



[Science Archives \(ISSN:2582-6697\)](https://doi.org/10.47587/SA.2023.4105)

Journal homepage: www.sciencearchives.org



<https://doi.org/10.47587/SA.2023.4105>

Research Article



The effect of resveratrol against sulfoxaflor–induced hepatotoxicity in adult male rats

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Received: Jan 20, 2023/ Revised: Feb 20, 2023/ Accepted: Feb 22, 2023

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Abstract

Resveratrol is a natural compound that exhibits many therapeutic properties including anti-inflammatory, antioxidant, and anticancer effects. Sulfoxaflor is a novel insecticide recently used in agriculture and pest control. The current study is an attempt to assess the vital role of resveratrol against sulfoxaflor-induced toxicity in the liver of adult male albino rats. Sixty adult male rats were randomly divided into six equal groups. In addition to the control group, one group received resveratrol alone (20 mg/kg). Two groups are treated with two selected doses of sulfoxaflor and two additional groups received combined treatment of resveratrol and sulfoxaflor. Treatment continued for four weeks. At the termination of the experiment, liver samples were collected for performing biochemical parameters. Liver function tests (serum ALT, AST, GGT, LDH, and albumin) and total antioxidants were also estimated. Histopathological studies, DNA fragmentation, and comet assay were performed. Our results indicated that, resveratrol reversed the significantly increased activities of antioxidant enzymes, albumin and decreased the levels of MDA, GSSG, NO_x of SFX treated rats. It also ameliorated the serum levels of ALT, AST, GGT, and LDH. Histopathological lesions in liver tissues and DNA damage attributed to SFX administration were enhanced by resveratrol. Thus, resveratrol has been the interest of our study as it protects cells from the toxic effects of SFX and oxidative damage. In conclusion, resveratrol can be used as a therapeutic agent to counteract SFX toxic effects on the liver tissues through the modulation of the antioxidant defense mechanism.

Keywords: Sulfoxaflor, Resveratrol, Hepatotoxicity, Oxidative stress, Rat.

Introduction

Registration of novel insecticides requires comprehensive mammalian toxicity evaluation to understand the severe effects to human health and approved utilizations for several crops. Unfortunately, most insecticides applied in agriculture are unspecified and during their application, they can affect non-target organisms, including humans. The non-judicious use of insecticides has led to increased environmental contamination. Occupational exposure to insecticides repeatedly occurs in some cases such as workers in the pesticide industry and farmers in open fields. In contrast, insecticide residues may be remains on vegetables and fruits in high levels, leading to destructive impact on human health (Cooper & Dobson, 2007) such as headaches and nausea (short-term effects), cancer, hepato-renal toxicity and

reproductive toxicity (long-term impacts) in addition various ecological problems (Bacci et al. 2018).

In comparison to traditional insecticides, Neonicotinoids popularity has been raised greatly over the past few decades. They have a low level of toxicity in mammals. Its insecticidal activity is due to their action on the receptors of nicotinic acetylcholine of insects that induces fast excitatory neurotransmission in the nervous system followed by paralysis and death (Ihara & Matsuda, 2018).

Sulfoxaflor, a new insecticide belongs to sulfoximine family, is considered as a fourth-generation neonicotinoid. It has a high level of effectiveness against a great variety of sap-feeding insects. Moreover, its commercial products are vital chemicals for controlling the predatory arthropod species. Sulfoxaflor traces can accumulate in plant nectar and pollen as

a systemic insecticide resulting in health hazards to both animals and humans (Whitehorn et al. 2012).

Sulfoxaflor binds to insects nAChRs more selective than those of mammals, a feature imparting their improved safety profile. Sulfoxaflor has destructive effects on the mitochondrial membrane transport in hepatic cells, so that it causes perturbation of cytochrome P450 system in human liver, also disturbs the antioxidants and elevates the markers of oxidative stress and free radicals damage the DNA, and protein (Bacci et al. 2018).

Toxicokinetic study in rats indicated that sulfoxaflor is absorbed directly after oral administration with little metabolism. Rats received sulfoxaflor orally for four weeks showed increased liver weight, hepatocellular hypertrophy, permeability of cell membranes, leading to a leakage of liver enzymes into bloodstream and decreased the synthesis of several coagulation factors resulted in a congestion of blood vessels. In addition, long-term administration of 750 ppm of Sulfoxaflor caused tumors of liver in rodents (LeBaron et al. 2014). Moreover, exposure to sulfoxaflor induces lipid peroxidation and oxidative stress as showed in earthworms that is indicated by the increase in the level of hydroxyl radical, malondialdehyde (MDA), and a significant decline in reduced glutathione(GSH) and total antioxidant enzymes (Said et al. 2021). Based on the fact that SFX leads to the generation of reactive oxygen species. The utilization of plant substances that can suppress free radicals and ameliorate the level of antioxidant enzymes can be used to reduce the oxidative stress of SFX.

In particular, resveratrol is an essential polyphenolic compound detected in extensive variety of different types of plants such as grapes, raspberries and peanuts (LeBaron et al. 2013). It has been noticed that resveratrol has health promoting effects in both experimental and clinical studies and has a diverse variety of biological activities involving antioxidant, and antiviral—effects (Dudka et al. 2012 and Benayahoum et al. 2013). Resveratrol declines the generation of free radicals, lipid peroxidation, and also improves the efficiency of antioxidant systems. The potential for resveratrol to act as a vital antioxidant may be attributed to several reasons such as the existence of its hydroxyl group that elevates the electron density at the hydroxyl group and a decrease in the energy of oxygen-hydrogen bond that leads to activating phenolic (Walle et al. 2004). Metabolism of resveratrol is carried out in by certain enzymes in the liver to trans-resveratrol-3-O-glucuronide and trans-resveratrol-3-sulfate (Jalili et al. 2019).

