Animal Health Research Journal Vol. 7, No. 3, September 2019 pp. 1-14

ISSN: 2356-7767

Protective Effect of Quercetin Against Oxidative Stress, Immunohistochemical and Histopathological Changes Induced by Flumethrin

Amany, M. Salama[*]; Ola, F.A. Talkhan[**]; Marwa, S. Khattab[***] and Fatma, M. Fakhry[*]

[*]Toxicology Unit, Biochemistry Dept., Animal Health Research Institute, Dokki, Egypt.

[**]Chemistry Department- Animal Health Research Institute- Shiben El-Kom, Egypt.

[***]Pathology Department, Faculty of Veterinary Medicine, Cairo University, Giza Square, Giza 12211, Egypt.

Received in 5/8/2019
Accepted in 9/9/2019

Abstract

The objective of the current study is to elucidate subchronic toxic effect of the synthetic pyrethroid, flumethrin, on redox status and histopathological changes in different tissues of male albino rats, as well as immunohistochemical detection of Caspase 3 in brain and testis, and the potential role of the natural polyphenolic flavonoid quercetin to alleviate oxidative stress due to flumethrin toxicity. Forty male albino rats weighed 140-150 gm were allocated into four equal groups: group (1) served as control, group (2) was administered flumethrin (1mg/kg BW), group (3) was orally administered Quercetin (50mg/kg BW) 2hours prior to insecticidal exposure then administered flumethrin (1mg/kg BW), group (4) was administered Quercetin only (50mg/kg BW). All treatments were orally administered 3 times/week for 4 weeks. Our results revealed a significant increase in Malondialdehyde (MDA), significant decrease in Catalase (CAT), Superoxide dismutase (SOD) and reduced glutathione (GSH) in livers and brains of rats treated with flumethrin only. In group administered flumethrin, liver, kidney and testis revealed minor histopathological lesions, however prominent brain lesions including neuronal degeneration and gliosis were observed. These lesions were attenuated by quercetin as evidenced in the group administered quercetin and flumethrin. Caspase 3 was highly expressed in brain and testis of flumethrin treated group, while it was poorly expressed in other groups. In conclusion, quercetin is shown to modulate the oxidative stress and histopathological lesions occurred due to subchronic exposure to flumethrin.

Keywords: *Pyrethroids, Flumethrin, Oxidative stress, MDA, Quercetin, Caspase 3.*

Introduction

Insecticides are widely used in veterinary medicine for controlling ticks, mites and lice. These agents are either chemical or biological derivation which result in killing the insect or preventing it from its behaviours ended with its destruction (**Ware and Whitacre 2004).** The pyrethroids are synthetic broad-spectrum insecticides similar to pyrethrins which are active substances extracted from flower, *Chrysanthemum cinerariaefolium***.** Pyrethroids are grouped into two classes: Type I and Type II according to its toxicological and physical properties. Flumethrin; (*RS*)-α-Cyano-4-fluoro-3-phenoxybenzyl 3-(β,4-dichlorostyryl)-2,2-dimethylcyclopropanecarboxylate; is a synthetic type II pyrethroids, which has cyano group (**Thatheyus and Selvam 2013)** and it is applied topically to cattle, goat and sheep for ectoparasite control. Pyrethroids toxicity to environment, mammals, humans and aquatic animals is well stated. Oxidation product is yielded as a result of pyrethroids reaction with the air. Moreover, pyrethroids type II with cyano group is more toxic than type I as it is incompletely excreted resulting in its bioretention in stomach and skin **(Patel and Patil 2016).** At high doses, neurotoxicity and liver

hypertrophy are the pronounced effects of pyrethroids in acute and sub-acute studies. Also, they are irritating to the eyes and skin with facial skin sensitization. On the other hand, chronic and sub-chronic toxicity revealed growth rate reduction, liver enlargement and an alteration of some liver enzyme activities (**Thatheyus and Selvam 2013)**. Also, nervous system functions and antioxidant enzymes disturbance due to pyrethroid compounds exposure were recorded (**Robea *et al.,* 2017)**. The neurotoxic effect of pyrethroid along its disruption of sodium channels normal functioning and interfering with the nervous impulse transmission, which lead to repetitive nervous influxes or depolarization was studied **(Soderlund, 2012).** Free radicals are produced normally as by-products along the physiological and biochemical processes in the cell beside that, the pollutants exposure and pesticides increase the free radicals overproduction that disturbs cellular redox balance leading to oxidative damage of cellular biomolecules **(Halliwell 2006)**. Oxidative stress induces a cellular redox imbalance which has been found in various cancer cells **(Valko *et al.,* 2006).** Cells are endowed with antioxidant enzymes: catalase, superoxide dismutase (SOD), glutathione-S- transferase (GST) and glutathione peroxidase (GPx) which maintain the redox balance. Non-enzymatic antioxidants as glutathione, vitamin E, vitamin C, polyphenols and natural flavonoids can scavenge these radicals **(Mytilineou *et al.,* 2002 and Uttara *et al.,* 2009).** The major thiol antioxidant is the tripeptide glutathione (GSH); the reduced form. GSH is a multifunctional intracellular non-enzymatic antioxidant. It is considered to be the major thiol-sulphide redox buffer of the cell. GSH is highly abundant in the cytosol, nuclei and mitochondria. The reduced form of glutathione is necessary for DNA repair and expression. GSH scavenges hydroxy radical and singlet oxygen directly, detoxifying hydrogen peroxide and lipid peroxides. Super oxide dismutase (SOD) catalyses the dismutation of superoxide anion($O_2^{\cdot-}$) to oxygen and water, also catalyzes H_2O_2**.** Catalase enzyme promotes the conversion of hydrogen peroxide to water and molecular oxygen **(Valko *et al.,* 2006).** Phenolic compound can regenerate or resynthesise GSH after neutralization of reactive oxygen species (ROS) **(Lakroun *et al.,* 2015).**

Programmed cell death (apoptosis) is mediated by endoproteases known as caspases. Caspases are classified according to their mode of action into either initiator caspases (caspase-8 and -9) or executioner caspases (caspase-3, -6, and -7). Caspase-3 is the mostly activated death protease which is vital in the process of apoptotic chromatin condensation and DNA fragmentation in all examined cell types **(Porter and Jänicke 1999; McIlwain *et al.,* 2015).**

Phenolic compound has the ability to neutralize the ROS which deplete the antioxidant defence **(Lakroun *et al.,* 2015).** Quercetin (3, 3′, 4′, 5, 7-pentahydroxyflavone) is a flavanol belongs to flavonoids under a class of polyphenol; with a neuroprotective effect toward neurotoxic chemicals and neurodegenerative diseases, in addition to its antioxidant effect through its direct antioxidant effect and cellular defence stimulation **(Costa *et al.,* 2016).** Quercetin is found in apples, onion, raspberries, red grapes, citrus fruit, cherries, broccoli and leafy greens. It inhibits angiogenesis through interaction with the Cox-2, lipoxygenase-5-enzymes, EGFR, HER intracellular signalling pathway and NF-KB nuclear transcription protein. Quercetin may enhance the anti-cancer effects of tamoxifen through anti-angiogenesis **(Ma *et al.,* 2004)**. In addition, quercetin able to decrease the number of vacuolated cells and the degree of vacuolation in liver tissue of diabetic rats **(Sirovina *et al.,* 2013)** and attenuates histopathology in a rat model of spinal cord injury **(Jiang *et al.,* 2016).** Quercetin is a natural antioxidant that acts by inhibiting lipid peroxidation through inhibition of xanthine oxidase enzyme, directly scavenging cytotoxic free radicals **(Takizawa *et al.,* 2003)**. It has a potent anti-inflammatory activity as it inhibits inducible nitric oxide synthase expression **(Comalada *et al.,* 2005)**. Therefore, this study aimed to inspect the possible protective effect of Quercetin against oxidative stress induced by flumethrin toxicity in rats.

