Neuroprotective Effect of Moringa Oleifera Extract on Acetamiprid Induced Neurotoxicity and Apoptosis in Albino Rats

Amr A. Abdallah[1], Marwa A. Ibrahim[2], Eman A. Ibrahim[3], Hossam El Din H. Abdelhafez[1], Naglaa F. Mahmoud[4]

[1]Associate Prof. of Mammalian Toxicology Department, Central Agricultural Pesticides Lab. (CAPL), Agricultural Research Center, Dokki 12618, Giza, Egypt, [2]Professor of Biochemistry and Molecular Biology Department, Faculty of Veterinary Medicine, Cairo University, Giza 12211, Giza, Egypt, [3]Associate Prof. of Plant Biochemistry Department, National Research Centre (NRC), 33 EL Bohouth st. (EL Tahrir st.), Dokki 12622, Giza, Egypt, [4]Associate Prof. of Forensic Medicine and Clinical Toxicology Department, Faculty of Medicine, Cairo University, El Manial 11956, Cairo, Egypt

Abstract

Acetamiprid (ACMP) can cause neurotoxicity and induces neuronal apoptosis. The ameliorative effects of Moringa Oleifera (MO) seed extract against the neurotoxicity induced by ACMP in adult male albino rats were investigated. Forty male albino rats were divided into 4 equal groups. The first group served as a control, the second group administered ACMP 26.8mg/kg b.w. (representing 1/10 LD50) by gavage. The third group was given combination of ACMP 26.8 mg/kg b.w. and MO seed extract 150 mg/kg b.w. the fourth group was given MO extract only 150 mg/kg b.w. for 28 consecutive days. Results proved the efficacy of MO extract as a neuroprotective agent through the reduction of the oxidant parameter malondialdhyde (MDA) content by 26.9%, significant increase of reduced glutathione content (GSH) by 21.4%, and elevation of catalase (CAT) and superoxide dismutase (SOD) activities by (62.2% and 40.8%) respectively, compared to ACMP intoxicated group. Consequently, gene expression analysis for Bax and NBN genes was downregulated by 54.5% and 36.3% respectively in MO treated rats (group III) compared with ACMP intoxicated group. Moreover, restoration of the pathological tissue injuries was noticed. In conclusion, MO proved to be an effective neuroprotective and anti-apoptotic agent against ACMP-induced toxicity.

Keywords: *Acetamiprid; Moringa Oleifera; Neurotoxicity; Apoptosis; Neuroprotection.*

Introduction

Acetamiprid (ACMP), is one of the recently developed neonicotinoid group of pesticides, widely used against different variety of insect pests [1]. It was the second manufactured insecticide in this group after imidacloprid [2]. Acetamiprid operates as a selective nicotinic acetylcholine receptor (nAChR) agonist in the central nervous system of insects [2]. Over the last ten years, the expanded usage of neonicotinoid insecticides has been criticized because of the hazard associated with their toxicity [3, 4]. ACMP was reported to have toxic effects on the thyroid, liver, respiratory and reproductive functions [5]. Acute poisoning after ingestion of acetamiprid in humans has been documented [6]. However, there is no satisfactory information on the neurotoxicity potential of acetamiprid on human health.

Corresponding author:
Naglaa F. Mahmoud
Associate Prof. of Forensic Medicine & Clinical Toxicology; Faculty of Medicine; Cairo University;
Mobile: Egypt- 00201226906767;
Email: naglafarid2000@yahoo.com

Acetamiprid may lead to oxidative damage by producing reactive oxygen species in the target tissues [7]. Exposure to acetamiprid induce imbalance of oxidative stress status, and disruption of mitochondrial membrane permeability in rat's brain through generating reactive oxygen species [8]. The brain is liable to the damage caused by oxidative stress imbalance because of its comparatively low antioxidant capacity, its abundance in peroxidable fatty acid and its high-energy needs; this irreversible molecular corruption is the main cause of neuronal cell death and neurodegenerative diseases [9].

