Quercetin ameliorates acetamiprid-induced hepatotoxicity and oxidative stress

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ABSTRACT

Introduction: Neonicotinoids are a new type of insecticides that have been introduced to the poison market during the last three decades. Acetamiprid (ACT) is a neonicotinoid and widely used for controlling pests. It targets the liver as a toxic agent and damages hepatic tissues through oxidative stress mechanisms. Quercetin is a flavonoid with potent antioxidant and hepatoprotective activity and protects tissues from oxidative damages. Thus, this study is aimed to assess the protective effect of quercetin on acetamiprid-induced hepatotoxicity.
Methods: Thirty-six Wistar rats were classified into six groups including control, DMSO, ACT 20, ACT 40, quercetin, and ACT40+quercetin. All treatments were administered orally with gavage for 28 days. Alanine amino transferase (ALT), aspartate amino transferase (AST), alkaline phosphatase (ALP) and lactate dehydrogenase (LDH) enzyme activity was measured in serum as biomarkers of hepatotoxicity. Lipid peroxidation, superoxide dismutase (SOD) enzyme activity and total thiol content were measured in hepatic tissues. Also, hepatic tissue sections were prepared and stained with hematoxylin and eosin and evaluated under optic microscope for any tissue injuries.
Results: Findings showed that ACT, especially in high dose (40mg/kg), induced hepatic tissue destruction associated with increased hepatic enzyme activity, except ALP activity, in the serum. Besides, ACT increased the lipid peroxidation and decreased total thiol content and SOD activity, which indicates ACT-induced oxidative stress in hepatic tissues. Also, hepatic tissue injuries were observed in ACT-treated group. All these changes in liver were prevented by quercetin.
Conclusion: Because of strong antioxidant properties, quercetin can cope effectively with ACT-induced hepatotoxicity.

Introduction

Neonicotinoids have been introduced into the market as a new class of insecticides three decades ago and have been widely used in agriculture, accounting for 10- 15% of the insecticide market worldwide (Tomizawa and Casida, 2005). Acetamiprid is a member of neonicotinoids that was introduced to the insecticide market in 1989. Following exposure to acetamiprid, it

is readily absorbed and is widely distributed into tissues. In addition, it is found with the highest concentration in the liver, adrenal gland and kidney following oral administration to the rats (Authority, 2016). It is initially believed that acetamiprid has low mammalian toxicity, but several studies have recently indicated that it has various toxic effects on the central nerve system, immune system and liver (Chakroun et al., 2016; Dhouib et al., 2017; Shakthi Devan et al., 2015). Sub-chronic exposure to acetamiprid results in changes in serum biochemical parameters, especially hepatic enzymes, which indicates that liver is the main target organ for acetamiprid toxicity (Authority, 2016; Chakroun et al., 2016). Many studies have reported that oxidative stress is the main mechanism of acetamiprid-induced organ toxicity in sub-chronic and chronic exposure. Oxidative stress is the consequence of inequality between free radical production and antioxidant defense. Metabolism of acetamiprid by the hepatic enzymes generates reactive oxygen species (ROS). Also, sub-chronic exposure to acetamiprid decreases the activity of antioxidant enzymes such as superoxide dismutase (SOD), catalase and glutathione peroxidase (GPx) in the liver. The acetamiprid-induced oxidative stress leads to massive oxidative damage in hepatic cells causing hepatic cells dysfunction and death (Wang et al., 2018; Zhang et al., 2011).

Flavonoids are a major class of polyphenolic compounds with a broad spectrum of clinical and biochemical functions (Panche et al., 2016; Samples et al., 2004). Quercetin is the best-known flavonoid which belongs to flavonol subclass widely found in fruits, vegetables, tea and red wine (Alrawaiq and Abdullah, 2014). Several biological properties such as anti-allergic, anti-proliferative, anti-oxidant, anti-inflammatory, neuroprotective and hepatoprotective have been attributed to quercetin (Selvakumar et al., 2012). Quercetin has hepatoprotective properties as indicated by several studies and protects the liver against several drugs, toxicants and chemical compounds-induced injuries by various mechanisms (Pingili et al., 2020). The antioxidant effect of quercetin has a crucial role in its hepatoprotective effect (Miltone prabu et al., 2017). Because of the wide range of hepatoprotective effects of quercetin, it is considered a potential nutraceutical product for the treatment of drug- and toxicant-induced hepatotoxicity. The possible beneficial effects of quercetin on acetamiprid-induced hepatotox-

icity have not been evaluated yet. Since hepatotoxicity caused by acetamiprid is a concern for the health of agricultural and pesticide manufacturing factory workers, we aimed to study the healing effects of quercetin on acetamiprid-induce injuries on hepatic cells.

