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Momordica foetida (Cucurbitaceae) prevents behavioral impairment, motor incoordination and brain oxidative stress induced by subchronic exposure to Parastar pesticide formulation

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Abstract

Background: Parastar is an agricultural insecticide formulation composed of two active ingredients; a pyrethroid lambda-cyhalothrin and a neonicotinoid imidachloprid used in Cameroon for vegetable protection. Previous studies showed reprotoxicity and neurotoxicity of this pesticide formulation. *Momordica foetida* Schumach. Et Thonn is a medicinal plant with potent antioxidant properties used traditionally in Cameroon for the treatment of several aliments. As farmers are currently exposed to Parastar pesticide formulation, this study was designed to evaluate the protective effect of *M. foetida* on behavioral impairment, motor incoordination and brain oxidative stress induced by subchronic administration of Parastar. **Methods**: The study was carried out using 40 Wistar male rats, divided into 5 groups of 8 rats each. The groups received a vehicle (distilled water; 10 mL/kg), Parastar alone (6.23 mg/kg), or concomitantly with *M. foetida* methanol extract (50, 100 and 200 mg/kg) for 60 days. The animals were submitted to behavioural tests including, beam walking test, open field test and forced swimming test. At end of the treatment, oxidative stress parameters and acetylcholinesterase activity were assessed in brain of each animal. **Results**: Parastar induced motor dysfunctions, anxiogenic like and depressive-like behaviours in the animals. The Parastar -induced alterations in behavior were all prevented by *M. foetida* extract. The plant extract alleviated Parstar-induced oxidative stress and alteration of cholinesterase activity, especially at the dose 100mk/kg. **Conclusion**: Results from this study suggest that, *M. feotida* methanol extract may prevent anxiety-like behavior, depressive-like behavior, motor incoordination induced by Parastar, possibly through it antioxidant properties and the preservation of acetylcholinesterase activity.

Keywords: acetylcholinesterase, *Momordica foetida*, neurotoxicity, oxidative stress, Parastar, rats

1. INTRODUCTION

A pesticide is a substance used to control organisms considered to be harmful and referred to as pests. It is designed to have a biocidal action and may be an insecticide, a fungicide, herbicide or parasiticide[1]. Insecticides are among the major classes of pesticides used in the agricultural field[2,3]. Though pesticide have integrally enhanced the economy by reducing losses in agricultural production, their intensive and inappropriate use has been associated with several toxicities and health problems including reproductive abnormalities, immune suppression, endocrine disruption, cancer and neurological pathologies in humans and other non-target animals[4-6]. Pesticides toxicity in humans generally results from occupational exposure during agricultural activities or consumption of food/water contaminated with residues of the agrochemicals [5,6].

Various classes of pesticides alter the nervous system through acute or chronic toxicity that may lead to behavioral and motor disorders including anxiety, depression, Parkinson's and Alzheimer's diseases[7,8]. Many studies attribute neurotoxic effect of pesticides mainly to their ability to promote oxidative stress to nervous[9-11] notably by inhibiting cholinesterase activity[6]. Parastar is an insecticide formulation composed of two active ingredients; a pyrethroid lambda-cyhalothrin and a neonicotinoid imidachloprid. This insecticide is generally used in Cameroon for vegetable protection[12,13]. Previous studies suggested neurotoxic effect of Parastar evidenced by its capacity to induce anxiogenic-like, depressive-like behavior as well as impaired motor coordination in Wistar rats. The neurotoxic effect was partly attributed to pro-oxidant effect of Parastar pesticide[14,3].

Regarding toxicity of pesticides, their constant increase use in various sectors, and their ubiquitous presence in the environment and human commodities, it is crucial to prevent pesticide toxicity on humans or to counteract their negative effects on untargeted organisms. Medicinal plants have been generally used to prevent and alleviate a wide variety of diseases empirically, including dysfunctions induced by chemical toxicity. Medicinal plants from Cameroon include *Momordica foetida* Schumach. Et Thonn (Cucurbitaceae), a perennial and widely distributed herb in tropical Africa. It is a trailing or climbing plant with simple or bifid tendrils that have both male and female flowers[15]. In many countries across Africa, *M. foetida* is used by local population to treat a number of diseases including hypertension, diabetes mellitus, fever, malaria, peptic ulcers, and as a purgative[16,17]. Curcubitane triterpenoids and polyphenolic compounds, alkaloids and glycosides have been isolated from the plant extracts. Previous studies have reported potent anti-oxidant and free radical scavenging capacity of *M. foetida*[18,19], which may counteract Parastar-induced neurotoxicity. This study therefore aimed at evaluating the protective effect of *M. foetida* against neurotoxicity induced by Parastar in adult male rats, focusing on behavioral impairment, motor incoordination and brain oxidative stress.