Sulfoxaflor has toxic effects, and resveratrol has beneficial properties. Moreover, no study has ever shed light on the impacts of resveratrol on the sulfoxaflor- caused hepatotoxicity. Hence, our study was accomplished to showing the ameliorative role of resveratrol against the toxicity of sulfoxaflor and also explored the short-term

toxicity that resulted by a daily oral treatment of 1/10 LD₅₀ and 1/20 LD₅₀ of sulfoxaflor on the liver of rats.

Materials and methods

Chemicals

Sulfoxaflor (50%) was bought from Agrimatco Company in Giza; the other ingredients are balance (25.9%) and kaolin (24.1%). Oral administration of kaolin did not have any toxic effects on the organisms (Trckova et al., 2004).

Trans-resveratrol was actually bought from Egyptian pharmacy; each capsule contains 100 mg of resveratrol. Other chemicals that were used were of the analytical grade and were bought by Sigm Chemical Co. (St. Louis, MO, USA).

Dose selection

The dose of RSV was detected based on previous findings that indicated the protective impacts of RSV on hepatic injury (Jalili et al., 2019) The doses of SFX was selected based on the calculation of LD₅₀ according to Miller & Tainter, (1944) LD₅₀ was estimated to be 3755.39 mg/kg.

Experimental groups and animals

A total of sixty adult male *Rattus rattus* rats weighing roughly 130±10 g in weight were purchased from the Egyptian Company for Production of Vaccines, Sera, and Drugs, Giza, Egypt. The animals were preserved in a laboratory at appropriate temperature under standard conditions of ventilation, humidity (60-70 %), with normal photoperiods (12hr dark/light). The animals were given with water *ad libitum* and fed with the appropriate pellet diet formulated that had been developed in accordance with composition authorized by Association of Official Analytical Chemists (A.O.A.C., 1990) After seven days of acclimation, the animals were randomly distributed into six different groups (10 rats / group) as follows:

Group I: Normal control group (C): Each animal was received dist. water daily by oral gavage for four weeks.

Group II: Resveratrol group (RSV): The rats were given RSV (20 mg/kg b.wt /day) by oral gavage for four weeks.

Group III: Low dose of sulfoxaflor (L-SFX): The rats were treated orally with SFX (187.7 mg/kg b.wt /day) dissolved in dist. water for four weeks.

Group IV: High dose of sulfoxaflor (H-SFX): The rats were treated with SFX (375.5mg/kg b.wt /day) and administered as previous.

Group V: (RSV+L-SFX group): The rats were given treatment of resveratrol (20 mg/kg b.wt) and Low dose of SFX (187.7mg/kg b. wt /day) as described in groups II &III.

Group VI: (RSV+H-SFX group): The rats were given treatment of resveratrol (20 mg/kg b.wt) and high dose of SFX (375.5mg/kg b.wt /day) as described in groups II & IV.

Tissue samples' collection

Each rat's liver tissues were removed and stored at -80°C for further analysis after being washed in an icy-cold 0.9% NaCl solution. Three groups of liver tissues were created (A, B, C). Weighing and homogenising group (A) in iced 70% methanol. And also, to remove debris and nuclei, the homogenate was centrifuged at 20,000 rpm for 10 minutes at 4°C. Malondialdehyde (MDA), reduced glutathione (GSH), oxidised glutathione (GSSG), and nitric oxide (NOx) were measured in the tissue homogenates' supernatant.

Group (B) was fixed in 10% phosphate-buffered formalin for histological studies while group (C) was stored at -20°C to analyze DNA fragmentation and comet test.

Hepatobiochemical Analysis

Under ketamine anesthesia, rats were killed at the ending of experimental periods by decapitation, were dissected and liver tissues collected. Blood samples were taken from the venous sinus of the retro-orbital.

Blood samples centrifuged at 3500 r.p.m for 10 minutes then sera were taken and then used for determination of alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma-glutamyl transferase (GGT), lactate dehydrogenase (LDH), and albumin.

Histopathological processing

The liver tissues were then cleared and embedded in soft paraffin after being fixed in 10% buffered formalin, dehydrated in an ascending graded series of ethanol, and clearing. 5- μ m-thick slices were prepared by using a microtome instrument from paraffin-embedded blocks. Sections were mounted on microscope slides. The haematoxylin and eosin were used for staining step. Finally, it assessed by using optical microscopy (Bancroft & Gamble, 2002).

DNA fragmentation assay

DNA fragmentation was done according to the method of Khalaf et al. (Khalaf et al., 2017) with minor modifications. Hepatic tissues were first homogenised, washed in PBS, suspended for 20 minutes on ice in 100 l of lysis buffer (10 mM Tris-HCl, pH 8.0, 100 mM NaCl, 5 mM EDTA, and 5% Triton X-100, 0.25% SDS), and then centrifuged. Tissues were then incubated in 50 g/ml proteinase K for an overnight period at 56 °C. Following that, 100 g/ml of the sample was treated with DNAase-free RNAase A. Then, after being incubated for 2 hours at 37°C, the tissues were extracted for 1 minute using an equal volume of phenol, chloroform, and

isoamyl alcohol (25: 24: 1). They were then centrifuged at 10,000 rpm for ten minutes. Additionally, the aqueous phase was also extracted with chloroform, and centrifuged. Two volumes of cold absolute alcohol involving 0.3 M sodium acetate was utilized to precipitate DNA at 4°C overnight. The precipitate was centrifuged at 10,000 rpm for ten minutes. After being cleaned with 70% alcohol, the DNA pellet was dissolved in TE buffer. They were electrophoresed in 1.5% agarose gel containing ethidium bromide at 80V. A 100-bp DNA ladder (Invitrogen, Waltham, MA, United States) was utilized as a molecular size marker. DNA fragments were seen and photographed using a UV light transilluminator (Stratagene, La Jolla, California, United States).