Several phytochemical expressed considerable protective effects against neurotoxicity in animal models by restoring the antioxidant capacity [10]. Many plants contain natural antioxidant compounds, which used as curative agents for neuronal diseases by increasing memory; alertness and brain function in general. Moringa oleifera (MO) named a miracle tree by consisting of phytochemical, vitamins, essential amino acids and mineral. It has many therapeutic benefits such as anti-inflammatory, antipyretic, antiulcer, antidiabetic, anticancer and antimicrobial agent [11]. MO extract has a role in preventing Alzheimer's disease by reducing hyperphosphorylation and amyloid-b pathology [11]. This study aimed to evaluate the neurotoxic effects induced by acetamiprid in terms of oxidant/antioxidant status, apoptosis markers and histopathological changes in male rats with studying the ameliorative effect of MO seed extract.

Materials and Methods

Chemicals:

Acetamiprid insecticide neonicotinoids (20% SP) was obtained from Starchem. Agro. Pesticides company, Egypt.

Preparation of Moringa olifera seed extract:

Powdered seed of moringa oleifera L (1kg) was extracted with hexane (1L×5) by soaking at room temperature. The hexane extracts were concentrated under reduced pressure at 60c. The total oil from hexane were weighed and stored at -10 until analysis. The residue of moringa oleifera after hexane extract was re-extracted with ethanol. Components of MO seeds extract determined by colour test method for phenols, flavonoids and tannins [12], carbohydrates and steroids [13].

Animals:

Forty albino rats aged 12-weeks (170±10g) were obtained from the breeding unit of the Toxicology and Forensic Medicine Department, Faculty of Veterinary Medicine, Cairo University. Animals were maintained at the animal care facilities of Central Agricultural Pesticides Laboratory (CAPL) in plastic cages under controlled temperature (23±2ºC), 12-h light/dark cycle and average humidity (50±5%). Water and food were available *ad libitum*. Rat's acclimatization to the environmental condition were done for two weeks preceded the experiment.

Experimental Design:

Animals were divided into four groups with 10 animals each. The first group was used as a control. The second group (group II) treated with acetamiprid 26.8mg/kg b. w. (representing 1/10 LD50). [14] by gavage. The third group (group III) given combination of acetamiprid (26.8 mg/kg b.w.) and MO seed extract (150 mg/kg b.w.), the fourth group (group IV) was given MO extract only (150 mg/kg b.w.). After treatment for 28 days, rats were anesthetized using diethyl ether, killed by capitation and the brain of each rat was collected, washed and kept for further analysis.

Lipid peroxidation and antioxidants parameters measurements:

Tissue preparation:

Brain were removed, washed with cold saline buffer, immediately stored at -80°C. For obtaining enzymatic extract, tissues were homogenized in ice cold 50mM sodium phosphate buffer (pH7.4) containing 0.1mM ethylendiaminetetraacetic acids (EDTA) yielding 10 % (W/V) homogenate. The homogenates were centrifuged at 2000 r.p.m for 30 min at 4°C. The supernatant samples were separated and used for measurement of

malondialdhyde (MDA) [15], reduced glutathione content (GSH) [16], catalase enzyme activity [17], superoxide dismutase (SOD) enzyme activity [18].

Quantitative real-time PCR for Bax and NBN genes:

Total RNA was extracted from fresh brain tissue using RNeasy mini kit (Qiagen) following the manufacturer's guidelines. cDNA synthesis was done by reverse transcription of 10 μg RNA samples. Real-time PCR performed using a Real-Time PCR System (Applied Biosystems, USA) by the following primers: forward 5′-CTTCAGGACAGCAGTGAGGA-3′ and reverse 5′-TCTTTCGAGCATGGTGACCT-3′ for NBN gene; forward ACCAAGAAGCTGAGCGAGTG and reverse CCAGTTGAAGTTGCCGTCTG for Bax gene. The cDNA amplification obtained by 40 cycles of denaturation at 95°C for 45 s, annealing at 63°C for NBN and 59°C for Bax gene for 45 s and extension at 72°C for 45 s. The 95°C step was extended to 5 min during the first cycle. 2% agarose gel electrophoresis stained with SYBR Safe DNA gel stain (Invitrogen) was used to confirm the size of the amplicons. As a reference gene, the β-actin gene amplified in the same reaction. Each measurement was repeated 3 times, and the values were used to calculate the gene/ β-actin ratio, with a value of 1.0 used as the control (calibrator). The normalized expression ratio was calculated using the method described by Livak and Schmittgen [19].

