissimum* contains active ingredient able to protect against stress-induced behavioural changes.

The forced swimming test is a behavioural test evaluating depression[5]. Depression is the state of feeling unhappy without hope or interest and is characterized by apathy, loss of energy and retardation of thinking and activities causing significant impairment in daily life such as suicidal ideation[29]. In the current work prenatal stress increased the immobility time in the forced swim parameter. Immobility in animal is interpreted as emotional despair and considered depression with cessation of all movements[30]. This effect may occur through oxidative stress[31,32], as several studies have shown the implication of oxidative stress in neurological pathologies such as neurodegenerative disorders (Alzheimer's disease, Huntington disease and Parkinson disease) and neuropsychiatric disorders (anxiety disorders and depression)[31,33]. Therefore, the results of this test suggest that exposure to prenatal stress results in depression-like symptoms in the litter rats.

Prenatal stress has significantly decreased brain activity of acetylcholinesterase. Acetylcholine is a neurotransmitter that plays a very vital role in the central and peripheral nervous systems. The action of this neurotransmitter is however terminated by the hydrolysis of the enzyme acetylcholinesterase ending impulse transmission. It ensures the transfer of signals between neurons in the central nervous system, while it relays nerve impulses to the muscles in the peripheral nervous system. In the current study, acetylcholinesterase activity was significantly inhibited in animals that were prenatally stressed and administered distilled water, an effect that may lead to behavioural, cognitive and motor dysfunctions. This could then be an additional mechanism of the neurotoxicity of prenatal stress in rats. The inhibitory effect of prenatal stress on acetylcholinesterase activity was significantly alleviated by the

administration of the aqueous extract of *O. gratissimum*. The dose of 100 mg/kg of plant extract showed better preventive effects against prenatal stress-induced neurotoxicity in all investigated parameters, as compared to the higher dose. Similar observations have been made in previous studies of Calabrese *et al*.[34] and Antoine *et al*.[35].

Oxidative stress is by far responsible for several neurotoxic and neurological pathologies, such as Parkinson's disease, epilepsy, depressive disorders and Alzheimer's disease. Prenatal stress has significantly decreased brain activity of superoxide dismutase, total antioxidant defense (TAD) and the level of glutathione while it increased the level of Thiobarbituric Acid Reactive Substance (TBARS) and total oxidative stress (TOS). It is known that ROS can damage cell structures[36] and GSH plays an important role in neuroprotection against oxidative stress in damaged neurons[37]. Therapeutic strategies that slow or stop the neurodegenerative processes of toxicity are expected to have a major impact on the treatment of nervous system toxicity[38,39].

Glutathione (GSH) is a pseudo-tripeptide formed by the condensation of glutamic acid, cysteine and glycine. Glutathione in its reduced form is the major antioxidant in cells, protecting them from free radicals. Glutathione level was significantly decreased in prenatally stressed rats, while it was alleviated by the aqueous extract of *O. gratissimum* with the dose of 100 mg/kg showing better preventive effect against prenatal stress-induced neurodevelopment in all investigated parameters, as compared to the higher dose. Low levels of GSH also noted by Sanda *et al*.[17] are accompanied by neurodegenerative diseases such as multiple sclerosis, Alzheimer's disease, Parkinson's disease and others .

Superoxide dismutases (SODs) are a group of metalloenzymes that are found in all kingdoms of life. The SODs form the front line of defense against reactive oxygen species (ROS)-mediated injury[33]. The SODs constitute a very important antioxidant defense against oxidative stress in the body. The inhibitory effect of prenatal stress on SOD activity was significantly alleviated by administration of the aqueous extract of *O. gratissimum* with the dose of 100 mg/kg showing a better preventive effect against prenatal stress-induced neurodevelopment, as compared to the higher dose. This finding coincided with that of Liaudat *et al*.[40].