Materials and methods

Animals

Experiments were carried out on 36 adult male Wistar rats (200±20g), obtained from the Faculty of Medical Sciences, Tarbiat Modares University. Animals were kept in standard conditions including clean plastic cages, controlled temperature of 22±1°C and a 12-h light/dark cycle with free access to *ad libitum* food and water. All experiments carried out on animals were approved by the ethics committee of Tarbiat Modares University (approval ID: IR.MODARES.REC.1397.114)

Chemicals

Quercetin, trichloroacetic acid (TCA), thiobarbituric acid (TBA), and malondialdehyde bis dimethyl acetal were purchased from Sigma-Aldrich. Acetamiprid technical grade was obtained from Golsam Gorgan Chemical Company (Iran). Alanine amino transferase (ALT), aspartate amino transferase (AST), alkaline phosphatase (ALP) and lactate dehydrogenase (LDH) kits were obtained from Pars Azmun (Iran) and SOD kit was purchased from Teb Pazhouhan Razi (Iran).

Treatment

Thirty-six rats were randomly allocated into six groups (6 rats in each group), including control group (without treatment), dimethysolfuxide (DMSO) treated group receiving DMSO as a vehicle (10% solution), acetamiprid treated groups receiving 20mg/kg (ACT20) or 40 mg/kg (ACT40) acetamiprid, quercetin treated group receiving 20mg/kg quercetin, ACT40-Quer treated group receiving 20mg/kg quercetin and 40mg/kg acetamiprid. All treatments were administered orally by gavage needle daily for 28 days. The rats, in ACT40-Quer treated group, were given 40mg/kg acetamiprid 30min after administration of 20mg/kg quercetin. The dose of quercetin was chosen based on previous studies (Ghahremani et al., 2018; Uzun and Kalender, 2013). Also, the doses of acetamiprid were chosen based on reported LD50, and 20 and 40mg/kg are approximately 1/10 and 1/5 LD50 value (Chakroun et al., 2016).

Twenty-four hours after the administration of the last dose, blood sample was collected by heart puncture under the ether anesthesia, then the liver tissue was immediately removed and rinsed with ice-cold normal saline.

Tissue preparation

To prepare liver tissue homogenate (10% w/v), 2g of the liver was homogenized in the phosphate buffer (pH: 7.4, 0.1M) and centrifuged at 3000g for 5min at 4°C. The supernatant was utilized for the estimation of biochemical parameters.

Measurement of serum ALT, AST, ALP and LDH activity

In the serum samples, ALT, AST, ALP and LDH activities were measured by kits according to the manufacturer's instructions (Pars Azmun Co. Iran). According to kit instruction, the activity of ALT, AST, and LDH enzymes was measured based on NADH consumption and reduction of NADH absorbance at 340nm was monitored for 3min by a plate reader (BioTek). The ALP activity was measured on the basis of P-nitrophenol production monitored at 405nm by a plate reader (BioTek).

Measurement of SOD activity

The SOD activity was measured by Teb Pazhouhan Razi kit that measures SOD activity based on Flohe method (Flohe, 1984). Briefly, xanthine-xanthine oxidase system produces the superoxide anion during the conversion of xanthine to uric acid. The superoxide anion causes a reduction in nitroblue tetrazolium to formazan dye, which has an absorbance at 450nm. The amount of enzyme which inhibits 50% of formazan dye production is considered as one unit.

Measurement of lipid peroxidation

Malondialdehyde (MDA) level as a marker of lipid peroxidation was estimated by the Satoh method (Kei, 1978). The TBA reagent consisting of 0.0925% TBA and 3.75% TCA (200µl) was mixed with liver homogenate (100µl) and then incubated at 90°C for 60min. After incubation, the mixture was immediately cooled on ice and was then centrifuged at 1000g for 10min. The optical density of supernatant was measured at 540nm by a plate reader (BioTek).