2. MATERIALS AND METHODS

2.1. Materials

2.1.1. Chemicals

The pesticide formulation Parastar 40WP containing imidachloprid (20 g/kg) and lambda-cyhalothrin (20 g/kg) was purchased from agricultural store in Santa, North West, Cameroon. This pesticide is manufactured by Elanco Novartis, imported and distributed in Cameroon by FIMEX international SABP, Douala, Cameroon. The dose of Parastar (6.23 mg/kg) used in this study was according to previous studies[3,14].

Other chemicals used in this study were analytical grade. S-Acetyl thiocholine iodide (ACTI), S-Butyrylthiocholine iodide (BCTI), 5,5′-dithiobis (2-nitrobenzoic acid) (DTNB) and trichloroacetic acid (TCA) were obtained from Sigma Aldrich (St Louis, MO, USA). Thiobarbituric acid (TBA) was gotten from Griffin and George (Wembly Middlesex, England). Methanol was purchased from LobaChemie Pvt. Ltd.107, Woodehouse (Mumbai, India).

2.1.2. Plant materials

The whole plant and fruit of *M. foetida* was harvested from Bambili, North West Region of Cameroon, on April 2018. It was then identified and compared with a specimen deposited at the Cameroon national herbarium under the identification number No 33420 HNC. *M. foetida* samples were cleaned by washing under running tap water, dried at room temperature, the leaves and stems were chopped and ground mechanically into fine powder. Four hundred grams of the obtained powder were macerated in 2 L of methanol, the mixture allowed to stand for 48 hours and then filtered using a Whatman N°3 filter paper. The solvent was thereafter dried out using an evaporator at 40°C, and 84 g of extract was obtained.

2.1.3. Experimental animals

Forty Wistar male rats (average weight of 191 ± 2g) were obtained from the animal house of the Department of Biochemistry (Faculty of Science, University of Bamenda). They were housed under standard conditions (temperature of 25°C) with light-dark natural cycle. They had free access to food and water. The animals were handled according to the guidelines and the authorization of the Cameroon National Ethical Committee on the use of laboratory animals for scientific research (Ref No.FW-IRB00001954). The study was done in conformation with the international regulation, minimizing the suffering and the number of animal use.

2.2. Methods

2.2.1 Animal treatment

The 40 male rats were assigned into five groups of eight rats each, and acclimatized for a period of two weeks. The groups were then administered a vehicle, plant extract or pesticide orally for 60 days as follows: group 1 (vehicle or control group) received distilled water (10 mL/kg), group 2 was given 6.23 mg/kg of Parastar, group 3 to 5 were exposed to same dose of Parastar (6.23 mg/kg) as well as different doses of the plant extract (50, 100 and 200 mg/kg, respectively).

2.2.2 Evaluation of the effect of Parastar and *M. foetida* on motor function, exploratory activity and depression

Behavioral tests were conducted from 58^{th} to the 60^{th} of the experiment. The Beam walking test was carried out on the 58^{th} day of the treatment, the Open field test on the 59^{th} day while the forced swimming test was done on the last day, i.e., day 60^{th} of the treatment. These experiments were carrying out different days to give enough time (24 hours) for animal to recover therefore to limit the influence of a given test on a subsequent one. All experiments were done from 05:30 PM local time in a quiet room under dim light.

2.2.2.1 Beam walking test (BWT)

This test is used for the assessment of motor coordination, particularly of the hind limb. Animals are placed in one corner of the narrow beam and allowed to walk across the narrow beam from one end to the other for at least three times. The narrow beam measures 3 cm wide and is elevated between a pole and their home cage. This training step was made before the test session and was useful to achieve a stable baseline measurement. Each animal was given 3 trials, and the average number of falls encountered, and time taken to cross the beam in each trial recorded. The number of falls expresses incoordination in rats[20].