Histopathological examination:

After routine histological laboratory procedures, sections of 5 μm paraffin-embedding sections were prepared and stained with hematoxylin and eosin (H&E) for histopathology [20].

Statistical Analysis

Data coded and entered using the statistical package for the Social Sciences (SPSS) version 26 (IBM Corp., Armonk, NY, USA). Data was summarized using mean and standard error of the mean (SEM) for quantitative variables. Comparisons between groups were done using analysis of variance (ANOVA) with multiple comparisons post hoc test. P-values less than 0.05 were considered as statistically significant.

Results

Phytochemical analysis of ethanol extract of Moringa olifera seeds:

The total oil of seed of moringa oleifera was 30%. Phenol, tannins, flavonoids, carbohydrates and steroids bioactive components are present in ethanol extract of seed moringa oleifera L (table 1).

Table (1): Components of MO seeds extract analysis

Ethanol phytochemical test	Results
Phenol test	+
Flavonoids Test	+
Tannins Test	+
Carbohydrates	+
steroids	+

+ Present, - absent

Effects on MDA level, GSH, SOD and catalase activities:

As shown in table 2, significant increase in the brain level of MDA in ACMP-treated rats was observed ($p \leq 0.05$) as well as significant reduction of enzymatic activity for SOD and CAT and GSH concentration compared to the control. MO extract treated group (group III) showed significant increase of all antioxidant parameters (GSH by 21.4%, CAT by 62.3%, and SOD by 40.8%) and reduction of oxidant parameter (MDA) by 26.9%, in comparison to ACMP-treated group.

Table 2: Ameliorative effects of MOLE against ACMP-induced oxidative stress in different experimental groups.

	GROUP I	GROUP II	GROUP III	GROUP IV
CAT	24.51±0.48 b	18.97±0.93a	30.81±2.11c	23.7±0.45 ab
SOD	5.66±0.05b	4.06±0.34a	5.72±0.45b	4.9±0.1ab
GSH	36.59±0.94b	30.87±0.52a	37.47±1.35b	30.02±2.23a
MDMA	24.41±0.67ab	31.49±1.14c	23±0.45a	27.52±1.49bc

Values are presented as mean ±SEM: Mean values with different superscript letters from(a-c) in the same raw are significant different P values between each 2 groups at ($P \leq 0.05$).

NBN and Bax genes mRNA expression:

Quantification of the mRNA expression of NBN and Bax genes by real-time PCR was performed to evaluate the proapoptotic activity of acetamiprid by controlling gene expression of apoptotic pathways. The apoptosis level was significantly elevated in acetamiprid intoxicated group with a significant up-regulation of mRNA expression level for BAX gene by more than five-folds (marker of cell apoptosis) and NBN gene by four-folds compared with the control group (figure 2 A). Gene expression analysis for Bax and NBN genes in the brain tissue of MO extract treated group (group III) revealed significant downregulation by 54.5% and 36.3% respectively compared to ACMP intoxicated group.