Total thiol concentration

The Ellman method was adopted for the measurement of total thiol in the liver (Ellman, 1959). The basis of the Ellman method is the reaction between thiols and DTND (as a reagent), and a formation of yellow ion of 5-thio-2-nitrobenzoic acid which has an absorbance at 405nm. Briefly, liver homogenate (20µl) was mixed well with 180µl of reaction buffer containing phosphate buffer (0.3M, pH: 8), DTNB (0.1mM) and sodium citrate (0.01%). After 5min, the absorbance was measured at 405nm by a plate reader (BioTek).

Histological examinations

Small pieces of the liver from three animals of each group were dissected and fixed in 10% formalin. The fixed tissues were dehydrated by immersing in a series of ethanol solution (from 70% until pure), then in xylene for clearing and were finally embedded in paraffin. The paraffin-embedded tissues were sectioned into 5-µm parts which were placed on the slides and stained with hematoxylin and eosin according to standard protocols. After coveslipping, stained slides were assessed for any change in the histological structure under the light microscope.

Statistical analysis

All values were expressed as mean±SEM. The data were analyzed using one-way ANOVA, followed by Tukey's multiple comparison test with GraphPad Prism 8 software. The $P<0.05$ was considered significant.

Results

Hepatic biomarkers in the serum samples

Table 1 shows the results of hepatic enzymes assay in the serum. Treatment of animals with acetamiprid for 28 days significantly increased ALT ($F_{5,30}=20.6$, $P<0.001$), AST ($F_{5,30}=35.06$, $P<0.001$) and LDH activity ($F_{5,30}=64.98$, $P<0.001$) in both 20 and 40mg/kg groups compared with the control. The increase in AST and LDH activity was dose-dependent. In the ACT40+Quer group, no increase in ALT, AST and LDH activity was observed which indicates that co-administration of quercetin with acetamiprid 40mg/kg significantly prevents ALT, AST and LDH activity increment. There was no significant difference in ALP activity between groups ($F_{5,30}=0.17$, $P=0.97$).

TABLE 1: Hepatic enzymes activity in the serum

	ALT activity (U/L)	AST activity (U/L)	LDH activity (U/L)	ALP activity (U/L)
Control	35.78±0.51	82.56±3.017	376.2±44.96	368.0±16.66
DMSO	37.33±0.73	83.30±2.42	415.5±25.8	377.5±29.21
ACT 20	54.25±3.31***	117.4±3.95***	557.4± 13.36***	390.0±24.39
ACT 40	52.50±2.99***	163.8±4.48***	1122±48.33***	364.3±12.84
ACT40+Quer	38.89±0.91+++	101.8±4.43+++	393.9±41.63+++	381.2±23.34
Quer	32.67±1.394	86.10±4.98	420±18.79	364.2±28.93