Single cell gel electrophoresis (Comet assay)

The comet assay was performed under alkaline conditions according to the protocol of Singh et al.¹⁸ with some modifications. To neutralise the slides, cold Tris buffer was used (0.4M, pH 7.5). Komet 5.5, a Kinetic imaging system connected to an Olympus fluorescent microscope, was used to evaluate the slides (CX41). One of the parameters used to assess the degree of nuclear DNA damage was comet tail length (μ m).

Assessment of the serum total antioxidant capacity

Total antioxidant capacity (TAC) was assessed in the serum by utilizing a Colorimetric assay kit (Biodiagnostic-Egypt). It was detected according to the protocol of Koracevic et al. 2001.

Measures of oxidative stress

The utilized apparatus in oxidative stress detection are Agilent HPLC 1260 series, UV detector, and quadrupole pump.

Assessment of MDA by HPLC

Standard preparation: 1, 1, 3, 3-tetraethoxypropane (10 μ l) in 10 ml of 0.1 M hydrochloric acid. The stock solution was heated, then cooling and diluted to 100 ml with water to form working standard. For preparation of samples, 0.1M HClO₄ was added to tissue homogenate samples. The addition of acid is required to precipitate proteins and release the MDA bound. Standard and samples then injected into HPLC by using C18 reversed-phase column (25 cm, 4.6mm, 5 μ m) at ambient temperature. At a wavelength of 254 nm, the mobile phase contained 30 mM of monobasic potassium phosphate (PH 3.6) and methanol in an 82.5:17.5 (v/v) ratio. (Said et al., 2021).

Assessment of GSH and GSSG by HPLC

Glutathione (GSH) and its disulfide (GSSG) were actually bought from Sigma (St. Louis, MO, USA). The determination of reduced and oxidised glutathione in liver homogenate was carried via a mobile phase made of methanol and 0.0025 M sodium phosphate buffer (pH 2.7), a C-18 column, and a UV

detector at 190 nm according to the method of Jayatilleke and Shaw, 1993.

Quantification of nitric oxide by HPLC

Sodium nitrite and sodium nitrate utilized for the reference standard were purchased from Sigma Aldrich. A standard mixture of nitrite and nitrate was utilized to detect the separation of the peaks at 230 nm with a mixture of 0.1 m NaCl–methanol, at a volume ratio 45:55. The concentrations of nitrite and nitrate were equal in the mixture solution; Nitrites and nitrate were assessed according to the method of Papadoyannis et al. 1999.

Statistical analysis

For statistical analysis, one-way analysis of variance (one-way ANOVA) was used, and Tukey's post hoc test was estimated

to assess group differences. The data analysis was done by utilizing SPSS version 25.

Results

Effect of RSV on oxidative stress markers (MDA, GSH, GSSG, and NO)

Oral administration of resveratrol (20 mg/kg b.wt) manifested a non-significant variation in MDA, GSH, GSSG, and NO in liver homogenate in comparison to the control group (Fig. 1). Treatment with SFX at both doses caused a significant ($p < 0.05$) elevation in MDA, GSSG, and NO. Further, administration of SFX showed a significant ($p < 0.05$) decrease in GSH against the control group. Co-administration of RSV and SFX at both doses showed a significant reduction in MDA, GSSG, and NO, but GSH was elevated as compared with SFX treated groups.