Thiobarbituric Acid Reactive Substances (TBARS) is a biochemical parameter used to measure lipid peroxidation products in cells, tissues and body fluids. Prenatal stress significantly increased the level of TBARS compared to the normal group while administration of the aqueous extract of *O. gratissimum* inhibited TBARS levels showing a preventive effect against prenatal stress. Previously, it has been reported that prenatal stress increased TBARS (estimated by TBARS level) in temporal lobe homogenates[41].

Total oxidative stress (TOS) and total antioxidant defense (TAD) are both parameters used to determine the level of stressful conditions. The level of total oxidative stress is usually higher in patients that have undergone prenatal stress without treatment (distilled water) and lower in patients with treatment and while the total antioxidant defense was higher in prenatally stressed rats that were treated with the aqueous extract of *O. gratissimum* and lower in prenatally stressed exposed rats. Similar findings were documented by Saha *et al*.[22].

In current study, the aqueous extract of *O. gratissimum* (100 mg/kg) restored the activity of the antioxidant enzyme SOD, GSH, TOS and TAD.

The results showed that prenatally stressed rats treated with the leaf aqueous extract of *O. gratissimum* had cortisol levels significantly reduced at almost to normal values while prenatally stressed rats treated with distilled water had cortisol levels significantly increased compared to normal. The HPA axis is made up of an assembly of stress responses mediated by the brain, pituitary and adrenal gland. The endocrine activity of the hypothalamus causes the production of the corticotropic releasing hormone (CRH), a compound that stimulates the production of Adrenocorticotropic Hormone (ACTH). The ACTH is liberated into the circulatory system and causes the adrenal cortex to secrete corticosteroid hormones, particularly cortisol. Cortisol increases the availability of refueling the body with substances necessary for the body's response to prenatal stress. Stress alleviation using a plant extract was also observed by Dornhorst *et al*.[42], Kyrou and Tsigos[43] and Fotsing *et al*.[44].

The cerebral cortex also known as the gray matter is the outer layer that lies on top of the cerebrum. It plays a key role in memory, thinking, learning, reasoning, problem-solving, emotions, consciousness and functions related to senses. The cerebral cortex is divided into; the frontal, parietal, temporal and occipital lobes. This confirmed the works of Amin *et al*.[45].

CONCLUSION

This study suggests that *O. gratissimum* prevent motor incoordination, anxiety-like behavior, depressive-like behavior and brain oxidative stress induced by prenatal stress. This protective effect may be attributed to the antioxidant

properties and the maintenance of acetylcholinesterase activity, cortisol and the brain cellular organization. Further study of *O. gratissimum* should be done identify the molecule(s) (the active ingredients) responsibles for its protective ability against prenatal stress toxicity. Also the specific mechanism of action of the aqueous extract of *O. gratissimum* in relation with prenatal stress should be conducted. The application of the results of this study is that the plant extract of *O. gratissimum* is a potential candidate of a source of new, safe and cheap molecule that can be use in the management of prenatal stress.

SIGNIFICANCE STATEMENT

Maternal stress during gestation causes numerous effects on infant physiology that extend well into adulthood. This study is coined at developing new molecules from the aqueous extract of *O. gratissimum* to solve the problem of motor coordination and behavioral impairment in the offspring of prenatally stressed rats. The results will support an adaptive life history perspective on maternal effects that is relevant for evolutionary biology, medicine and psychology.

ACKNOWLEDGMENTS

The authors gratefully acknowledged the graduate student Ahmad Yaya, Stanley Tashi for their assistance in conducting the experiments and Mr. Boubga Cliford for biochemical analyses.