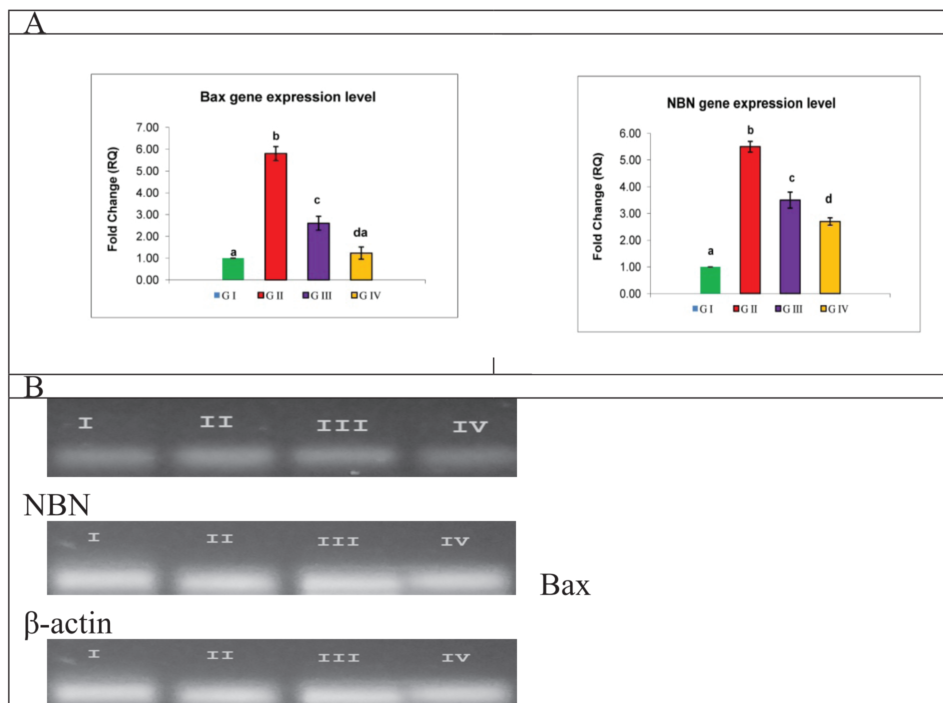

Figure 1: Effect of MO extract on NBN and Bax genes expression level in brain rats intoxicated with acetamiprid pesticide. (A) Fold change of mRNA expression of Bax and NBN genes in different experimental groups using qPCR. Data showed as mean ± SEM. Groups having different superscripts letters are significantly different from each other. (B) The results of qPCR analysis were proved by agarose gel electrophoresis analysis of the PCR product compared to β-actin.

Histopathological examination:

As illustrated in figure 3, no histopathological changes of the neurons in the hippocampus were recorded in the control group (A). Degeneration and nuclear pyknosis were noticed in the neurons of group II (B). Degeneration and nuclear pyknosis were reported in few neurons of group III (C). Neurons of group IV (D) showed no histopathological abnormalities.