Material and Methods

Animals

Forty male albino rats with body weight 140-150 g were purchased from Laboratory Animals Breeding Unit, National Research Center, Dokki, Egypt. Rats were maintained under standard hygienic conditions with daily 12 h light/dark cycle and free access to food and water. The rats were accommodated to laboratory conditions for two weeks before beginning the experiment.

Chemicals

Flumethrin1% pour-on solution was obtained as Bayticol from KVP Pharma, GmbH.

Quercetin powder was received as Quercetin hydrate 95% through ACROS Company (Morris, New Jersey, USA) and dissolved in saline in a concentration of 50mg/kg BW according to **(Zaafan *et al.*, 2013).**

Experimental design

Rats were randomly grouped to four groups each with ten rats. Group (1) kept as control and administered saline orally by stomach tube, group (2) received field oral dose of flumethrin (1mg/kg BW), group (3) (flum.+ Quer.) received Quercetin (50mg/kg BW) then flumethrin (1mg/kg BW) with 2 hours apart, group (4) was given Quercetin (50mg/kg BW). All treatments were continued for 28 days and administered 3 times/week orally by stomach tube.

Sampling

All rats were euthanized the day after the end of experiment. Livers and brains were immediately removed and divided into two parts; one part kept at -4 ^{0}C for the biochemical analysis. The other part from brain, liver in addition to kidney, and testis were preserved in 10% neutral formalin buffer for histopathological and/ or immunohistochemical examination.

Biochemical analysis

Brain and liver tissues were perfused with phosphate buffer saline solution pH 7.4 containing 0.16 mg/ml heparin to remove any blood cells and clots. The tissue was then homogenized in 5-10 ml cold buffer per gram tissue and centrifuged at 4000 r.p.m for 15 minutes. After that the supernatant was collected for estimation of catalase according to method described by **Aebi, (1984),** superoxide dismutase (SOD) activities according to **Nishikimi *et al.*, (1972),** reduced glutathione (GSH) according to **Beutler *et al.*, (1963)** and malondialdehyde (MDA) concentrations according to **Satoh (1987)**, All tests were performed using commercial kits obtained through Bio diagnostic company.

Immunohistochemical study

Caspase-3 was immunohistochemically stained in the brain and testis to detect apoptotic cells in different groups using paraffin-embedded tissue sections. All procedures were carried out according to the manufacturer protocol. Deparaffinization and antigen retrieval using enzymatic method were performed followed by the application of the primary antibodies against caspase-3 prepared in rabbits (Abbexa, UK). To detect primary antibody, Horse Raddish Peroxidase (HRP) conjugated anti-rabbit antibodies were used. Diaminobenzidine (DAB) was used as a substrate for colour development. Harris Hematoxylin was used as a counter stain (**Ramos-Vara, 2005)**. Neurons and glia cells and spermatogonia in testis staining brown were considered positive for caspase 3. Using image J software, the area percent of caspase-3 positive cells was measured in three images/rat in all groups.

Histopathological examination

Tissue samples were collected from brain, liver, kidney, and testis of examined rats after PM examination and fixed in 10% neutral formalin buffer. Samples were then processed by paraffin embedding technique **(Suvarna *et al.*, 2012)**. Tissue sections about 3 microns thick were made using microtome (leica 2135, Germany) and stained by haematoxylin and eosin stain. Tissue sections were examined using light microscope and photographs of histopathological lesions were captured using digital camera (Olympus XC30, Tokyo, Japan).

Statistical analysis

Obtained data were interpreted by one –way ANOVA using the SPSS statistical package program, and difference among the individual means were compared using LSD range test **(SPSS, 2007).**

Results

Biochemical analysis

The obtained data hereunder in table (1) and (2) revealed a significant difference between different groups and the flumethrin was able to harm redox status of brain and liver tissues as it induced an elevation of MDA with decrement of catalase, SOD and GSH. On the other hand, Quercetin administration with Flumethrin was able to repair the redox status. Also, a non-significant change between quercetin group and control one was clear.

Table (1). The effect of Quercetin against oxidative status in brain tissue induced by Flumethrin

Parameters / Group	Catalase (Unit/g)	SOD (Unit/g)	GSH (Unit/g)	MDA (n.mol/g)
Control	2.31± 0.03^{b}	80.40±3.06^{bc}	1.76±0.04^{b}	13.73±0.82^{a}
Flumethrin	1.84± 0.11^{a}	60.57±3.69^{a}	1.57±0.03^{a}	18.87±1.14^{b}
Flum. + Quer.	2.21±0.04^{b}	74.20±3.16	1.70±0.04^{b}	15.40±0.77^{a}
Quercetin	2.36±0.06^{b}	91.73±3.96^{c}	1.82±0.04^{b}	12.68±0.67^{a}

Values are expressed as mean ± SE with different alphabetical superscript along column are significantly different at ($p < 0.05$)

Table (2). The effect of Quercetin against oxidative status in liver tissue induced by Flumethrin

Parameters / Group	Catalase (Unit/g)	SOD (Unit/g)	GSH (Unit/g)	MDA (n.mol/g)
Control	2.61± 0.15^{bc}	93.27±5.87^{b}	3.20±0.05^{bc}	9.68±0.66^{ab}
Flumethrin	1.84± 0.06^{a}	65.98±5.71^{a}	2.61±0.07^{a}	15.88±0.95^{c}
Flum. + Quer.	2.32±0.07^{b}	85.97±4.65^{b}	3.06±0.09^{b}	12.23±0.88^{b}
Quercetin	2.80±0.12^{c}	95.10±3.16^{b}	3.40±0.06^{c}	7.99±0.59^{a}

Values are expressed as mean ± SE with different alphabetical superscript along column are significantly different at ($p < 0.05$)

Histopathological findings

The livers in the control group were of normal histological structure (Fig. 1a) whereas in the group treated with flumethrin there was multifocal areas of necrotic hepatocytes which were infiltrated with mononuclear cells (Fig. 1b). In the group treated with flumethrin and quercetin (Fig. 1c) and the group treated with quercetin alone (Fig. 1d), there were no histopathological lesions detected.

The kidneys in the control group demonstrated a normal histological structure (Fig. 1e) whereas it exhibited hypertrophy of glomerular tuft and swelling of the renal tubular epithelium in the group treated with flumethrin (Fig. 1f). The group treated with flumethrin and quercetin (Fig. 1g) was almost comparable to control and group treated with quercetin alone (Fig, 1h).

Histopathological examination of the testis revealed minor changes such as vacuolation of sertoli cells in the group treated with flumethrin compared to control (Fig. 2a, b). In the group treated with flumethrin and quercetin, the vacuolation of Sertoli cells was less prominent (Fig. 2c) compared to the group treated with flumethrin alone. The quercetin treated group exhibited normal histological structure of seminephrous tubules (Fig. 2d).