2.2.2.2 Open-field test (OFT)

The open field test provides a useful method to simultaneously measure of the exploratory behavior, total locomotor activity levels and anxiety-like behavior in rodents. The test is based on the tendency of the rodents to explore the novel area and avoid bright light. The open field is an enclosed space, with surrounding walls that prevent the animal from escaping. The field is marked with a grid and square crossings. The center of the field is marked with a different color to differentiate from the other squares[21]. On the 59^{th} of experiment, the animals were subjected to OFT 30 min after administration of the respective treatments. The animals were placed in the open field and observed for 5 minutes.. The behavior assessments included duration at the central square; line crossings referring the rate with which the animals cross a grid line with all four paws; and rearing referring to the number of times the animals stood on their hind legs. These behaviors indicated change in exploratory behavior[22,23]. Other parameters assessed included stretch attended posture referring to the number of times the animal showed forward elongation of the head and forelimbs and then pulling back to the original position, and grooming indicating the duration of time the animal spent licking or scratching his body[22]. High stretch attended posture frequency indicates high levels of anxiety[24] while motionless and freezing represents the duration of time for which the animal remains completely stationary[22].

2.2.2.3 Forced swimming test (FST)

The force swimming test (FST) is widely used to assess depressive-like behavior in animal models and to screen potential antidepressant drugs. The test is based on the principle that animals develop an immobile posture in a non-escapable water filled cylinder[25]. As reported in 1978[26], animals were forced to swim individually for 15 minutes in a transparent cylinder filled with water during the pre-test session[26,27]. Twenty four hours later, the rat was forced to swim in the identical condition and environment for 5 minutes and its duration of immobility as well as struggling and swimming were measured. Increased floating is interpreted as a behavioral despair, correlate of negative mood, representing a kind of depression in the animal[25].

2.2.3 Evaluation of the effect of Parastar and *M. foetida* on brain oxidative stress parameters and acetylcholinesterase activity

After 60 days of exposure to different treatments, animals were anesthetized (using 10 mg/kg diazepam and 50 mg/kg ketamine), sacrificed the brain dissected out and weighed. A 20% of the brain homogenate was then prepared in phosphate buffer (pH 7.2, 50 mM), centrifuged (3000 rpm, 15 min, 4°C) and the supernatant collected and for biochemical analyses.

reduced glutathione (GSH) levels and catalase activity were estimated by the methods of Ellman[28], Sinha[29] and Misra and Fridovich[30]. The tissue concentration of nitrite (a stable oxidized product of nitric oxide) and the acetylcholinesterase activity were also determined as reported by Manfo et al[6]. All enzyme activities were normalized against protein concentrations determined by the method of Gornal et al[31].

2.2.4 Statiscal analysis

Data were expressed as mean ± Standard Error of Mean (SEM). The data were analyzed by one-way analysis of variance, and differences between groups assessed using Student–Newman–Keuls' test. Differences were considered statistically significant at $p<0.05$. All analyses were performed using Graphpad Instat software version 3.10.

3. RESULTS

3.1 The beam walking test

The effect of co-treatment of Parastar and the methanol extract of *M. foetida* on the parameters of motor coordination on the Beam walking test is presented in table 1. The insecticide significantly ($p<0.05$) decrease the distance travelled, and turns by rats, while significantly increased the number of falls/5 min when compared to the vehicle group. But in the dose 100 mg/kg of the methanol extract of *M. foetida* significantly ($p<0.05$) prevented the increasing number of falls and the decreasing distance travelled induced by Parastar on the beam walking parameters. The other doses (50 and 200 mg/kg) of the methanol plant extract showed sub-protective activity against Parastar -induced negative effects on the investigated behavioural parameters in rats.

3.2 Open-field test results

On the decreasing effect of Parastar on open-field experiment, the presence of the methanol extract of *M. foetida* at all the doses relieved the effects, by inducing a significant increase in the number of center square entries (table 2). When compared with the control group the dose 100 mg/kg of *M. foetida* extract also prevented ($p<0.05$) Parastar-induced alteration on the center square duration, total distance travelled, number of line crossed, number of rearing against the wall, number of stretch attained posture as well as freezing time experienced by the animals. The grooming duration was not altered by the different treatments.