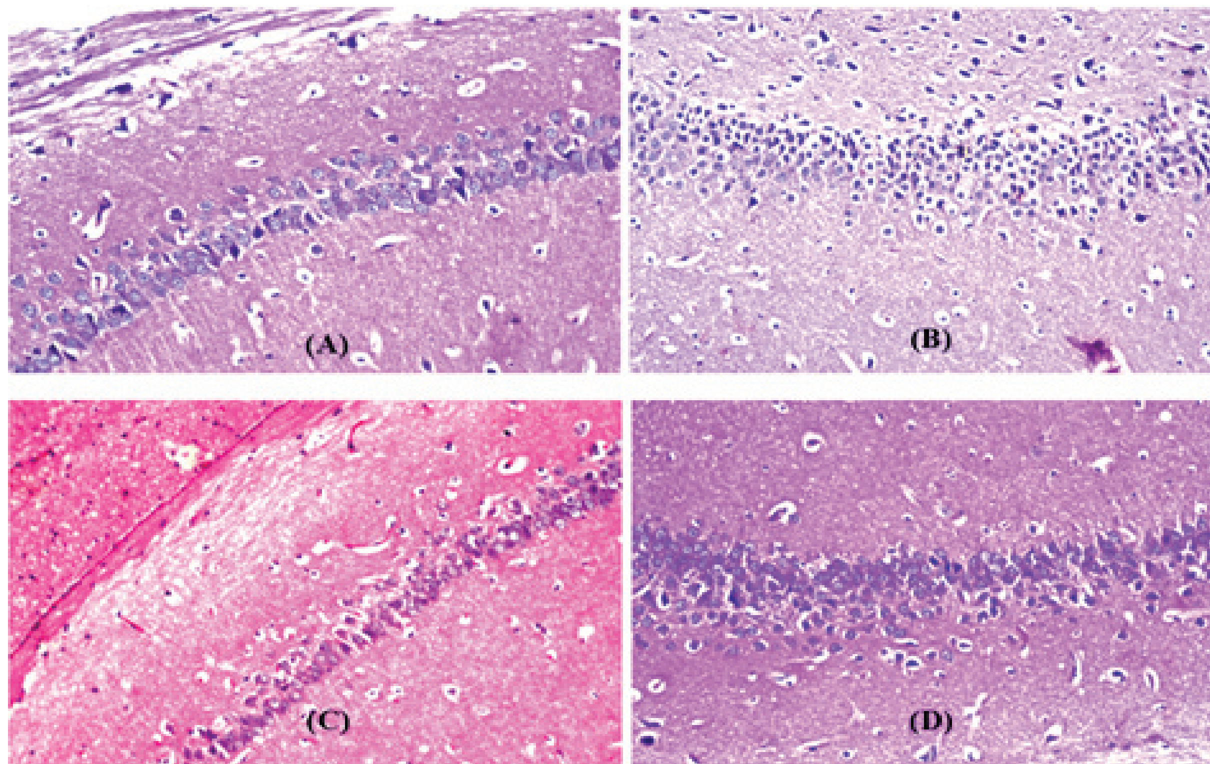

Figure 2: (A). Photomicograph of neurons of the control group showed no histopathological changes and normal histological structure (B). Photomicograph of neurons in ACMP treated group (II) showed degeneration and nuclear pyknosis in the neurons of subiculum of the hippocampus (C). Photomicrograph of neurons of ACMP + MO extract treated group (III) showed nuclear pyknosis and degeneration in few neurons of subiculum of the hippocampus (D). Photomicrograph of neurons of MO extract treated group (IV) showed no histopathological abnormalities and preserved normal structure of the neurons.

Discussion

The present study proved that the dried seeds of MO are great source of polyphenol compounds, such as phenolic acids and flavonoids. The flavonoids contained in MOLE are quercetin, catechin and kaempferol as reported by others [21] in addition to tannins, flavonoids, carbohydrates, steroids bioactive components and vitamins [22]. Therefore, MOLE has a role as antioxidant, anti-apoptosis and anti-inflammatory.

ACMP –induced oxidative stress damage evaluated by quantification of MDA content, GSH, SOD and CAT activity. In the present work, Oxidative stress mediated by ACMP induced depletion in SOD, GSH, and CAT activities and induction of lipid peroxidation. This finding reflects one of the causative mechanism implicated in ACMP-induced toxicity [23]. Acetamiprid can cause increased reactive oxygen species (ROS) production in the cells. Excess ROS lead to increased lipid peroxidation (LPO) of the cell membrane followed by cellular damage, accumulation of MDA level and antioxidant depletion [24, 25]. Several studies have reported the imbalance between oxidant / antioxidant status after Acetamiprid exposure [26, 27]. The observed improvement

in the oxidative stress parameters in ACMP treated group in this study attributed to the coadministration of MO extract. The antioxidant activity of MO extract because of its content of bioactive polyphenols and flavonoids as (quercetin, catechin and kaempferol) against oxygen free radicals and its ability to prevent oxidative damage, polyphenols components of moringa seed extract such as catechin can act as an antioxidant by scavenging the free radicals and chelating the sulprus metal ions [(28)]. Moreover, quercetin contains a hydroxyl group with antioxidant capacity and other flavonoids, which suppress the production reactive oxygen species [(29)].

Determination of mRNA expression level of NBN and Bax genes was performed to assess ACMP role as apoptotic and neuronal cell death provoking factor in the neurodegenerative diseases [(30)]. As revealed in figure 1A, ACMP intoxication induced significant upregulation of NBN and Bax genes expression level. However, there was a significant decrease of their expression level in MO extract treated group. Apoptosis is regulated by Bcl-2 family and caspase family of proteins [(31)]. Bax gene is a pro-apoptotic factor of the Bcl-2 gene family; encodes BCL2L4 protein that, upon activation of Bax protein, its function is to bind and induce mitochondrial outer membrane (MOM) permeabilization, leading to release of cytochrome c, followed by caspase pathway activation, hence, apoptotic cell death is accelerated in response to cell death signals [(32-34)].

NBN gene plays an important role in genomic stability and repair of DNA double strand breaks. The *NBN* gene encodes for a protein called nibrin. This protein is responsible for several crucial cellular processes, including the repair of damaged DNA. Increased NBN gene expression indicates DNA damage [(35, 36)]. As proved by other authors [(37, 38)], exposure to ACMP mediates apoptosis through disruption of the oxidative stress pathway. Similarly, recent studies [(39, 40)] reported that, acetamiprid treatment leads to loss of mitochondrial membranes integrity and cell death through the induction of necrosis concomitantly with the generation of ROS. As shown in our results, ACMP induced neuronal cell death was significantly reduced by MO extract treatment, as revealed by significant downregulation of mRNA expression for both NBN and Bax genes in the brain tissue (figure1A). Moringa extract used in this work as a protective agent against ACMP neurotoxicity. Treatment with MO extract significantly reduces apoptosis markers induced by ACMP; it improves mitochondrial functions, the flavonoids content of MO extract as luteolin has a powerful antioxidant activity and a protective role against DNA damage. Moringa extract contains tannins, steroids and phenols, which have free radical scavenging and anti-inflammatory capabilities [(41, 42)]. Moreover, MO extract contains Vit E (a-tocopherol) which inhibit programmed cell death by disrupting the activation of free radical cascade reactions in the lipid layer of the outer cell membrane [(43)]. Recently, Khan et al. [(44)] stated that, quercetin content in MO extract could control apoptosis pathway in the mitochondria by interrupting the activation of caspases-3 and cytochrome-c.