The brain was the most affected organ in which there was multi focal gliosis in the cerebrum in addition to neuronal degeneration and neurophagia in the group treated with flumethrin compared to control (Fig. 3a, b). The group treated with flumethrin and quercetin exhibited less lesions (Fig. 3c) compared to group treated with flumethrin alone. There were less areas of gliosis and degenerated neurons in this group. The quercetin treated group exhibited normal histological structure of brain (Fig. 3d).

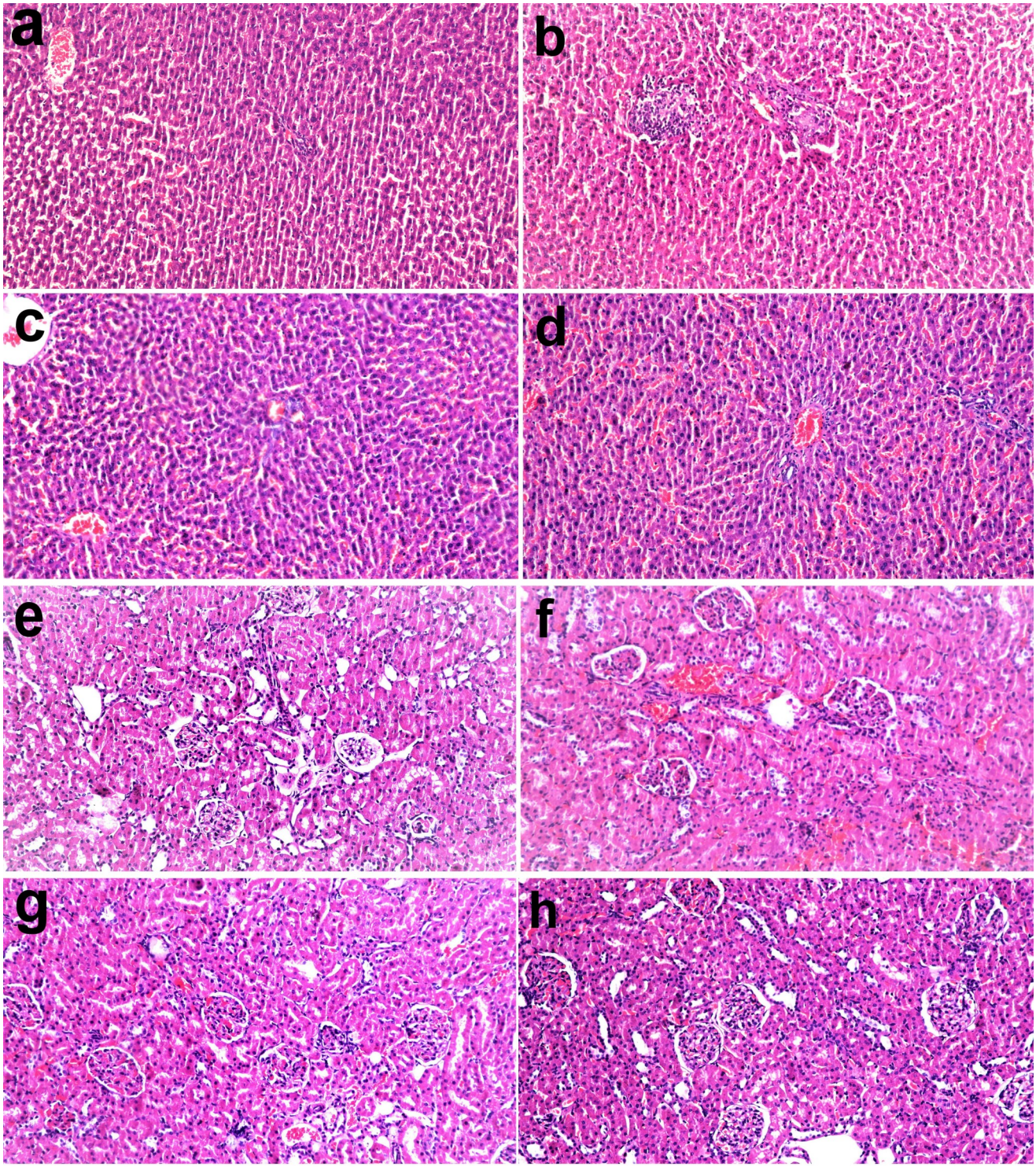

Figure (1). Histopathology of liver and kidney of rats. Liver: (a) Normal histological structure of liver in control group. (b) Focal area of necrosis with mononuclear cell infiltration (arrow) in group treated with flumethrin. (c, d) normal histological structure of liver in both groups treated with quercetin with or without flumethrin. Kidney: (e) Normal histological structure of kidney in control group. (f) hypertrophy of glomerular tuft (arrow) and swelling of renal tubular epithelium in the group treated with flumethrin (g) which was less severe in the group treated with flumethrin and quercetin. (h) Normal histological structure in the group treated with quercetin. Haematoxylin and Eosin stain X 200.

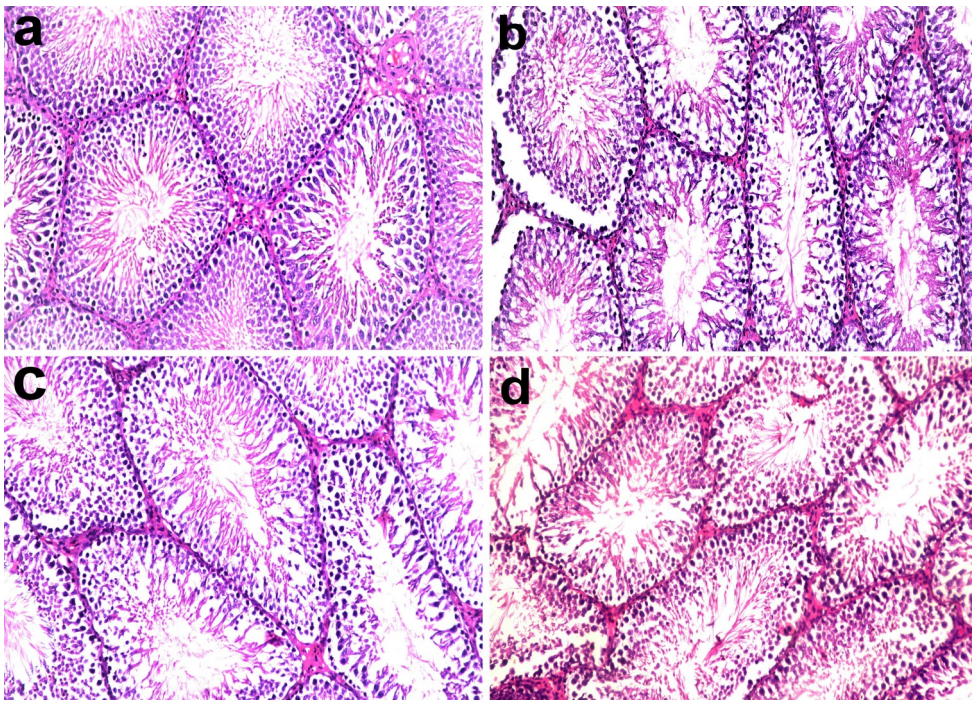

Figure (2). Histopathology of testis of rats (a) normal histological structure of seminepherous tubules in control group. (b) Sertoli cell vacuolation (arrow) in the group treated with flumethrin which (c) was less severe in the group receiving both flumethrin and quercetin. (d) Normal histological structure of seminepherous tubules in the group treated with quercetin only. Haematoxylin and Eosin stain X 200)