3.3 Forced swimming test results

Forced swimming test parameters after co-treatment of rats with Parastar and the methanol extract of *M. foetida* are shown in Table 3. Parastar significantly ($P < 0.01$) increased (181.52%) immobility time when compared to the vehicle or distilled water. However, concomitant exposure to 6.23 mg/kg insecticide and plant extract at the doses of 100 and 200 mg significantly prevented the immobility time induced by the insecticide with the immobility duration similar to that of the control group. The number of defecation remained similar across different treatments.

3.4 Brain oxidative stress parameters and acetylcholinesterase activity

As shown in table 4, Parastar significantly reduced ($P < 0.05$) glutathion, catalase and actylcholinesterase activity while it increased ($P<0.05$) nitrites levels when compared to the control group treated with distilled water. These parameters were alleviated by the plant extract of *M. foetida*. It should e noted that the dose 100 mg/kg of *M. foetida* displayed comparable effects with the distilled water treated control rats.

Table 1: Effect of the co-treatment of Parastar and the methanol extract of *M. foetida* on the beam walking test parameters

Parameters / Treatments	Distance travelled (cm)	Number of falls/5 min	Number of turns
Distilled water (5mL/kg)	47.13 ± 1.77[a]	45.65 ± 2.23[a]	4.77 ± 0.28[a]
Parastar (6.23 mg/kg)	14.38 ± 3.71[c]	85.37 ± 2.56[b]	1.25 ± 0.16[c]
Parastar (6.23 mg/kg) + *M. foetida*(50 mg/kg)	28.75 ± 0.25[b]	55.40 ± 1.67[c]	1.62 ± 0.18[c]
Parastar (6.23 mg/kg) + *M. foetida*(100 mg/kg)	43.00 ± 2.83[a]	40.98 ± 3.07[a]	3.85 ± 0.17[b]
Parastar (6.23 mg/kg) + *M. foetida* (200 mg/kg)	30.00 ± 3.45[b]	58.00 ± 2.48[c]	2.01 ± 0.01[c]

Values in the table represent means ± standard deviation, n=8 rats per group. Values not sharing a common letter differ significantly with each other ($P < 0.05$, Student–Newman–Keuls).

Table 2: Effect of the co-treatment of Parastar and the methanol extract of *M. foetida* on the open field test parameters.

Parameters / Treatments	Center square entries	Duration of the center square (s)	Total distance travelled (cm)	Lines crossed	Rearing against the wall	Stretch attained posture	Grooming (s)	Freezing time (s)
Distilled water (5 mL/kg)	20.25±1.16ᵃ	24 ± 1.02ᵃ	340.38±7.45ᵃ	38.13±0.98ᵃ	15.25±0.62ᵃ	6.76±0.34ᵃ	3.54±0.13	23.00±0.19ᵃ
Parastar (6.23 mg/kg)	6.24±1.06ᶜ	7.13 ± 0.19ᶜ	126.92±8.34ᶜ	14.25±0.65ᶜ	6.32±0.80ᶜ	23.31±0.71ᶜ	2.98±0.16	81.75±1.00ᶜ
Parastar + *M. foetida* (50 mg/kg)	17.49±2.75ᵃ	14.13 ± 0.29ᵇ	319.00±7.22ᵃ	23.00±0.33ᵇ	6.38±0.18ᶜ	20.00±0.53ᶜ	4.00±0.27	75.13±1.04ᵇ
Parastar + *M. foetida* (100 mg/kg)	23.41±2.34ᵃ	28.30 ± .27ᵃ	326.75±5.15ᵃ	35.10±0.46ᵃ	16.41±0.49ᵃ	7.50±0.80ᵃ	3.50±0.28	21.13±0.85ᵃ
Parastar + *M. foetida* (200 mg/kg)	17.51±1.28ᵃ	20.25±1.16ᵃ	324.00±7.79ᵃ	21.88±1.52ᵇ	13.54±0.37ᵃ	8.00±0.53ᵃ	3.63±0.18	30.25±0.45ᵃ

Values in the table represent means ± standard deviation, n=8 rats per group. Values not sharing a common letter differ significantly with each other ($P < 0.05$, Student–Newman–Keuls).

Table3: Effect of the co-treatment of Parastar and the methanol extract of *M. foetida* on forced swimming test parameters.