The present study showed histopathological changes of the brain cells including nuclear pyknosis, shrinkage and degeneration in response to ACMP. The generation of ROS induced by acetamiprid result into damage of different membrane components of the cell. On the other hand, the histopathological changes were improved with MO extract treatment in comparison with ACMP intoxicated group. Results obtained by **Kou et al**, proved the neuronal protection effect of moringa extract [(45)]. Moringa Oleifera components can cross the blood brain barrier so it have beneficial effects on the neuronal system of the intoxicated-rats [(45)].

Conclusion

The current findings revealed that, ACMP exposure might induce neurodegenerative disorders by induction of oxidative stress damage and apoptosis. The present study explored the role of MOLE to alleviate the ACMP-toxic effects. The protective role of MOLE is mediated through the regulation of antioxidant, and antiapoptotic signaling pathways.

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Conflict of Interest: The authors declare that there are no conflicts of interest.

References

1. Honda H, Tomizawa M, Casida JE. Neonicotinoid metabolic activation and inactivation established with coupled nicotinic receptor-CYP3A4 and -aldehyde oxidase systems. Toxicol Lett. 2006; 161: 108-114.
2. Yamamoto I, Casida JE. Nicotinoid insecticides and the nicotinic acetylcholine receptor. 1st edition. 1999; Tokyo: Springer-Verlag. P:149-177.
3. Houchat JN, Cartereau A, Mau AL, Taillebois E, Thany SH. An Overview on the Effect of Neonicotinoid Insecticides on Mammalian Cholinergic Functions through the Activation of Neuronal Nicotinic Acetylcholine Receptors. Int J Environ Res Public Health. 2020; 17(9): 3222.
4. Zhou Q, Ding Y, Xiao J. Sensitive determination of thiamethoxam, imidacloprid and acetamiprid in environmental water samples with solidphase extraction packed with multiwalled carbon nanotubes prior to high-performance liquid chromatography. Anal Bioanal Chem 2006; 385:1520–1525.
5. Ford KA, Gulevich AG, Swenson TL, Casida JE. Neonicotinoid insecticides: oxidative stress in planta and metallo-oxidase inhibition. J Agric Food Chem. 2011; 59: 4860-4867.
6. Imamura T, Yanagawa Y, Nishikawa K, Matsumoto N, Sakamoto T. Two Cases of Acute Poisoning with Acetamiprid in Humans. Clin Toxicol. 2010; 48, 851–53.
7. Zhang JJ, Wang Y, Xiang HY, Li MX, Li WH, Ma KG, Wang XZ, Zhang JH. Oxidative stress: role in acetamiprid-induced ımpairment of the male mice reproductive system. Agric Sci China. 2011; 10: 786-796.
8. Devan RS, Mishra A, Prabu P, Mandal T, Panchapakesan S. Sub-chronic oral toxicity of acetamiprid in wistar rats. Toxıcol Environ Chem. 2015; 97: 1236-1252.
9. Adams J, Vorhees CV, Middaugh LD. Developmental neurotoxicity of anticonvulsants: human and animal evidence on phenytoin. Neurotoxicol Teratol. 1990;1(12):203-14.
10. Khalaf AA, Galal MK, Ibrahim MA, Abd Allah AA, Afify MM, Refaat R. The Terminalia laxiflora modulates the neurotoxicity induced by fipronil in male albino rats. Bioscience Reports. 2019; 39(3): BSR20181363.
11. Mahaman YA, Huang F, Wu M, Wang Y, et al. Moringa Oleifera Alleviates Homocysteine-Induced Alzheimer's Disease-Like Pathology and Cognitive Impairments. Journal of Alzheimer's Disease. 2018; 63(3):1141–1159.
12. Roghini, R. and Vijayalakshmi, K. (2018) PHYTOCHEMICAL SCREENING, quantitative analysis of flavonoids and minerals in ethanolic extract of citrus paradisi. International Journal of Pharmaceutical Sciences and Research Vol. 9(11): 4859-4864.
13. Gul, R., Jan, S.U., Faridullah, S., Sherani,S., and Jahan, N. (2017). Preliminary Phytochemical Screening, Quantitative Analysis of Alkaloids, and Antioxidant Activity of Crude Plant Extracts from Ephedra intermedia Indigenous to Balochistan.The Scientific World Journal,2017:1-7.
14. Weil , C.S. 1952: Tables for convenient calculation of median effective dose (ED50 OR LD50) and instructions in their use. Biometrics, 8: 249.
15. Okhawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction, Anal. Biochem. 1979; 95: 351–358.
16. Beutler ED, Duron O and Kelly MB. Improved method for the determination of blood glutathione. J. Lab and Clin. Med. 1963; 61: 882-888.
17. Aebi H. Catalase in vitro. Methods in Enzymology. 1984; 105:121–126.
18. Marklund S, Marklund G. Involvement of the superoxide anion radical in the autoxidation of