Data represented as mean±SEM (n=6). ***$P<0.001$ compared to control group, +++$P<0.001$ compared to ACT40 group. ALT: alanine amino transferase, AST: aspartate amino transferase, ALP: alkaline phosphatase, LDH: lactate dehydrogenase, DMSO: dimethysolfuxide, ACT: acetamiprid, Quer: quercetin.
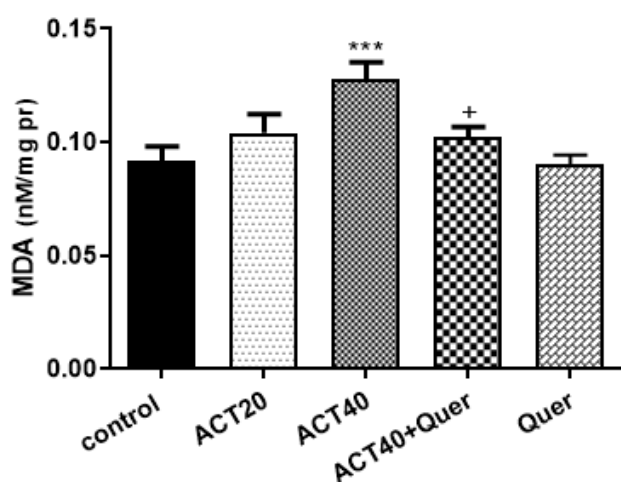
FIGURE 1. Comparison of liver's malondialdehyde (MDA) level as a lipid peroxidation marker between studied groups. Rats received all treatments orally through gavage for 28 consecutive days, then MDA level was measured in liver homogenate. ACT: acetamiprid, Quer: quercetin. Values represent mean±SEM (n=6). ***$P<0.001$ compared to control, +$P<0.05$ compared to ACT40.
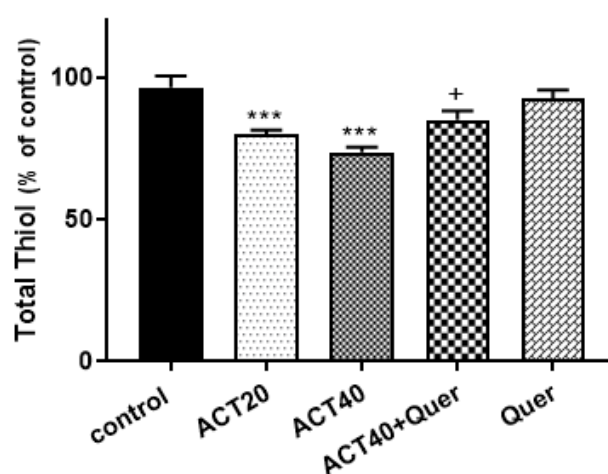
FIGURE 2. Comparison of liver's total thiol content between studied groups. Rats received all treatments orally through gavage for 28 consecutive days, then total thiol content was measured in liver homogenate. ACT: acetamiprid, Quer: quercetin. Values represent mean±SEM (n=6). ***$P<0.001$ compared to control, +$P<0.05$ compared to ACT40.

Oxidative stress biomarkers in the liver

Lipid peroxidation

Figure 1 compares the MDA content of the liver tissue between groups. One-way ANOVA analysis indicated significant differences between groups ($F_{4, 25}= 6.08$, $P<0.001$). The Tukey's multiple comparisons test indicated that the MDA level was significantly elevated in the ACT40 group compared with the control. However, the MDA level was significantly decreased in the ACT40+Quer group compared with the ACT40 group.

Total thiol content

Figure 2 shows the total thiol content of the liver tissue in the studied groups. One-way ANOVA procedure indicated significant differences between groups ($F_{4,25}=$

11.15, $P<0.001$). In acetamiprid-treated groups (ACT20 and ACT40), a significant decrease was observed in the amount of liver total thiol in comparison with the control. Also, in the ACT40+Quer group, no significant difference was observed compared with the control, but there was a significant difference with the ACT40 group.