Parameters / Treatments	Immobility time (s)	Number of defecations
Distilled water (5mL/kg)	71.55±3.23ᵃ	5.75±0.41
Parastar (6.23 mg/kg)	201.43 ± 4.87ᶜ	5.13 ± 0.35
Parastar+ *M. foetida* (50 mg/kg)	118.63 ± 3.54ᵇ	4.94± 0.50
Parastar+ *M. foetida* (100 mg/kg)	68.00 ± 5.26ᵃ	5.25± 0.41
Parastar+ *M. foetida* (200 mg/kg)	65.34±3.61ᵃ	4.88± 0.35

Values in the table represent means ± standard deviation, n=8 rats per group. Values not sharing a common letter differ significantly with each other ($P < 0.05$, Student–Newman–Keuls).

Table 4: Effect of the co-treatment of Parastar and the methanol extract of *M. foetida* on brain oxidative stress parameters and acetylcholinesterase activity

parameters / Treatments	GSH (mmole/mg protein)	CAT (UI/mg protein)	Nitrites (µM/mL of homogenate)	Acetylcholinesterase activity (UI/mg protein)
Distilled water (5mL/kg)	1.75 ± 0.03ᵃ	40.00 ± 5.64ᵃ	1.35 ± 0.07ᵃ	10.55 ± 1.70ᵃ
Parastar (6.23 mg/kg)	0.35± 0.003ᵇ	18.00 ± 4.33ᵇ	3.15 ± 0.16ᵇ	3.58 ± 0.38ᵇ
Parastar (6.23 mg/kg) + *M. foetida* (50 mg/kg)	0.40 ± 0.02ᵇ	16.42 ± 3.67ᵇ	1.33 ± 0.05ᵃ	4.05 ± 2.06ᵇ
Parastar (6.23 mg/kg) + *M. foetida* (100 mg/kg)	1.54 ± 0.05ᵃ	36.85 ± 3.27ᵃ	1.41 ± 0.06ᵃ	9.59 ± 1.26ᵃ
Parastar (6.23 mg/kg) + *M. foetida* (200 mg/kg)	1.15 ± 0.02ᵃ	20.38 ± 4.42ᵇ	1.29 ± 0.05ᵃ	6.01 ± 1.33ᵇ

Values in the table represent means ± standard deviation (SE), n=8 rats per group. Values not sharing a common letter differ significantly with each other ($P < 0.05$, Student–Newman–Keuls).

4. DISCUSSION

This study was aimed at evaluating the protective effect of the methanol of extract of *M. foetida* on Parastar induced neurotoxicity focusing on behavioral parameters like anxiety, exploration, and depression as well as motor coordination and balance.

The beam walking assay helps assessing the status of motor coordination and balance[32]. Subchronic exposure of rats to Parastar resulted into decreased distance travelled, number of foot slip and number of animal turns compared to the control group. These results suggest that the pesticide alter motor coordination and balance in the rats. Similar results were previously reported by Kada et al[3]. Interestingly, the motor incoordination induced by Parastar was prevented by *M. foetida* extract, suggesting that the extract contains bioactive compounds with protective potentials vis-à-vis of Parastar-induced neurotoxicity. This result is similar to results of Abdel-Tawab al[33] 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 is an evidence of increasing reports stating protective effect of some medicinal on neurotoxicity[34,35].

Anxiety is a state of uncomfortable feelings, nervousness or worry characterized by unpleasant and uncomfortable feelings of inner turmoil, often accompanied by nervous behavior such as pacing back and forth, somatic complaints, and rumination. Anxiety is amongst the most common psychiatric disorders[36]. The OFT has been appropriate for evaluation of the basal activity in rodents as well as its evolution in response to novelty or to an anxiety-inducing environment, pharmacological treatment, lesions or genetic modification[37]. The number of center square entries, duration in the center square and the number of line crossed and rearing were all dropped in Parastar-exposed animals. These parameters measure exploratory behavior and anxiety and their low frequency indicate low exploratory and high anxiety induced by the insecticide[24,34,3]. Treatment of animals with Parastar also significantly increases other anxiety related parameters such as stretch attained posture. Consistently, previous investigation demonstrated that Parastar induced anxiety-like behaviour in rats[38,3]. Similarly, de Souza[39] reported the anxiogenic effects of fenvalerate, a type II pyrethroid insecticide chemically composed of α cyano-3-phenoxybenzyl α-(4-chlorophenyl) isovalerate. Administration of the methanol extract of *M. foetida* effectively prevented the changes induced by Parastar in animals. This result suggests that the methanol extract of *M. foetida* contained active ingredient able to protect against Pararstar-induced behavioral changes. The *M. foetida* active ingredients prevent anxiety probably by enhancing the response to GABA or facilitating the opening the GABAA-activated chloride channels.