pyrogallol and a convenient assay for superoxide dismutase. Eur J Biochem; 1974; 47:469–74.

19. Livak KJ, Schmittgen TD. Analysis of Relative Gene Expression Data Using Real-Time Quantitative PCR and the $2^{-\Delta\Delta C}{}_{T}$ Method. Methods. 2001; 25(4):402-408.
20. Banchroft JD, Stevens A, Turner DR. Theory and practice of histological techniques. Fourth Ed. 1996, Churchil Livingstone, New York, London, San Francisco, Tokyo.
21. Gaafar AA, Ibrahim EA, Asker MS, Moustafa AF, Salama ZA. Characterization of polyphenols, polysaccharides by HPLC and their antioxidant, antimicrobial and antiinflammatory activities of defatted moringa (Moringa oleifera L.) meal extract. International Journal of Pharmaceutical and Clinical Research. 2016; 8(6): 565-573.
22. Fowoyo P.T. and Oladoja E.O(2017) Phytochemical Screening, Nutritional Composition and Antimicrobial Activity of Moringa Oleifera Seed and Leaf Extract against Selected Gastrointestinal Pathogens. Journal of Pharmacy and Biological Sciences,10(6):114-116.
23. Mossa AH, Refaie AA, Raan A. Effect of exposure to mixture of four organophosphate insecticides at no observed adverse effect level (NOAEL) dose on rat liver: the protective role of vitamin C. Res. J. Environ. Toxicol. 2011; 5, 323–335.
24. Gasmi S, Rouabhi R, Kebieche M, Salmi A, Boussekine S, Toualbia N, Taib C, Henine S, Bouteraa Z and Djabri B Neurotoxicity of Acetamiprid in Male Albino Rats and the Opposite Effect of Quercetin Biotechnol Ind J. 2016;12(7):113.
25. Devan RKS, Mishra A, Prabu PC, Mandal TK, Panchapakesan S. Sub-chronic oral toxicity of acetamiprid in Wistar rats. Toxicol Environ Chem. 2015; 97:1236–1252.
26. Emre YA, Damla GK, Bahar UK, Tuğçe B, Narin O, Alper O, Feriha E, Gül Ö. Reproductive effects of subchronic exposure to acetamiprid in male rats. Scientific RepoRtS. 2020; 10:8985.
27. Sana C, Lobna E, Intissar G, Emna K, Fadoua N, Rakia B, Amira S, Mohamed FN, Mohssen H, Meriem M, Zohra H, Hassen B. Hematological, biochemical, and toxicopathic effects of subchronic acetamiprid toxicity in Wistar rats. Environ Sci Pollut Res. 2016; 23:25191–25199.
28. Ratheesh, G, Tian L, Venugopal JR, Ezhilarasu H, Sadiq A, Fan T. et al. Role of medicinal plants in neurodegenerative diseases. Biomanuf Rev. 2017; 2(2): p1-16.
29. Omotoso, G.O; Gbadamosi, I.T., Olajide,O.J., Dada-Habeeb, S.O., Arogundade,T.T., Yawson, E.O: Moringa oleifera phytochemicals protect the brain against experimental nicotine-induced neurobehavioral disturbances and cerebellar degeneration Pathophysiology Volume 25, Issue 1, March 2018, Pages 57-62.
30. Radi E, Formichi P, Battisti C, Federico A. Apoptosis and oxidative stress in neurodegenerative diseases. J. Alzheimers Dis. 2014; 42: S125–S152.
31. Liang Y, Yan C, Schor NF. Apoptosis in the absence of caspase 3. Oncogene. 2001; 20: 6570 – 6578.
32. Kuwana T, Newmeyer DD. Bcl-2-family proteins and the role of mitochondria in apoptosis. Curr Opin Cell Biol. 2003;15(6):691-9.
33. Wang C, Youle RJ. The role of mitochondria in apoptosis. Annu. Rev. Genet.2009; 43: 95–118.
34. Wang XQ, Li YG, Zhong S, Zhang H, Wang XY, Qi PP. et al. Oxidative injury is involved in fipronil-induced G2/M phase arrest and apoptosis in Spodoptera frugiperda (Sf9) cell line. Pest. Biochem. Physiol. 2013; 105: 122–130.
35. Zhang Y, Chen X, Gueydan C, Han J. Plasma membrane changes during programmed cell deaths. Cell Res. 2018;28(1):9-21.
36. Kobayashi J, Antoccia A, Tauchi H, Matsuura S, Komatsu K. NBS1 and its functional role in the DNA damage response. DNA Repair (Amst). 2004;3(8-9):855-61.
37. Ivkovic B, Kitanovic N, Fa S, Andric N. Effects of acetamiprid on gene-specific DNA methylation