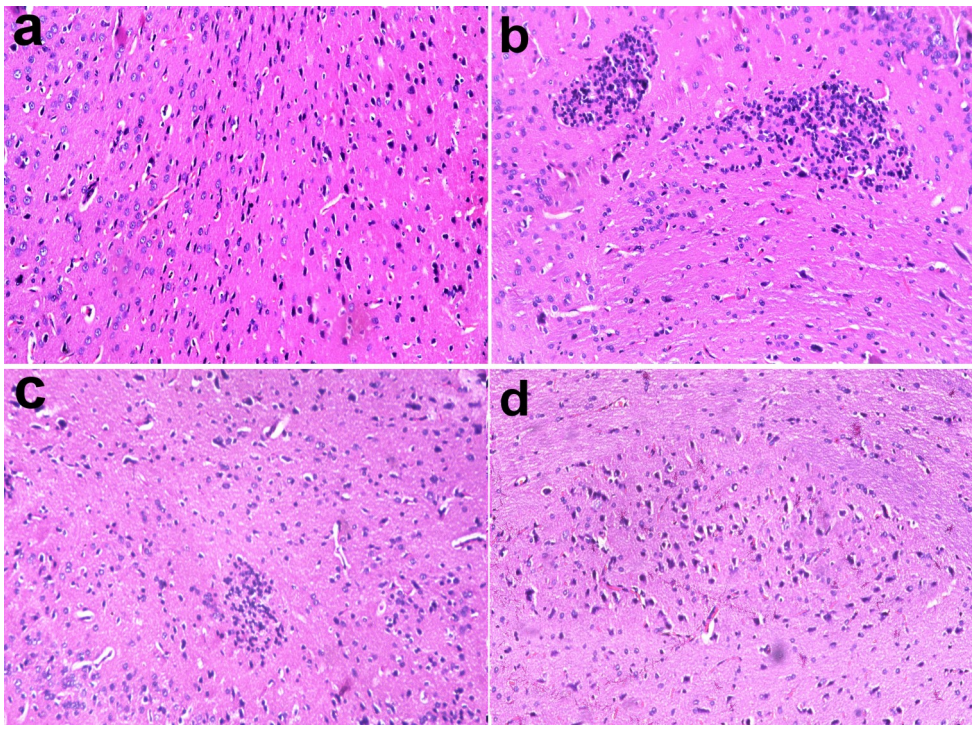

Figure (3). Histopathology of brain of rats. (a) Normal histological structure in the cerebrum of control group. (b) Multifocal areas of gliosis (arrow) in the cerebrum of flumethrin treated group. (c) Minute area of gliosis in the group treated with flumethrin and quercetin. (d) Normal histological structure of brain in the group treated with quercetin.

Immunohistochemical findings

Caspase-3 was found to be expressed in neurons and even more in glia cells. Neurons in the hippocampus region demonstrated a prominent and significant difference between groups in which the caspase-3 was poorly expressed in the control group and group treated with quercetin alone. It was moderately expressed in the group treated with flumethrin whereas it was poorly expressed in the group treated with flumethrin and quercetin (Fig. 4). In the testis, caspase-3 was also expressed in few spermatogonia in the group treated with flumethrin however it recorded no significant difference with the control and other groups (Fig.5, 6).

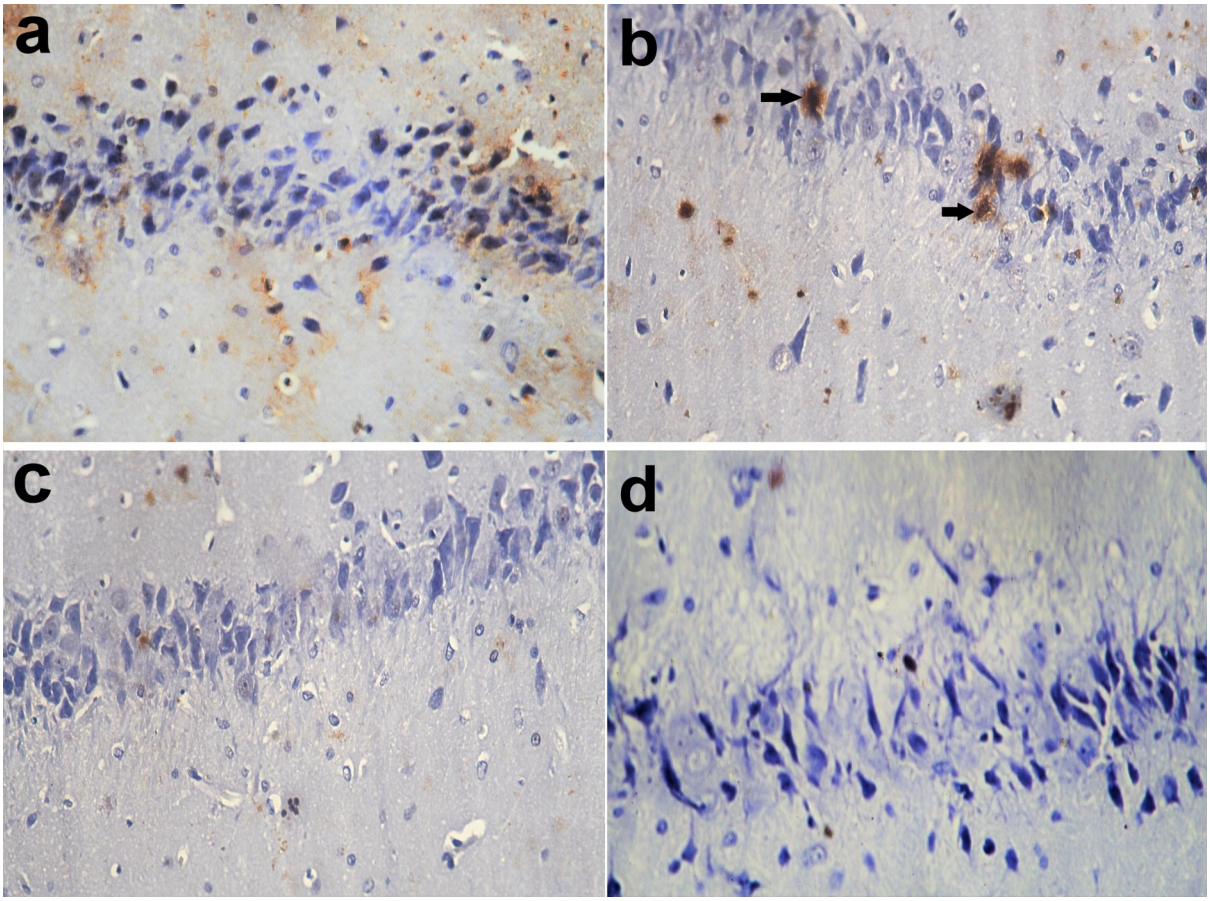

Figure (4). Immunohistochemical staining of caspase-3 in hippocampus region of brain of rats. (a) Negative staining of neurons in brain of control group. (b) Multiple positive brown staining of neurons and glia cells. (c) Few positively stained neurons in group treated with flumethrin and quarecetin. (d) Negative staining of neurons in brain of group treated with quercetin. Immunoperoxidase stain and Haematoxylin counterstain X 400.