REFERENCES

1. Liaudat, A.C., P. Bosch and N. Rodriguez, 2018. Stress versus immunity. MOJ Anat. Physiol., 5: 131-132.
2. de Kloet, E.R., M. Joels and F. Holsboer, 2005. Stress and the brain: From adaptation to disease. Nat. Rev. Neurosci., 6: 463-475.
3. Ryu, J.R., C.J. Hong, J.Y. Kim, E.K. Kim, W. Sun and S.W. Yu, 2016. Control of adult neurogenesis by programmed cell death in the mammalian brain. Mol. Brain, Vol. 9. 10.1186/s13041-016-0224-4.
4. Mark, H. and G. Peter, 2011. Developmental origins of noncommunicable disease: Population and public health implications. Am. J. Clin. Nutr., 94: S1754-S1758.
5. Foyet, H.S., D.E. Tsala, A.A. Bouba and L. Hritcu, 2012. Anxiolytic and antidepressant-like effects of the aqueous extract of *Alafia multiflora* stem barks in rodents. Adv. Pharmacol. Sci., Vol. 2012. 10.1155/2012/912041.
6. McEwen, B.S. and P.J. Gianaros, 2010. Central role of the brain in stress and adaptation: Links to socioeconomic status, health and disease. Ann. N. Y. Acad. Sci., 1186: 190-222.
7. Orafidiya, L.O., A.O. Oyedele, A.O. Shittu and A.A. Elujoba, 2001. The formulation of an effective topical antibacterial product containing *Ocimum gratissimum* leaf essential oil. Int. J. Pharm., 224: 177-183.
8. de los Angeles, G.A.M., R.O.M. del Carmen, G.A. Sonia and R.M. Socorro, 2017. Prenatal stress reduces learning and memory in pre-pubertal, young, and adult rats of both sexes. Global J. Zool., 2: 13-20.
9. Schöpper, H., T. Klaus, R. Palme, T. Ruf and S. Huber, 2012. Sex-specific impact of prenatal stress on growth and reproductive parameters of guinea pigs. J. Comp. Physiol. B, 182: 1117-1127.
10. Meneses, J.O., M.V.S. do Couto, N.C. Sousa, F. dos S. Cunha and H.A. Abe *et al*., 2018. Efficacy of *Ocimum gratissimum* essential oil against the monogenean *Cichlidogyrus tilapiae* gill parasite of Nile tilapia. Arquivo Bras. Med. Vet. Zootecnia, 70: 497-504.
11. Akah, P.A. and A.I. Nwambie, 1994. Evaluation of Nigerian traditional medicines: 1. Plants used for rheumatic (inflammatory) disorders. J. Ethnopharmacol., 42: 179-182.
12. Matasyoh, L.G., J.C. Matasyoh, F.N. Wachira, M.G. Kinyua, A.W.M. Thairu and T.K. Mukiama, 2007. Chemical composition and antimicrobial activity of the essential oil of *Ocimum gratissimum* L. growing in Eastern Kenya. Afr. J. Biotechnol., 6: 760-765.
13. Rabelo, M., E.P. Souza, P.M.G. Soares, A.V. Miranda, F.J.A. Matos, and D.N. Criddle, 2003. Antinociceptive properties of the essential oil of *Ocimum gratissimum* L. (Labiatae) in mice. Braz. J. Med. Biol. Res., 36: 521-524.
14. Ojewumi, M.E., O.R. Obanla and D.M. Atauba, 2021. A review on the efficacy of *Ocimum gratissimum*, *Mentha spicata*, and *Moringa oleifera* leaf extracts in repelling mosquito. Beni-Suef Univ. J. Basic Appl. Sci., Vol. 10. 10.1186/s43088-021-00176-x.
15. Braastad, B.O., 1998. Effects of prenatal stress on behaviour of offspring of laboratory and farmed mammals. Appl. Anim. Behav. Sci., 61: 159-180.
16. García-Vargas, D., L. Juárez-Rojas, S.R. Maya and S. Retana-Márquez, 2019. Prenatal stress decreases sperm quality, mature follicles and fertility in rats. Syst. Biol. Reprod. Med., 65: 223-235.
17. Sanda, A.K., A.E. Nantia, T.F.P. Manfo, R.T. Toboh and R.E. Abende *et al*., 2022. Subchronic administration of parastar insecticide induced behavioral changes and impaired motor coordination in male Wistar rats. Drug Chem. Toxicol., 45: 426-434.