Superoxide dismutase activity

The results showed significant differences between groups ($F_{3, 20}= 11.34$, $P<0.001$). A significant reduction in the SOD activity was observed in the ACT40 group compared with the control. Notably, a significant difference in the SOD activity between the ACT40-Quer group and control was not observed, whereas the SOD activity in the ACT40-Quer group was significantly increase in compared to the ACT40 group (Figure 3).
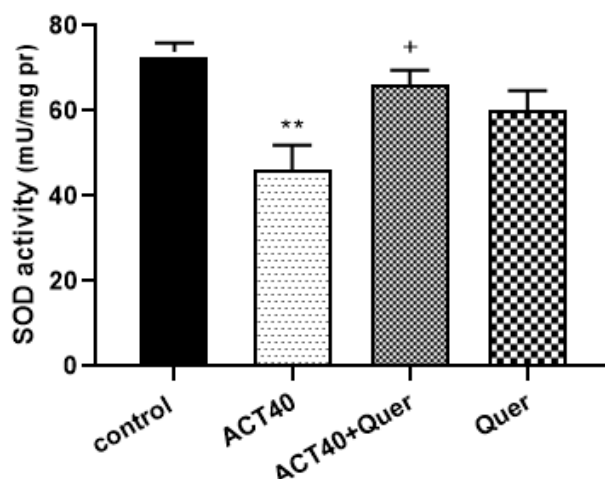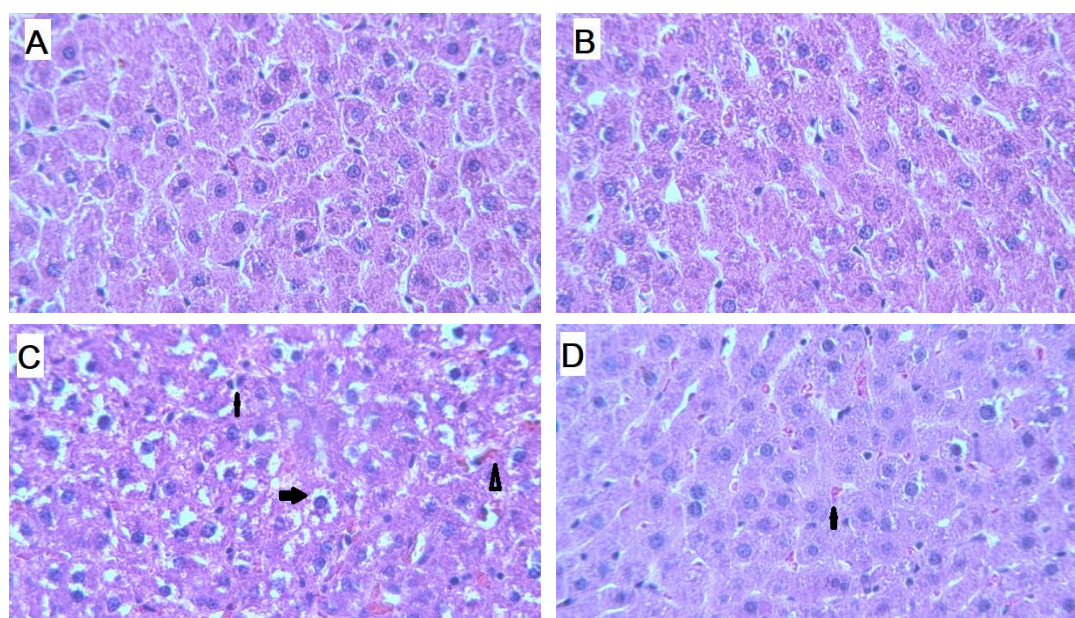
FIGURE 4. Liver section stained with the hematoxylin & eosin (400X). Normal architecture of liver tissue is observed in the control group (A) and quercetin-treated group (B). In the 40mg/kg acetamiprid treated group, vacuolation of hepatocytes (thick arrow), pyknotic nuclei (thin arrow) and congestion (arrow head) are observed (C). Normal architecture of liver tissue with only mild congestion is observed in 40mg/kg acetamiprid + quercetin-treated group (D).

Histological evaluation

As shown in Figure 4, liver sections of rats in the control and quercetin-treated groups have normal architecture (Figures 4A and B), but severe liver injuries as pyknosis, vacuolation of hepatocytes and congestion are observed in the tissue section of the rats in ACT40 group (Figure 4C). However, in the ACT40-Quer group, a normal histologic feature of hepatic tissues was observed with only mild congestion (Figure 4D).

Discussion

Results of our study indicated that sub-acute exposure to acetamiprid, especially at 40mg/kg, caused elevated level of hepatic enzymes in serum and sever histological changes in hepatic tissue, which confirmed severe hepatic injury. The central role of the liver is to metabolize xenobiotics and is therefore highly vulnerable to the damage from toxic xenobiotics (Gulati et al., 2018). Serum levels of the hepatic enzymes are the markers for the assessment of liver integrity and function. Generally, the elevation of serum levels of the hepatic enzymes is an indicator of the loss of liver structural integrity and severe damage to liver cell membranes (Chakroun et al., 2016). The most reliable biomarker for liver injury, especially necrosis, is ALT. Since ALT is predominantly found in the liver, it is a more specific

marker for detecting liver abnormalities (Cohn, 1975). AST and LDH are other enzymes for the assessment of hepatotoxicity. Higher level of AST is observed in liver injury and other organs such as heart, muscles, kidneys and brain (Tennant, 1997). Also, increased level of serum LDH is regarded as a possible marker for hepatotoxicity. Hepatic enzymes are released into the bloodstream following liver cell membrane damage and cell death (Chakroun et al., 2016). Therefore, it is suggested that following 28 days of oral exposure to acetamiprid, its concentration in the hepatic tissue raises so high as to cause cell death and induce hepatotoxicity. This finding is in line with previous studies revealing acetamiprid-induced hepatotoxicity in sub-chronic and chronic exposure (Devan et al., 2015).