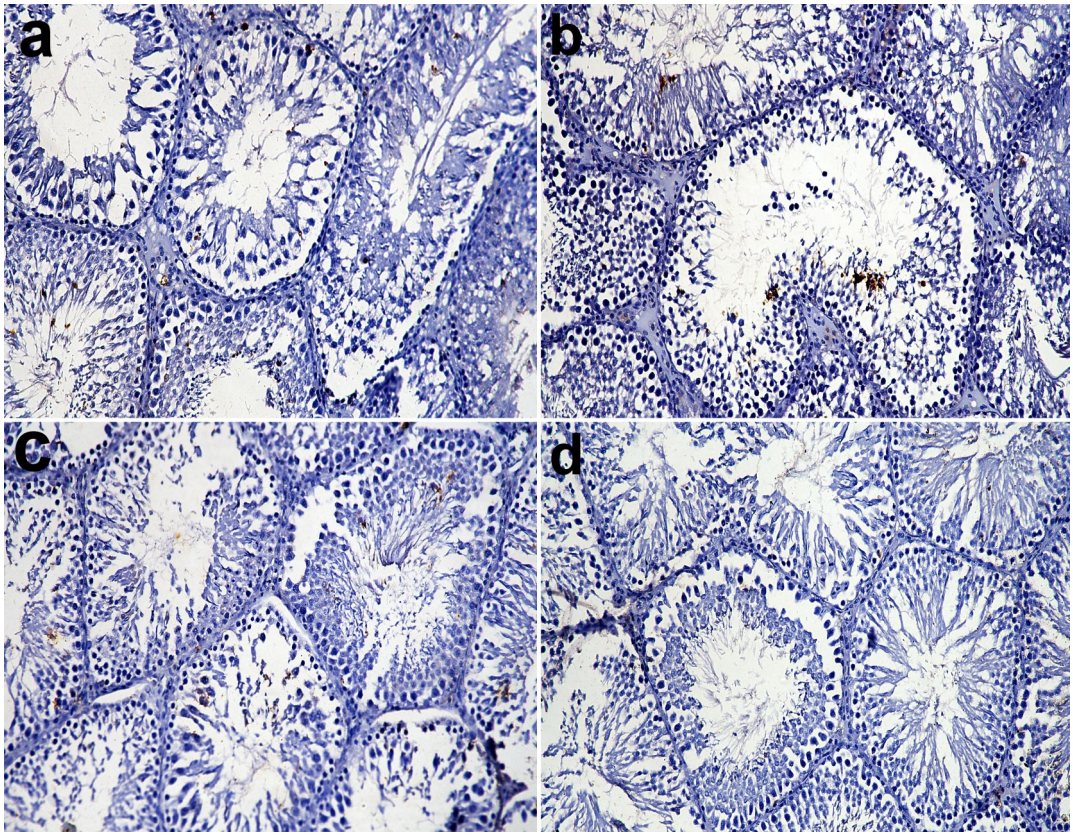

Figure (5). Immunohistochemical staining of caspase-3 in testis of rats. (a) Poorly stained caspase-3 in spermatogonia in control group. (b) Few positively stained cells in seminepherous tubule in group treated with flumethrin. (c) negatively stained cells in flumethrin and quercetin treated group and (d) in quercetin treated group. Immunoperoxidase stain and Haematoxylin counterstain X 200.

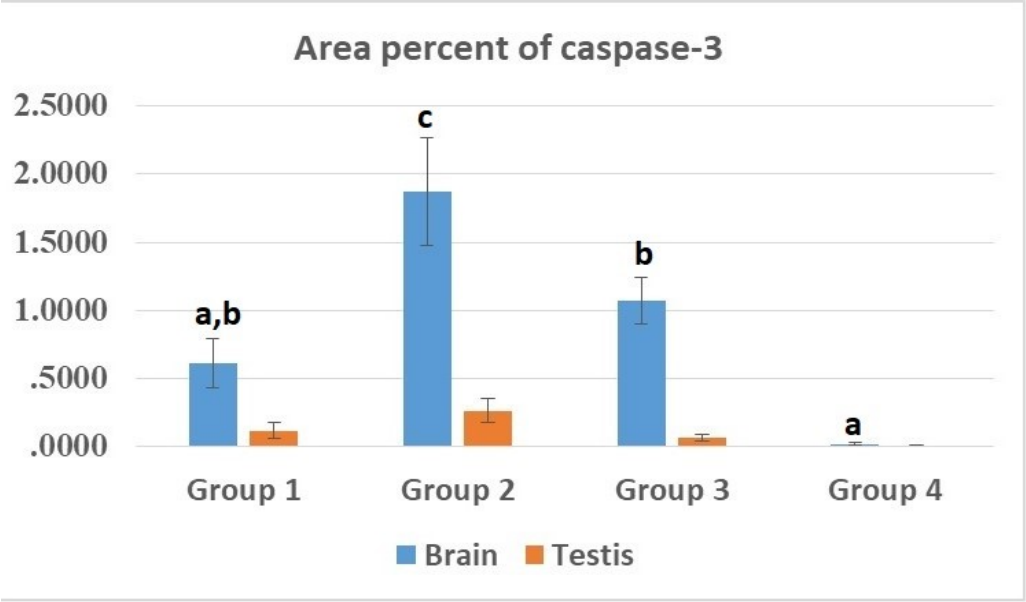

Figure (6). Area percent of caspase-3 positive cells in brain and testis.