Depression is a health condition characterized in a particular great sadness, feeling of hopelessness (depressed mood), loss of motivation and decision-making faculties, decrease in the feeling of pleasure, eating and sleep disorders, morbid thoughts and the feeling of having no value as an individual. It is generally associated with decreased functional amine-dependent synaptic transmission[40]. The immobility displayed by rats in the forced swimming test reflects a state of despair or lower mood, which is characterized depressive disorders in humans[41]. The immobility time increased following Parastar exposure, suggesting a depressive-like effect induced by this pesticide. Aldridge et al[42] obtained similar results following exposure of rats to the insecticide Chlorpyrifos suggesting a behavioral alterations and development of depression-like symptoms in rats. The methanol extract of *M. foetida* has counteracted the depressive-like symptoms induced by Parastar in rats suggesting its possible richness in bioactive constituents able to protect amine-dependent neurons implicated in the process of depression. This dysfunction and other related neurological disorders are generally caused by oxidative stress[43,44] that was also investigated.

Oxidative stress is by far responsible for several neurotoxic and neurological pathologies, including Parkinson's disease, epilepsy, depressive disorders and Alzheimer's disease[11,45]. Parastar has significantly decreased brain level of reduced glutathione, the activity of catalase and increased level of nitrites. Glutathione is a pseudo-tripeptide formed by the condensation of glutamic acid, cysteine and glycine. Glutathione in reduced form is the major antioxidant in cells, protecting them from free radicals. On the other hand, the brain is particularly susceptible to oxidative insults and its antioxidant defense system is dependent on its GSH content. Low levels of GSH are associated with neurodegenerative diseases such as multiple sclerosis, Alzheimer's disease, Parkinson's disease and others[46]. Catalase is a heme-containing tetrameric enzyme with the potential to directly dismutate the highly oxidant molecule H_2O_2 into H_2O and O_2. This enzyme is indispensable for reactive oxygen species detoxification during oxidative stress conditions[47], while nitrite is the stable metabolites of NO[48], implicated in the generation of free radicals and oxidative damage in brain. Notably, NO has been proposed to play a role in the neurotoxic actions of glutamate and trans-synaptic regulation, as well as in learning and memory processes[48]. As all these parameters were altered in the rats exposed to Parastar, it can be suggested that the neurotoxic effects of the pesticide formulation occur at least in part through oxidative stress in brain. More importantly, all investigated oxidative stress parameters were maintained by the *M. foetida* extract at the levels close to that of the control animals receiving the vehicle. This implies that the plant extract, thanks to its antioxidant properties[19], could protect against neurotoxicity of Parastar.

Acetylcholine is neurotransmitter that plays a very vital roles in the central and peripheral nervous systems. In the central nervous system, it ensures the transfer of signals between neurons while in the peripheral nervous system, it relays nerve impulses to the muscles[49]. The action of this neurotransmitter is however terminated by the hydrolysis by the enzyme acetylcholinesterase ending impulse transmission[49]. In the current study, Parastar significantly inhibited acetylcholinesterase activity in animals, an effect that can lead to behavioral, cognitive and motor dysfunctions. This could then be an additional mechanism of the neurotoxicity of Parastar in rats. Administration of the methanol extract of *M. foetida* significantly alleviated the inhibitory effect of Parastar on acetylcholinesterase activity. The dose 100 mg/kg of plant extract showed better preventive effect against Parastar-induced neurotoxicity in all investigated parameters, as compared to the higher dose. This is referred to as hormesis, i.e., a biphasic dose response of *M. foetida* extract characterized by a low dose beneficial effect and a high dose inhibitory or toxic effect. Similar observations have been made in previous studies[50,51].

In conclusion, results from this study suggest that *M. feotida* methanol extract prevented anxiety-like behavior, depressive-like behavior, motor incoordination and brain oxidative stress induced by Parastar in rats. This protective effect may be attributed to the antioxidant properties and the maintenance of acetylcholinesterase activity, at least in part. However, the current findings suggest further neuro-chemical, histological and neurohistochemical studies to better elucidate the action mechanism of both Parastar and the methanol extract of *M. feotida*.

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Conflict of Interests

The authors have non-financial interests to disclose

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