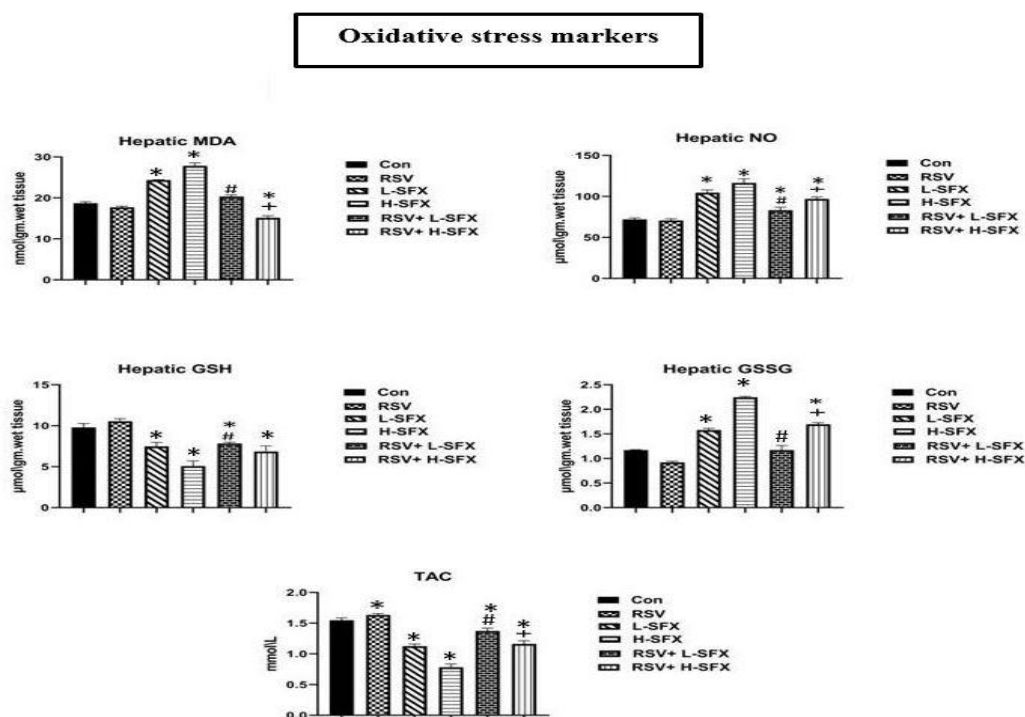


Fig. 1 Influence of Resveratrol (RSV) supplementation on oxidative stress markers (MDA, GSH, GSSG, and NO) and total antioxidant (TAC) of male rats treated with different doses of sulfoxaflo. Data expressed by means ± SEM, (n = 10). Significant difference from control group (*: $p < .05$), significant difference between L-SFX and RSV + L-SFX treated groups (+: $p < .05$), significant difference between H-Sulfx and RSV + H-SFX treated groups (#: $p < .05$).

Effect of RSV on liver enzymes (ALT, AST, GGT, LDH and albumin) and on total antioxidant capacity (TAC) level

Treatment of male albino rats with RSV alone resulted in a non-significant change in ALT, AST, GGT, LDH, and albumin regarding to control group as shown in Fig. 2.

Administration of SFX at both the low and high doses leads to a ($p < 0.05$) elevation in ALT, AST, GGT and LDH and decrease in the level of albumin in comparison to the control group. Moreover, co-administration of RSV and SFX improved liver functions and resulted in a significant ($p < 0.05$) decrease in ALT, AST, GGT and LDH and a significant

($p < 0.05$) increase in albumin level in comparison with SFX treated groups.

Total antioxidant capacity (TAC) was substantially reduced in both of SFX treated groups as compared with the control

group. Both doses of SFX treatment lead to a reduction of TAC in comparison to the normal rats ($p < 0.05$). Treatment with RSV reversed this increase in comparison with the corresponding group of SFX treated rats.

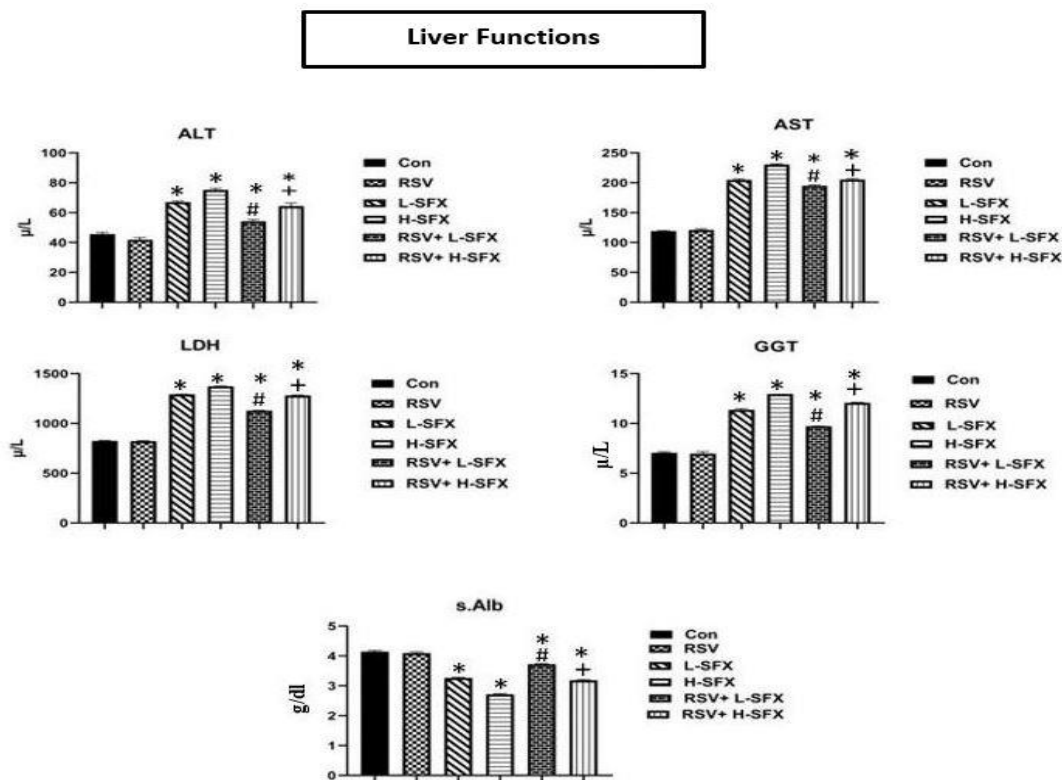


Fig. 2 Effect of Resveratrol(RSV) treatment on liver function (ALT, AST, LDH, GGT, and Alb) of male rats treated with different doses of sulfoxaflo. Data expressed by means \pm SEM, ($n = 10$). Significant difference from control group (*: $p < .05$), significant difference between L-SFX and RSV + L-SFX treated groups (\ddagger : $p < .05$), significant difference between H-Sulfx and RSV + H-SFX treated groups ($\#$: $p < .05$).

Histopathological evaluation

Fig. 3 showing that liver section of control group exhibited normal central vein at the center of lobule, regularly arranged hepatic cords, kupffer cells (K) and normal blood sinusoids (S). RSV group showed also normal central vein, regularly arranged hepatic cords, kupffer cells (K) and normal blood sinusoids (S). Sulfoxaflo low dose (L-SFX) group showed showing damage in hepatic tissue including dilated central vein (CV) surrounded by fibrocytes, necrosis (N), and dilated blood sinusoids (S) filled with hypertrophied kupffer cells (K). Sulfoxaflo high dose (H-SFX) showing congested central vein (CV), hepatocytes with fat vacuoles, necrosis(N), dilated sinusoid (S) with hypertrophied kupffer cells (K). Co-administration of RSV and SFX at both doses reduced the liver damage resulted by SFX toxicity [H&E., X 400].