Discussion

The present study examined the protective effect of querectin against the toxicity of flumethrin. Here the study assessed that flumethrin disturbs the oxidative status of liver and brain tissues with nerve cell death. Either little or much apoptosis is considered a factor in many conditions including ischemic damage, neurodegenerative diseases, autoimmune disorders and some types of cancer **(Elmore, 2007)**. The disrubtion of oxidative stress occurs as a result of imbalance between the free radical production and the cell scavenger process, which lead to unsteadiness in apoptosis and cell death **(Sun, 1990).** Oxidative stress causes autoimmune or neurodegenerative diseases along with altered cell growth, chronic infections leading to neoplasia, metastatic cancer and angiogenesis **(Valko *et al.,* 2006)**. Oxidative stress acts as precancerous state, leading to the initiation of genetic mutations, genetic errors, epigenetic abnormalities, wrongly coded genome and impaired gene expression **(Babbar and Gerner 2011). Halliwell, (2006)** reported that pollutants exposure and pesticides increase the free radical production. Pyrethroids have adverse effects on nervous system, respiratory and haematological systems. Pyrethroids inhibit microsomal enzymes and inhibit Na, K-ATPases. In the present study, significant elevation of MDA with significant decrease of catalase, SOD and GSH in brain and liver tissues with flumethrin group support that its toxicity includes free radical generation then oxidative damage. This result came in accordance with **Mishra *et al.,* (2012),** who recorded that flumethrin has hepato-toxic effect and oxidative stress in animal. They reported a significant increase of AST, ALT and MDA; which is second toxic messenger increases initial free radical actions and act as signalling mediators, involved in cell signal transduction **(Este-rbauer *et al.,* 1991)**. Moreover, MDA induces an increase in 47 key proinflammatory molecules, its effect can be induced via p38MAPK and protein kinase C pathways **(Raghavan *et al.,* 2012).** MDA can react with DNA bases guanine (G), adenine (A) and cytosine (C) to form adducts M_1G, M_1A and M_1C respectively **(Valko *et al.,* 2006)**. Flumethrin decreases catalase and SOD of rats liver after receiving it for 14 days which indicates damage of liver tissue and necrosis **(Manna *et al.,* 2004).** On the other hand, according to **Floyd and Carney (1992)** the brain tissue has large amounts of polyunsaturated fatty acids and when exposed to free radicals lead to formation of lipids peroxides. Lipid peroxides decompose to form by-product malonaldehyde **(Mishra *et al.,* 2012)**. Flumethrin is lipophilic (**Costa, *et al.,* 2016)** and the increase of flumethrin accumulation in the tissues increases free radical production and disturbs the antioxidant system. Free radicals accumulation involved in damage of macromolecules as lipids and proteins and this is suggested to be the cause of many neurodegenerative diseases **(Huang *et al.,* 2007).** In addition, the decrease of SOD indicates inability of liver tissue to get rid of toxic radicals leading to their accumulation and its cytotoxicity **(Mishra *et al.,* 2012)**. They added that catalase level decreases during oxidative stress causing cytotoxicity. Moreover, GSH is a vital intracellular and extracellular protective antioxidant, which plays important roles in the control of signalling processes, detoxifying certain xenobiotics**.** Also, it considered as one of the most important free radical scavengers in the cell **(Zitka *et al.,* 2012).** Depletion of GSH evokes oxidative stress initiating cell death **(Jaswinder and Christopher, 1997).** It enhances neuronal degeneration **(Franco *et al.,* 2009).** Low levels of brain GSH associated with its oxidation to GSSG occur under oxidative conditions **(Zhu *et al.,* 2006).**

Our histopathological findings revealed that flumethrin results in minor lesions in all organs except in the brain it induced neuronal degeneration and gliosis. Pyrethroids, are lipophilic, which allow them to cross the blood-brain barrier at concentrations considered to be neurotoxic **(Nasuti *et al.,* 2013, Nasuti *et al.,* 2014, Costa, 2015).** Severe gliosis with astrocytes and olingodendrocytes were reported previously in the cerebrum and cerebellum of rats exposed to pyrethroids **(Igho and Afoke, 2014).** The extensive proliferation of glia cells may lead to the formation of glial scar which in turn would hinder the normal neuronal conduction in the nervous system (**Nasuti *et al.,* 1999**). Other organs were minimally affected by flumethrin similar to a previous study that

showed that flumethrin had a minor histopathological lesion in liver of goat exposed to flumethrin **(Dewangan *et al.,* 2012).** Caspase 3 is one of the executioner proteases in the process of apoptosis. The activation of caspase 3 is triggered via an extrinsic pathway involving the activation of death receptor, intrinsic pathway comprising mitochondrial changes and perforin/granzyme pathway. Each pathway trigger its own initiator caspase which at the end will activate the executioner caspase-3 **(Elmore, 2007).** Flumethrin induced nerve cells death and more commonly in the glia cells as evidenced by the increased expression of caspase-3 in the current study. Pyrethroid type II family such as deltamethrin was reported to induce an increase in Bax, p38 MAPK expressions, and caspase-3 activity in isolated splenocytes from mice in addition to decreasing the protein expression of Bcl-2; antiapoptotic gene; **(Kumar *et al.,* 2016).** Also, It was demonstrated that deltamethrin; a pyrethroid type II, induces apoptotic cell death via its interaction with Na^+ channels, causing calcium overload and activation of the endoplasmic reticulum (ER) stress pathway and by the activation of caspase-3 and nuclear condensation. **(Hossain and Richardson, 2011; Ko *et al.,* 2016;).** Endoplasmic reticulum and mitochondria are connected through mitochondria-ER associated membranes with a characteristic set of proteins. These domains does not only transmit Ca2+ but also ROS-mediated signals to the mitochondria after ROS-based ER stress. Therefore when the ROS formation is favoured by redox balance, mitochondrial dysfunction occurs with subsequent cell death **(Marchi *et al.,* 2014).**

Quercetin prevents preneoplastic lesions at normal cells and induce DNA damage to cancer cells, enhances DNA repair mechanism through modulation of DNA enzymes **(Vazhappilly, 2017).** On the other hand, the use of quercetin; a lipid soluble antioxidant; was able to attenuate the histopathological findings of flumethrin in examined organs. Quercetin was reported to have an inhibitory effect on progression of oxidative stress and inflammation through the reduction of NFκB; nuclear transcription factor; and stabilization HIF-1α in lungs of rats under hypoxia **(Tripathi *et al.,* 2019).** Therefore, the decreased brain gliosis and apoptosis observed in brain and testis could attributed to the antioxidant potential of quercetin, also, neuroprotective effects of quercetin were investigated by **(Yao *et al.,* 2010).** In addition, **Costa *et al.,* (2016)** clarified that the neuroprotective effect of quercetin on neurotoxic chemicals and neurodegenerative diseases is by stimulating cellular defences against oxidative stress. Two pathways include induction of Nrf-2-ARE and induction of antioxidant/anti-inflammatory enzyme paraoxonase 2. Quercetin activates sirtuins (SIRT I) to induce autophagy and act as phytoestrogen. All mechanisms provide its neuroprotection. **Sun *et al.,* (2013)** showed that querectin reduces H_2Ax and P53 phosphorylation in human fibrosarcoma cells. In addition, **Yao *et al.,* (2007**) revealed that quercetin protects hepatocytes from ethanol-induced cellular damage through a pathway involving P38, ERK and Nrf2, with the initiation of HO-1 expression. Moreover, Quercetin is potent free radical scavenger **(Boots *et al.,* 2008)** as it can modulates antioxidant defence mechanism of the cell **(Fraga *et al.,* 2010)** by its prooxidant and antioxidant effect **(Halliwell 2013),** protects brain mitochondria from lipid peroxidation and consequently oxidative stress **(Lakroun *et al.,* 2015).** The present study clarify quercetin effect in reducing the brain and the liver oxidative damage by maintaining the levels of Catalase, SOD, GSH and MDA. The result came in accordance with **Farombi *et al.,* (2012)** who reported the ability of quercetin to inhibit cadmium adverse effects on the oxidative status in rats by improving the antioxidant system and protecting its enzymes. Quercetin elevates cellular defences against free radicals as glutathione which prevents the death of brain cell **(Arredondo *et al.,* 2010)** also it is the most effective among other nutrient antioxidants (vitamin E, vitamin C and β-carotene) **(Ferrali *et al.,* 2000).** In addition to its role in the increment of brain expression; protective paraoxonase2 results in scavenging the free radicals and protecting mitochondrial membranes (**Costa *et al.,* 2014).** Also, quercetin acts as Nrf-2 activator and consequently improves GSH synthesis **(Tripathi *et al.,***

2019), which boosts up the oxidative status.

Conclusion

The current study concluded that quercetin with its prooxidant and antioxidant effects can modulate the flumethrin adverse effects. To prevent oxidative stress related cancer, exposure to endogenous and exogenous sources of oxidative stress must be minimised. Daily intake of natural flavonoid polyphenols is recommended as their effects against oxidative stress, beside their roles in genome stability and health benefits.