Our results indicated elevated lipid peroxidation, oxidation of thiol groups and decrease of SOD activity in hepatic tissues of acetamiprid- treated groups, implying the occurrence of oxidative stress in hepatic cells. Thus, it is indicated that acetamiprid-induced hepatotoxicity was mediated through oxidative stress mechanism. These findings agree with previous studies that have shown oxidative stress in hepatic tissue due to over-production of ROS and disturbance in antioxidant defense system following acetamiprid exposure. The cells have enzymatic and non-enzymatic antioxidant defense mechanism: antioxidant enzyme such as catalase, GPx and SOD play a key role in the enzymatic antioxidant defense and the thiol-containing proteins have the main role in the non-enzymatic antioxidant defense (Verma and Srivastava, 2001). The reduction in antioxidant enzyme activities and the oxidation of thiol groups were reported following exposure to acetamiprid in the rat liver (Chakroun et al., 2016). On the other hand, the metabolism of acetamiprid by hepatic enzymes induces over-production of ROS in the liver that causes oxidative damages such as lipid peroxidation, protein degradation and DNA damage (Selvakumar et al., 2012). Therefore, it is suggested that over-production of ROS due to massive acetamiprid metabolism in the liver results in the depletion of non-enzymatic and enzymatic antioxidant defense, together with massive oxidative damage in hepatic cells that leads to hepatic cell death.

In the present study, we investigated the possible protective effect of quercetin against acetamiprid-induced hepatotoxicity. Our result indicated that the administration of 20mg/kg quercetin prevented

acetamiprid-induced liver injury. Animals that received acetamiprid in combination with quercetin had normal level of hepatic enzymes in serum and mitigated histological changes in hepatic tissues. In addition, we found that quercetin alleviated the acetamiprid-induced oxidative stress; therefore, it is suggested that hepatoprotective effect of quercetin against acetamiprid-induced toxicity is mainly mediated through its antioxidant features. Quercetin is a flavonoid with potent antioxidant and hepatoprotective effects. Previous studies indicated that quercetin protects against drug- and chemicals-induced oxidative damages in hepatic tissues. The antioxidant effect of quercetin is mediated through direct and indirect mechanisms. Quercetin can scavenge free radicals directly, especially in high concentrations (Procházková et al., 2011). Based on the assessment of quercetin distribution in the rat tissues, there was a relatively high concentration of quercetin in the liver (de Boer et al., 2005). Hence, it is assumed that in hepatocytes of animals that received acetamiprid and quercetin together, quercetin has been able to scavenge over-produced ROS due to acetamiprid exposure, protecting them from oxidative damages and death. The indirect antioxidant effect of quercetin is associated with the activation of the cellular antioxidant signaling pathway such as nuclear factor erythroid2-related factor2 (Nrf2) pathway. The Nrf2 is a transcription factor which is activated by quercetin. The activated Nrf2 transfers into the nucleus and up-regulates the expression of antioxidant enzymes such as heme oxygenase-1, glutathione S-transferases, GPx and glutamate-cysteine ligase, hence enhancing cellular antioxidant defense against oxidative insults (Valentová et al., 2014). Another mechanism that can be postulated for the hepatoprotective effect of quercetin in acetamiprid-induced toxicity is that quercetin activates the cellular antioxidant signaling pathway and potentiates the hepatocyte antioxidant defense so that hepatocytes can counteract oxidative stress caused by acetamiprid.

Conclusion

In conclusion, our study showed the hepatoprotective effect of quercetin against acetamiprid-induced hepatotoxicity, which is mediated through attenuation of acetamiprid- induced oxidative stress. Both direct and indirect antioxidant mechanism of quercetin may

be involved in its hepatoprotective effect. Accordingly, quercetin can be considered as a potential nutraceutical product for the prevention of hepatotoxicity in persons who are at high exposure of acetamiprid, such as agricultural and pesticide manufacturing factory workers.

Conflict of interest

The author declares no conflict of interest.

Acknowledgments

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