Impact of RSV on hepatic DNA fragmentation in SFX-treated animals

The hepatic DNA fragmentation patterns of various groups were shown in Fig. 4. As seen in Fig. 4, treatment with RSV did not result in any DNA fragmentation (lane 3), as its banding pattern was similar to the pattern of control group (lane 2) and appeared without a DNA ladder or a smear on the gel. In contrast to the intact banding pattern of the DNA of the control group, treatment with SFX at both the low and high dose caused DNA fragmentation, as evidenced by the appearance of its banding as laddered and smeared DNA fragments (lanes 3 and 4). A very slight smear of DNA demonstrated the combined treatment with RSV and SFX administration at both the low and high doses reduced the DNA damage (lanes 5,6).

Effect of RSV on hepatic DNA damage of liver of SFX-treated animals

Fig. 5 exhibited the effects of SFX, RSV+ SFX treatments on DNA damage (tail length, DNA% tail, % of damage, tail moment and olive tail moment) in rat hepatic tissues. SFX

groups at both doses showed a significant elevation in all tested parameters in comparison with control group.

Otherwise, co-administration of RSV and SFX at both doses showed a significant reduction in all parameters and ameliorated the level of DNA damage resulted by SFX.

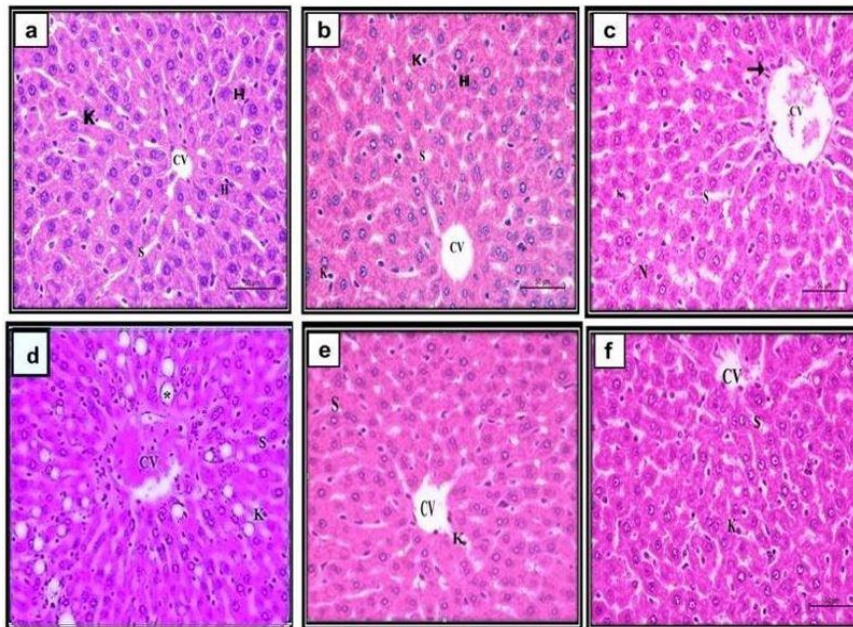


Fig. 3 (A) Effects of Resveratrol on Histopathological cross-section of liver of male rats treated with different doses of sulfoxaflor (H&E X400). (A) Liver of Control and (B) Resveratrol (RSV) treated rats showing normal central vein (CV) surrounded by the hepatocytes (H) are arranged into hepatic strands running radiantly from the central vein and are separated by adjacent sinusoids (S) which contain kupffer (K); (C) Liver of Sulfoxaflor low dose (L-SFX) treated rats showing damage in hepatic tissue including dilated central vein (CV) surrounded by fibrocytes (black arrow), necrosis (N), and dilated blood sinusoids (S) filled with hypertrophied kupffer cells (K); (D) Sulfoxaflor high dose (H- SFX): treated liver showing congested central vein (CV), hepatocytes with fat vacuoles (*), necrosis(N), dilated sinusoid (S) with hypertrophied kupffer cells (K); (E) RSV + L-SFX: treated liver showing nearly more or less normal histological architecture, normal central vein (CV), slightly dilated hepatic sinusoids (S) with kupffer cells (K).; (F) RSV + H-SFX: treated liver showing nearly more or less normal histological architecture, normal central vein (CV), slightly dilated hepatic sinusoids (S) with kupffer cells (K).

Discussion

Neonicotinoids have destructive effects on different organs of rat due to numerous factors such as generation of ROS, alteration in cellular antioxidant defense system. Various reports suggested several toxicity outcomes are resulted from neonicotinoids utilization such as hepatic toxicity, immunotoxicity, cytotoxicity, renal toxicity, and neurotoxicity (Whitehorn et al. 2012). Sulfoxaflor (SFX) is considered an essential member of sulfoximine that is a vital class of insecticides. This class is commonly used in agriculture as it controls a variety of piercing and sucking insects (Mansour & Mossa, 2010). The liver is crucial organ affected by pesticides (Wang et al. 2006).

Oxidative stress is the most commonly indication in numerous studies performed on insecticides. Numerous studies were

done on the other neonicotinoids such as imidacloprid, Clothianidin, and acetamiprid exhibited that oxidative stress played an essential role in their toxic actions. Therefore, oxidative stress is considered as a possible mechanism for different neonicotinoids toxicity, such as SFX. Moreover, MDA is a crucial indicator for the level of lipid peroxidation and essential evidence for tissue damage (Draper & Hadley, 1990).