References

Aebi, H. (1984). Methods Enzymol 105, 121-126.

Arredondo, F.; Echeverry, C. and Abin-Carriquiry, J.A. (2010). After cellular internalization, quercetin causes Nrf2 nuclear translocation, increases glutathione levels, and prevents neuronal death against an oxidative insult. Free Radic Biol Med. 49(5):738 -47.

Babbar, N. and Gerner, E.W. (2011). Targeting polyamines and inflammation for cancer prevention: Recent Results in Cancer Research. 188: 49- 64.

Beutler, E., Duron O., Kelly M.B. (1963). J. Lab Clin. Med. 61, 882.

Boots, A.W.; Haenen, G.R. and Bast, A. (2008). Health effects of quercetin: from antioxidant to nutraceutical. European Journal of Pharmacology, 585: 325–337.

Comalada, M.; Camuesco, D.; Sierra, S.; Ballester, I.; Xaus, J. and Galvez, J. (2005). In vivo quercitrin anti-inflammatory effect involves release of quercetin, which inhibits inflammation through down-regulation of the NF-kappa B pathway. Eur J Immunol; 35: 584–592.

Costa, L.G. (2015). The neurotoxicity of organochlorine and pyrethroid pesticides. Handb. Clinical Neurology, 131: 135-148.

Costa, L.G.; de Laat, R. and Dao, K. (2014). Paraoxonase-2 (PON2) in brain and its potential role in neuroprotection. Neurotoxicology, 43: 3-9.

Costa, L.G.; Garrick, J.M.; Roquè, P.J. and Pellacani, C. (2016). Mechanisms of Neuroprotection by Quercetin: Counteracting Oxidative Stress and More. Oxidative Medicine and Cellular Longevity, http://dx. doi. org/10. 1155/2016/2986796.

Dewangan, G.; Patra, P.H.; Mishra, A.; Singh, A.K.; Datta, B.K.; Sar, T.K.; Chakraborty, A.K. and Mandal, T.K. (2012). Hematobiochemical, immunological, antioxidant status, and residues of flumethrin following weekly dermal application in goats. Toxicological & Environmental Chemistry, 94: 2, 377-387, DOI: 10. 1080/ 02772248.2011.641968.

Elmore, S. (2007). Apoptosis: A Review of Programmed Cell Death Toxicol Pathol.; 35 (4): 495–516.

Esterbauer, H.; Schaur, R.J. and Zollner, H. (1991). Chemistry and biochemistry of 4-hydroxynonenal, malonaldehyde and related aldehydes. Free Radic. Biol. Med. 11, 81–128.

Farombi, E.O.; Adedara, I.A.; Akinrinde, S.A.; Ojo, O.O. and Eboh, A.S. (2012). Protective effects of kolaviron and quercetin on cadmium-induced testicular damage and endocrine pathology in rats. Andrologia, 44: 273–284.

Ferrali, M.; Signorini, C. and Ciccoli, L. (2000). Protection of erythrocytes against oxidative damage and autologous immunoglobulin G (IgG) binding by iron chelator fluor-benzoyl-pyridoxal hydrazone. Biochem Pharmacol 59: 1365–1373.

Floyd, R.A. and Carney, J.M. (1992). Free radical damage to protein and DNA: mechanisms involved and relevant observations on brain undergoing oxidative stress**.** Annals of Neurology, 32: S22-S27.

Fraga, C.G.; Galleano, M.; Verstraeten, S. V. and Oteiza, P.I. (2010). Basic biochemical mechanisms behind the health benefits of polyphenols. Molecular Aspects of Medicine, 31(6): 435–445.

Franco, R.; Sanchez-Olea, R.; Reyes-Reyes, E.M. and Panayiotidis, M.I. (2009). Environmental toxicity, oxidative stress and apoptosis: menage a trois. Mutat Res 674: 3-22.

Halliwell, B. (2006). Oxidative stress and neurodegeneration: where are we now? J Neurochem 97: 1634–1658.

Halliwell, B. (2013). The antioxidant paradox: less paradoxical now? British Journal of Clinical Pharmacology, 75 (3): 637– 644.

Hossain, M.M. and Richardson, J.R. (2011). Mechanism of Pyrethroid Pesticide–Induced Apoptosis: Role of Calpain and the ER Stress Pathway. Toxicological Sciences, 122(2): 512–525.

Huang, Y.L.; Yu, F.Z.; Si, Y.W. and Shi, N. (2007). NF_E2 related factor 2 activation and heme oxygenase-1 induction by tert-butylhydroquinone protect against deltamethrin-mediated oxidative stress in PC12 cells. Chemical Research in Toxicology, 20(9): 1242-1251.

Igho, O.E. and Afoke, I.K. (2014). A histomorphologic analysis of pyrethroid pesticide on the cerebrum and cerebellum of adult albino rats. Journal of Experimental and Clinical Anatomy, 13: 54-9.

Jaswinder, S.B. and Christopher, A.S. (1997). Neurodegenerative disorders in humans: the role of glutathione in oxidative stress-mediated neuronal death. Brain Res Rev 25: 335–358.

Jiang, W.; Huang, Y.; Han, N.; He, F.; Li, M.; Bian, Z.; Liu, J.; Sun, T. and Zhu, L. (2016). Quercetin suppresses NLRP3 inflammasome activation and attenuates histopathology in a rat model of spinal cord injury. Spinal Cord, 54(8): 592-596. doi: 10. 1038/ sc.2015.227. Epub 2016 Jan 12.

Ko, J.; Park, J.H.; Park, Y.S. and Koh, H.C. (2016). PPAR-γ activation attenuates deltamethrin-induced apoptosis by regulating cytosolic PINK1 and inhibiting mitochondrial dysfunction. Toxicology Letters, 260: 8-17.

Kumar, A.; Sasmal, D.; Bhaskar, A.; Mukhopadhyay, K.; Thakur, A. and Sharma, N. (2016). Deltamethrin-induced oxidative stress and mitochondrial caspase-dependent signaling pathways in murine splenocytes. Environmental Toxicology, 31 (7): 808-819**.**

Lakroun, Z.; Kebieche, M.; Lahouel, A.; Zam, D.; Desor, F. and Soulimani, R. (2015). Oxidative stress and brain mitochondria swelling induced by endosulfan and protective role of quercetin in rat. Environ Sci Pollut Res DOI 10.1007/s11356-014-3885-5.

Ma, Z.S.; Huynh, T.H.; Ng, C.P.; Do, P.T.; Nguyen, T.H. and Huynh, H. (2004). Reduction of CWR22 prostate tumor xenograft growth by combined tamoxifen-quercetin treatment is associated with inhibition of angiogenesis and cellular proliferation. International Journal of Oncology, 24(5): 1297-304.

Manna, S.; Bhattacharya, D.; Mandal, T.K. and Das, S. (2004). Repeated dose toxicity of alpha cypermethrin in rats. Indian Journal of Pharmacology, 47 (3): 161 – 164.

Marchi, S.; Patergnani, S. and Pinton, P. (2014). The endoplasmic reticulum-mitochondria connection: One touch, multiple functions. Biochimica et Biophysica Acta—Bioenergetics, 1837(4): 461-469.