in zebrafish (Danio rerio) embryos. In Serbian Biochemical Society Eighth Conference. 2018; p. 133.

38. KARA M, ÖZTAŞ E, ÖZHANA G. Acetamiprid-induced Cyto- and Genotoxicity in the AR42J Pancreatic Cell LineTurk J Pharm Sci. 2020;17(5):474-479.

39. Dhouib IB, Annabi A, Doghri R, Rejeb I, Dallagi Y, Bdiri Y, Lasram MM, Elgaaied A, Marrakchi R, Fazaa S, Gati A. Neuroprotective effects of curcumin against acetamiprid-induced neurotoxicity and oxidative stress in the developing male rat cerebellum: biochemical, histological, and behavioral changes. Environ Sci Pollut Res Int. 2017; 24(35):27515-27524.

40. Gasmi S, Kebieche M, Rouabhi R, Touahria C, Lahouel A, Lakroun Z, Henine S, Soulimani R. Alteration of membrane integrity and respiratory function of brain mitochondria in the rats chronically exposed to a low dose of acetamiprid. Environ Sci Pollut Res Int. 2017; 24:22258-22264.

41. El-Hawary SS, El-Sofany RH, Abdel-Monem AR, Ashour RS, Sleem AA. Polyphenolics content and biological activity of Plectranthus amboinicus (Lour) Spreng growing in Egypt (Lamiaceae). Pharmacognosy J. 2012; 4 (32):45–54.

42. Khalaf A.A.; Marwa A. Ibrahim; Mona K. Galal; Amr A. Abdallah; Ragaa Mansour; and Mostafa M. Afify (2020): The protective effects of Terminalia laxiflora extract on hepato-nephrotoxicity induced by fipronil in male rats. Environmental Science and Pollution Research. 2020; 27(31):39507-39515.

43. Roy C, Das SK. Role of Moringa oleifera on brain electrical activity in colchicine induced experimental rat model of Alzheimer's disease: possible involvement of antioxidants. Int. J. Curr. Pharm. Res. 2013; 5 (4): 40–45.

44. Khan A, Ali T, Rehman SU, Khan MS, Alam SI, Ikram M, Muhammad T, Saeed K, Badshah H, Kim MO. Neuroprotective effect of quercetin against the detrimental effects of LPS in the adult mouse brain. Front Pharmacol. 2018; 9: 1383.

45. Kou X, Li B, Olayanju JB, Drake JM. Nutraceutical or Pharmacological Potential of Moringa oleifera Lam Nutrients. 2018; 10(3): 343.