The recent study showed that daily treatment of SFX at low and high doses for 4 weeks showed hepatotoxic impacts elevated the hepatic MDA, GSSG, and NOx levels while reduced GSH level in a dose-dependent manner. These results are in line with the recent study of Piner Benli et al. 2021 who showed that oral administration of sulfoxaflor at dose 1/50 of LD₅₀ caused alteration in hepatic oxidative stress by inducing lipid peroxidation and reducing in GSH content.

The elevated level of hepatic MDA following SFX administration was ascribed to the extreme production of ROS. Mitochondria have major role in ROS formation (Bergamini et al. 2004 and Wang et al. 2006). The excess production of ROS causes oxidative damage of essential cellular macromolecules such as lipids, proteins, and nucleic acids. Also, the reduction level of TAC, and the antioxidant such as GSH in SFX treated group in the current study resulted from the oxidative stress (Lohiya et al. 2017).

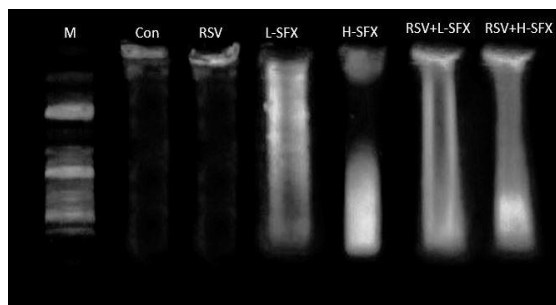


Fig. 4 A representative photo of agarose gel electrophoresis of total genomic DNA extracted from the liver of rats of different experimental groups. Lane M represents DNA molecular weight marker (bp); lane (2): control (Con), lane (3): Resveratrol treated rats (20 mg/kg b. wt., RSV). lane (4); low dose sulfoxaflor treated rats (187.7 mg/kg b. wt., L- SFX); lane (5); high dose sulfoxaflor treated rats (375.5 mg/kg b. wt., H- SFX); lane (6); Resveratrol + low dose sulfoxaflor treated rats (187.7 mg/kg b. wt., L- SFX +RSV); and lane (7) Resveratrol + high dose sulfoxaflor treated rats (375.5 mg/kg b. wt., H-SFX +RSV). The experiment was repeated at least three times.

This is in line with the previous study of Said et al. (2021) reported that male Wister rats were administered sulfoxaflor at doses 1/7th of LD₅₀ and LOAEL for 28 days caused an elevation in testicular oxidative stress parameters (NO, MDA, GSSG) accompanied by reduction in GSH content. Furthermore, NO level in liver tissue increased in fipronil (a systemic insecticide) treated group for 45 days (Mossa et al., 2015).

On the other hand, our results revealed that a noticeable elevation in the liver enzymes (ALT, AST, LDH and GGT) and depletion in albumin level in SFX treated group as compared to control group. Our end findings matched with the results of Piner Benli et al. (2021) who concluded that oral administration of sulfoxaflor at dose 1/50 of LD₅₀ for 7 days caused marked elevation in AST and LDH levels while Shalaby et al. 2010 concluded that injection of 1/10 LD₅₀ of thiamethoxam is combined with an elevation of (ALT, AST, LDH, and GGT) and marked decline in albumin level resulted from damage of cell membranes. Furthermore, our data are agreed with Soujanya et al. 2012 that revealed that the administration of imidacloprid over 4 weeks caused a marked

elevation in ALT and AST activities in rats. High levels of liver enzymes are crucial indicators of liver cell damage.

The decreased levels in serum albumin may be resulted from a change in the formation of protein in the liver. Further, the low level of protein is due to a loss of protein that may be resulted from an increase of proteolytic activity. Albumin level is diminished in the case of liver disorders. In accordance with our results, Zama et al. (2007) and Ambali et al. (2010) showed that the injection of female albino rats with chlorpyrifos (20 mg/kg) resulted in a significant decline in the albumin level.

In our study, histopathological studies of the liver revealed that SFX treatment leads to a notable congestion of central vein, enlargement of sinusoids, necrosis and pyknosis.

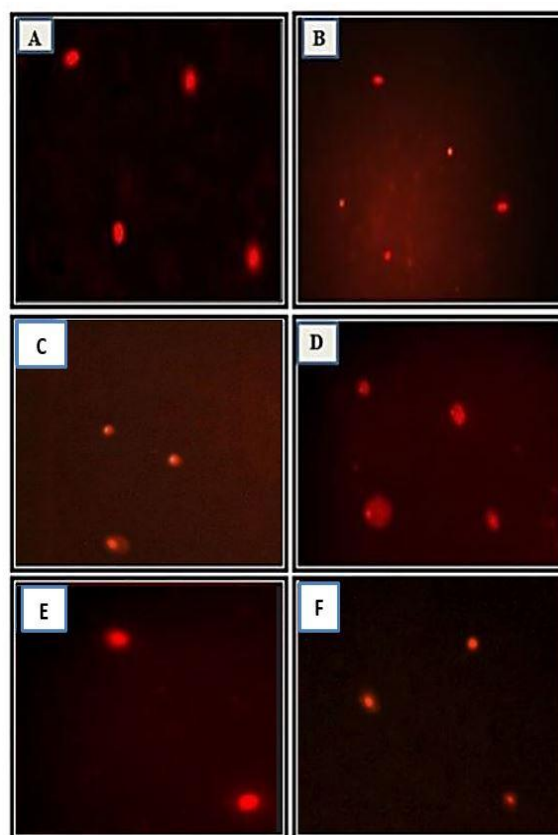


Fig. 5 Photomicrographs representative the different degrees of DNA damage in the liver of rats as evaluated by the comet assay after treatment by SFX and the co-treatment of RSV and SFX. (A) Control group, (B) RSV-treated group, (C) Sulfoxaflor low dose (L-SFX) group, (D) Sulfoxaflor high dose (H- SFX), (E) RSV + L-SFX, (F) RSV + H-SFX.