McIlwain, D.R.; Berger, T. and Mak, T.W. (2015). Caspase functions in cell death and disease. Cold Spring Harbor Perspectives in Biology, 1: 7(4).

Mishra, A.; Dewangan, G.; Mahajan, V. and Mandal, T.K. (2012). Effect of flumethrin on tissue biochemistry following oral admin-

istration in wistar albino rats. International Journal of Pharma and Bio Sciences, 3(2): 191-200.

Mytilineou, C.; Kramer, B.C. and Yabut, G.A. (2002). Glutathione depletion and oxidative stress. Parkinsonism Related Disorder 8: 385–387.

Nasuti, C.; Carloni, M.; Fedeli, D.; Gabbianelli, R.; Di Stefano, A.; Serafina, C.; Silva, I.; Dominques, V.; Fawcett, J.W. and Asher, R.A. (1999). The glial scar and central nervous system repair. Brain Research Bulletin, 49 (6): 377-91.

Nasuti, C.; Carloni, M.; Fedeli, D.; Gabbianelli, R.; Di Stefano, A.; Cerasa, L.S.; Silva, I.; Domingues, V. and Ciccocioppo, R. (2013). Effects of early life permethrin exposure on spatial working memory and on monoamine levels in different brain areas of pre-senescent rats. Toxicology., 303: 162-168.

Nasuti, C.; Fattoretti, P.; Carloni, M.; Fedeli, D.; Ubaldi, M.; Ciccocioppo, R. and Gabbianelli, R. (2014). Neonatal exposure to permethrin pesticide causes lifelong fear and spatial learning deficits and alters hippocampal morphology of synapses. Journal of Neurodevelopmental Disorder, 6(1): 7.

Nishikimi, M.; Roa, N.A. and Yogi, K. (1972). Biochem. Bioph. Res. Common., 46, 849- 854.

Patel, M. and Patil, P. (2016). Synthetic Pyrethroids: Toxicity and Metabolism. IOSR Journal of Agriculture and Veterinary Science, 9(10): 55-60.

Porter, A.G. and Jänicke, R.U. (1999). Emerging roles of caspase-3 in apoptosis. Cell Death and Differentiation, 6(2): 99-104.

Ramos-Vara, J.A. (2005). Technical Aspects of Immunohistochemistry. Veterinary Pathology 42: 405-426.

Raghavan, S.; Subramaniyam, G. and Shanmugam, N. (2012). Proinflammatory effects of malondialdehyde in lymphocytes. J. Leukoc. Biol. 92: 1055–1067.

Robea, M.; Nicoară, M. Plăvan, G.; Ciobîcă, A. and Strungaru, S. (2017). An overview on toxicological effects of pyrethroid insecticide deltamethrin. Analele Științifice ale Universității,, Alexandru Ioan Cuza" din Iași, s. Biologie animală, 63: 97-104.

Satoh, K. Clinica Chimica Acta (1987). 90, 37.

Sirovina, D.; Orsolić, N.; Koncić, M.Z.; Kovacević, G.; Benković, V. and Gregorović, G. (2013). Quercetin vs chrysin: effect on liver histopathology in diabetic mice. Human and Experimental Toxicology, 32 (10): 1058-66. doi: 10. 1177/ 0960327112472993.

Soderlund, D.M. (2012). Molecular mechanisms of pyrethroid insecticide neurotoxicity: recent advances, Archives of Toxicology, 86 (2): 165-181.

SPSS, (2007). SPSS for Windows. Release, 16.0.1 Standard Version. SPSS INC.

Sun, Y. (1990). Free radicals, antioxidant enzymes, and carcinogenesis. Free Radical Biology and Medicine, 8: 583-599.

Sun, B.; Ross, S.M.; Trask, J.; Carmichael, P.L.; Dent, M. and White, A. (2013). Assessing dose-dependent differences in DNA-damage, P53 response and genotoxicity for quercetin and curcumin. Toxicol. In vitro 27 (6): 1877-1887.

Suvarna, K.S.; Layton, C. and Bancroft, J.D. (2012). Bancroft's Theory and Practice of Histological Techniques, UK: Elsevier Health Sciences.

Takizawa, S.; Fukuyama, N.; Hirabayashi, H.; Kohara S., Kazahari S. and Shinohara, Y. (2003). Quercetin, a natural flavonoid, attenuates vacuolar formation in the op-

tic tract in rat chronic cerebral hypoperfusion model. Brain Res. 980: 156–160.

Thatheyus, A.J. and Selvam, A.D.G. (2013). Synthetic Pyrethroids: Toxicity and Biodegradation. Applied Ecology and Environmental Sciences, 1(3): 33-36.

Tripathi, A.; Kumar, B. and Sagi, S.S.K. (2019). Prophylactic efficacy of Quercetin in ameliorating the hypoxia induced vascular leakage in lungs of rats. PLoS ONE 14(6): e0219075. https://doi.org/10.1371/journal.pone.0219075

Uttara, B.; Singh, A.V.; Zamboni, P. and Mahajan, R.T. (2009). Oxidative stress and neurodegenerative diseases: a review of upstrea3m and downstream antioxidant therapeutic options. Current Neuropharmacology 7: 65–74.

Valko, M.; Rhodes, C.J.; Moncol, J.; Izakovic, M. and Mazur, M. (2006). Free radicals, metals and antioxidants in oxidative stress-induced cancer. Chem. Biol. Interact. 2006 Mar 10;160(1):1-40. Epub 2006 Jan 23.

Vazhappilly, C.G.; Dellaire, G. and Rupasinghe, H.P.V. (2017). Plant flavonoids in cancer chemoprevention: Role in genome stability. J. Nut. Biochem. 45:1-14.

Ware, G.W. and Whitacre, D.M. (2004). The Pesticide Book, 6th Ed. 496 pp. Meister Media Worldwide, Willoughby, Ohio. (ISBN 1892829-11-8).

Yao, P.; Nussler, A.; Liu, L.; Hao, L.; Song, F.; Schirmeier, A. and Nussler, N. (2007). Quercetin protects human hepatocytes from ethanol-derived oxidative stress by inducing heme oxygenase-1 via the MAPK/Nrf2 pathways. J Hepatol 47: 253–261.

Yao, Y.; Han, D.D.; Zhang, T. and Yang, Z. (2010). Quercetin improves cognitive deficits in rats with chronic cerebral ischemia and inhibits voltage-dependent sodium channels in hippocampal CA1 pyramidal neurons. Phytother Res 24: 136–140.

Zaafan, M.A.; Zaki, H.F.; El-Brairy, A.I. and Kenawy, S.A. (2013). Protective effects of atorvastatin and quercetin on isoprenaline-induced myocardial infarction in rats. Bull Fac Pharm 51: 35–41.

Zhu, Y.; Carvey, P.M. and Ling, Z. (2006). Age-related changes in glutathione and glutathione-related enzymes in rat brain. Brain Research, 1090: 35-44.

Zitka, O.; Skalickova, S.; Gumulec, J.; Masarik, M.; Adam, V.; Hubalek, J.; Trnkova, L.; Kruseova, J.; Eckschlager, T. and Kizek, R. (2012). Redox status expressed as GSH:GSSG ratio as a marker for oxidative stress in paediatric tumour patients. Oncology Letters, 4: 1247-1253.