18. Abramova, O., V. Ushakova, Y. Zorkina, E. Zubkov, Z. Storozheva, A. Morozova and V. Chekhonin, 2021. The behavior and postnatal development in infant and juvenile rats after ultrasound-induced chronic prenatal stress. Front. Physiol., Vol. 12. 10.3389/fphys.2021.659366.
19. Ellman, G.L., 1959. Tissue sulfhydryl groups. Arch. Biochem. Biophys., 82: 70-77.
20. Misra, H.P. and I. Fridovich, 1972. The generation of superoxide radical during the autoxidation of hemoglobin. J. Biol. Chem., 247: 6960-6962.
21. Sanda, A.K., E. Aneng, M. Mbouh, F.P.T. Manfo, S.N. Ngimgoh and E.A. Nantia, 2021. Testicular antioxidants and testosterone enhancing effects of the hydro-ethanolic extract of *Rauvolfia vomitoria* (Apocynacaea) in male Wistar rats. Invest. Med. Chem. Pharmacol., Vol. 4. 10.31183/imcp.2021.00054.
22. Saha, P., P. Banerjee, L. Auddya, P. Pal and M. Das *et al*., 2015. Simple modified colorimetric methods for assay of total oxidative stress and antioxidant defense in plasma: Study in diabetic patients. Arch. Med., Vol. 7.
23. Sanda, A.K., D. Fotsing, O. Mahamat, V. Natesan and J. Rajendran *et al*., 2021. Effect of *Allanblackia floribunda* stem bark aqueous extract in male albino wistar rats: Androgenic activities and penile nitric oxide (NO) production. Asian J. Pharm. Pharmacol., 7: 63-73.
24. Luong, T.N., H.J. Carlisle, A. Southwell and P.H. Patterson, 2011. Assessment of motor balance and coordination in mice using the balance beam. J. Vis. Exp., Vol. 10. 10.3791/2376.
25. Mossa, A.T.H., F.M. Ibrahim, S.M. Mohafrash, D.H. Abou Baker and S. El Gengaihi, 2015. Protective effect of ethanolic extract of grape pomace against the adverse effects of cypermethrin on weanling female rats. Evidence-Based Complementary Altern. Med., Vol. 2015. 10.1155/2015/381919.
26. Mainul Haque and A.R. Abubakar, 2019. Anxiolytic testing of medicinal plants in Nigeria: Frequently used experimental models. Medeniyet Med. J., 34: 83-98.
27. Taïr, K., O. Kharoubi, O.A. Taïr, N. Hellal, I. Benyettou and A. Aoues, 2016. Aluminium-induced acute neurotoxicity in rats: Treatment with aqueous extract of *Arthrophytum* (*Hammada scoparia*). J. Acute Dis., 5: 470-482.
28. Abushouk, A.I., A. Negida, H. Ahmed and M.M. Abdel-Daim, 2107. Neuroprotective mechanisms of plant extracts against MPTP induced neurotoxicity: Future applications in Parkinson's disease. Biomed. Pharmacother., 85: 635-645.
29. Ślusarczyk, J., E. Trojan, K. Głombik, B. Budziszewska and M. Kubera *et al*., 2015. Prenatal stress is a vulnerability factor for altered morphology and biological activity of microglia cells. Front. Cell. Neurosci., Vol. 9. 10.3389/fncel.2015.00082.
30. Jellinger, K.A., B. Janetzky, J. Attems and E. Kienzl, 2008. Biomarkers for early diagnosis of Alzheimer disease: 'Alzheimer associated gene'-A new blood biomarker? J. Cell. Mol. Med., 12: 1094-1117.
31. Godoy, L.D., M.T. Rossignoli, P. Delfino-Pereira, N. Garcia-Cairasco and E.H. de Lima Umeoka, 2018. A comprehensive overview on stress neurobiology: Basic concepts and clinical implications. Front. Behav. Neurosci., Vol. 12. 10.3389/fnbeh.2018.00127.