Similar to our findings, Toor et al. (2013) indicated that histopathological examination of liver tissues of albino rats injected with 1/10 of LD₅₀ (45 mg/kg bw) of imidacloprid

revealed congestion of central vein, enlargement of blood sinusoids and parenchymatous degeneration. Also, [Toś-Luty et al. \(2003\)](#) revealed that injection male wistar rats with different doses of malathion resulted in destructive alterations in the liver such as enlargement of blood sinusoids and necrosis in several of animals. Moreover, [Kaliwal and Ksheerasagar, \(2006\)](#) indicated that the injection of albino mice with carbosulfan for twenty and thirty days resulted in congestion of central vein, necrosis and lymphocytic infiltration.

The recent study showed that the administration of SFX resulted in DNA fragmentation. [Vohra and Khera, \(2015\)](#) & [Elhamalawy et al. \(2022\)](#) showed DNA damage by increasing ROS and MDA in rats after the administration of imidacloprid and in mice after the administration of Thiamethoxam, respectively.

DNA is one of the primary targets of ROS in cells. The released free radicals may cause high levels of DNA damage leading to mutations and apoptosis and eventually cell death. Neonicotinoids cause alterations in the structure of DNA ([Taha et al. 2021](#)).

DNA fragmentation was observed by [Said et al. \(2021\)](#) in male Wister rats after receiving sulfoxaflor at doses 1/7th of LD50 and LOAEL for 28 days.

Comet assay is a sensitive, essential, and vital tool that is used to detect DNA damage. ROS have negative impact on DNA as they react with DNA and damage the DNA backbone besides MDA is considered as a mutagenic compound ([Martinez et al. 2003](#)). The results of comet assay indicated that showed the deleterious effect of SFX on DNA demonstrated by a sharp elevation in tail length, DNA% tail, % of damage, tail moment and olive tail moment. DNA damage causes apoptosis leading to cell death ([Sharma et al. 2009](#)).

In a line with these findings, imidacloprid (neonicotinoid insecticide) caused elevations in the levels of tail length, extent tail moment and % DNA in tail ([Ahmed & Nasr, 2015](#)).

Several plant products are utilized to be crucial factors in reducing the effects of neonicotinoid by squelching free radicals and ameliorating antioxidant activities involving lipoic acid and resveratrol ([Wang et al., 2018](#)). RSV has strong free radical suppressing properties ([Mehri et al. 2020](#)). In our findings, RSV show an effective mitigation impact against SFX toxicity resulted from its antioxidative activity. Treatment with RSV reduces the hepatic MDA, GSSG, and NO_x levels and enhancing GSH levels. Moreover, RSV lowers the hepatic DNA damage. RSV also improves the histological structure of liver.

Similar with our findings, [Mehri et al. \(2020\)](#) showed that resveratrol reduces the oxidative stress by reducing the levels of MDA, GSSG which is associated with an elevation in the level of GSH. In this respect, [Akbel et al. 2018](#) reported that

adjuvant treatment of RSV restores the levels of GSH and MDA to normal levels in tested liver tissue and prevents the oxidation of LDL by scavenging ROS; this explains the decrease in the MDA levels. Also, [Wadsworth & Koop \(1999\)](#) showed that RSV inhibits the efficacy of the iNOS enzyme that declines NO.

[Atmaca et al. \(2014\)](#) indicated that RSV has a curative impact on the liver in adult rats by showing a decrease in the levels of ALT, AST, LDH, GGT and increased albumin as well as TAC after oral treatment.

In our data, RSV co-treatment declined ALT, AST, LDH and GGT levels which confirm its curative effect on liver diseases. our data are in a line with [Akbel et al. \(2018\)](#) who indicated that RSV regulates the activity of ALT and AST.

In the present study, hepatic tissue of the RSV/SFX group showed notable amelioration in its histological structure where the hepatocellular alteration was diminished. It was similar to the that of control rats. RSV improved histological structures of liver. It elevated the number of intact hepatocytes with regular arrangements and decrease size of central veins and blood sinusoids. In accordance with our findings, [Jalili et al. \(2019\)](#) established that resveratrol elevated the number of intact hepatocytes and enhanced liver structure.

The current study revealed that RSV concurrent treatment with SFX showed that RSV reduces the level of DNA fragmentation compared to SFX-treated groups. In this respect, [Aydin et al. \(2016\)](#) suggested that RSV reduces apoptosis in hepatic cells and suppresses DNA damage.

Furthermore, the administration of RSV significantly inhibits the apoptotic index and hepatic DNA fragmentation ([Elmore, 2007](#)).

In the current study, RSV concurrent treatment with SFX protected DNA from damage as showed by decrease in the levels of tail length, DNA% tail, % of damage, tail moment and olive tail moment.

The therapeutic impact of RSV may be due to its capability to squelching free radicals and to preserve cells from oxidative stress ([Uguralp et al. 2005](#)). The previous studies documented that RSV treatment diminishes MDA levels, increases GSH levels and declines oxidative stress parameters ([Jalili et al. 2019](#)).

Our results coincide with that of [Attia, 2012](#) who documented that RSV declines the production of ROS that are major reasons for the DNA fragmentation. who documented that RSV declines the production of ROS that are major reasons for the DNA fragmentation.