32. Meli, R., A. Monnolo, C. Annunziata, C. Pirozzi and M.C. Ferrante, 2020. Oxidative stress and BPA toxicity: An antioxidant approach for male and female reproductive dysfunction. Antioxidants, Vol. 9. 10.3390/antiox9050405.
33. Foyet, H.S., A.E. Asongalem, E.K. Oben, O. Cioanca, M. Hancianu and L. Hritcu, 2016. Effects of the methanolic extract of *Vitellaria paradoxa* stem bark against scopolamine-induced cognitive dysfunction and oxidative stress in the rat hippocampus. Cell. Mol. Neurobiol., 36: 1139-1149.
34. Calabrese, E.J. and L.A. Baldwin, 2003. Hormesis: The dose-response revolution. Annu. Rev. Pharmacol. Toxicol., 43: 175-197.
35. Antoine, K.S., M. Pierre, P.D.D. Desire, F.N.N. Mesmer, W. Pierre, D. Théophile and K. Pierre, 2012. Effect of aqueous extract of allanblackia floribunda (oliver) stem bark on sexual behaviour in adult male rats. World J. Pharm. Pharm. Sci., 1: 585-600.
36. Prokai, L., K. Prokai-Tatrai, P. Perjési and J.W. Simpkins, 2005. Mechanistic insights into the direct antioxidant effects of estrogens. Drug Dev. Res., 66: 118-125.
37. Leret, M.L., J.A.S. Millán, E. Fabre, R. Gredilla and G. Barja, 2002. Deprenyl protects from MPTP-induced Parkinson-like syndrome and glutathione oxidation in rat striatum. Toxicology, 170: 165-171.
38. Meissner, W., M.P. Hill, F. Tison, C.E. Gross and E. Bezard, 2004. Neuroprotective strategies for Parkinson's disease: Conceptual limits of animal models and clinical trials. Trends Pharmacol. Sci., 25: 249-253.
39. Hritcu, L., H.S. Foyet, M. Stefan, M. Mihasan, A.E. Asongalem and P. Kamtchouing, 2011. Neuroprotective effect of the methanolic extract of *Hibiscus asper* leaves in 6-hydroxydopamine-lesioned rat model of Parkinson's disease. J. Ethnopharmacol., 137: 585-591.
40. Liaudat, A.C., N. Rodríguez, A. Vivas, H.F. Gauna and N.I. Mayer, 2012. Effect of early stimulation on some immune parameters in a model of prenatally stressed rats. Int. J. Psychol. Stud., 4: 73-82.
41. Ciobica, A., L. Hritcu, V. Artenie, B. Stoica and V. Bild, 2009. Effects of 6-OHDA infusion into the hypothalamic paraventricular nucleus in mediating stress-induced behavioural responses and oxidative damage in rats. Acta Endocrinol., 5: 425-436.
42. Dornhorst, A., D.E. Carlson, S.M. Seif, A.G. Robinson, E.A. Zimmerman and D.S. Gann, 1981. Control of release of adrenocorticotropin and vasopressin by the supraoptic and paraventricular nuclei. Endocrinology, 108: 1420-1424.

43. Kyrou, I. and C. Tsigos, 2009. Stress hormones: Physiological stress and regulation of metabolism. Curr. Opin. Pharmacol., 9: 787-793.
44. Fotsing, D., G.T. Ngoupaye, A.C. Ouafo, S.K.J. Njapdounke, Y.A. Kenneth and E.N. Bum, 2017. Effects of *Gladiolus dalenii* on the stress-induced behavioral, neurochemical, and reproductive changes in rats. Front. Pharmacol., Vol. 8. 10.3389/fphar.2017.00685.
45. Amin, S.N., S.S. Hassan, A.S. Khashaba, M.F. Youakim, N.S. Abdel Latif, L.A. Rashed and H.D. Yassa, 2020. Hippocampal and cerebellar changes in acute restraint stress and the impact of pretreatment with ceftriaxone. Brain Sci., Vol. 10. 10.3390/brainsci10040193.