Conclusion

According to the results of a recent study, oxidative stress is thought to be the mechanism by which SFX causes hepatic

damage. RSV can protect the liver from the toxicity caused by SFX as it can decrease Ros generation and stop the apoptosis. RSV also greatly reduced the DNA damage and histopathological changes caused by SFX in the liver. As a result, people who are exposed to SFX should consume enough RSV in their diets to reduce the hepatic damage.

Conflict of Interest

The author hereby declares no conflict of interest.

Consent for publication

The author declares that the work has consent for publication.

Ethical Considerations

The study was approved by the institutional ethical committee.

Funding support

The author declares that they have no funding support for this study.

References

A.O.A.C., A. (1990). Official methods of analysis. *Association of the Official Analytical Chemists, 15th Ed. Association of Official Analytical Chemists, Arlington, Virginia.*

Ahmed, M., & Nasr, S. (2015). Protective effect of broccoli and ferulic acid on imidacloprid-induced hepatotoxicity in rat. *The Egyptian Journal of Biochemistry and Molecular Biology, 33*(1–2), 1–15.

Akbel, E., Arslan-Acaroz, D., Demirel, H. H., Kucukurt, I., & Ince, S. (2018). The subchronic exposure to malathion, an organophosphate pesticide, causes lipid peroxidation, oxidative stress, and tissue damage in rats: The protective role of resveratrol. *Toxicology Research, 7*(3), 503–512. <https://doi.org/10.1039/c8tx00030a>

Ambali, S. F., Abubakar, A. T., Shittu, M., Yaqub, L. S., Anafi, S. B., & Abdullahi, A. (2010). Chlorpyrifos-induced alteration of hematological parameters in Wistar rats: ameliorative effect of zinc. *Research Journal of Environmental Toxicology, 4*(2), 55–66.

Atmaca, N., Yıldırım, E., Güner, B., Kabakçı, R., & Bilmen, F. S. (2014). Effect of resveratrol on hematological and biochemical alterations in rats exposed to fluoride. *BioMed Research International, 2014*.

Attia, S. M. (2012). Influence of resveratrol on oxidative damage in genomic DNA and apoptosis induced by cisplatin. *Mutation Research - Genetic Toxicology and Environmental Mutagenesis, 741*(1–2), 22–31. <https://doi.org/10.1016/j.mrgentox.2011.10.008>

Aydin, S., Şahin, T. T., Bacanlı, M., Taner, G., Başaran, A. A., Aydin, M., & Başaran, N. (2016). Resveratrol protects sepsis-induced oxidative DNA damage in liver and kidney of rats. *Balkan Medical Journal, 33*(6), 594–601.

Bacci, L., Convertini, S., & Rossaro, B. (2018). A review of sulfoxaflor, a derivative of biological acting substances as a class of insecticides with a broad range of action against many insect pests. *Journal of Entomological and Acarological Research, 50*(3).

Bancroft, J. D., & Gamble, M. (2002). Theory and practice of histological techniques. 5th London Edinburgh New York Philadelphia St. *Louis Sydney Toronto, 53*, 5143–5147.

Benayahoum, A., Amira-Guebailia, H., & Houache, O. (2013). A DFT method for the study of the antioxidant action mechanism of resveratrol derivatives. *Journal of Molecular Modeling, 19*(6), 2285–2298.

Bergamini, C. M., Gambetti, S., Dondi, A., & Cervellati, C. (2004). Oxygen, reactive oxygen species and tissue damage. *Current Pharmaceutical Design, 10*(14), 1611–1626.

Cooper, J., & Dobson, H. (2007). The benefits of pesticides to mankind and the environment. *Crop Protection, 26*(9), 1337–1348.

Draper, H. H., & Hadley, M. (1990). [43] Malondialdehyde determination as index of lipid Peroxidation. In *Methods in enzymology* (Vol. 186, pp. 421–431). Elsevier.

Dudka, J., Gieroba, R., Korga, A., Burdan, F., Matysiak, W., Jodlowska-Jedrych, B., Mandziuk, S., Korobowicz, E., & Murias, M. (2012). Different effects of resveratrol on dose-related doxorubicin-induced heart and liver toxicity. *Evidence-Based Complementary and Alternative Medicine, 2012*.

Elhamalawy, O. H., Al-Anany, F. S., & El Makawy, A. I. (2022). Thiamethoxam-induced hematological, biochemical, and genetic alterations and the ameliorated effect of *Moringa oleifera* in male mice. *Toxicology Reports.*

Elmore, S. (2007). Apoptosis: a review of programmed cell death. *Toxicologic Pathology, 35*(4), 495–516.

Ihara, M., & Matsuda, K. (2018). Neonicotinoids: molecular mechanisms of action, insights into resistance and impact on pollinators. *Current Opinion in Insect Science, 30*, 86–92.

Jalili, C., Farzaei, M. H., Roshankhah, S., & Salahshoor, M. R. (2019). Resveratrol attenuates malathion-induced liver damage by reducing oxidative stress. *Journal of Laboratory Physicians, 11*(03), 212–219. https://doi.org/10.4103/jlp.jlp_43_19.

Khalaf, A. A., Ibrahim, M. A., Tohamy, A. F., Allah, A. A. A., & Zaki, A. R. (2017). Protective effect of vitazinc on chlorsan induced oxidative stress, genotoxicity and histopathological changes in testicular tissues of male rats. *International Journal of Pharmacology, 13*(1), 22–32.

LeBaron, M. J., Geter, D. R., Rasoulpour, R. J., Gollapudi, B. B., Thomas, J., Murray, J., Kan, H. L., Wood, A. J., Elcombe, C., & Vardy, A. (2013). An integrated approach for prospectively investigating a mode-of-action for rodent liver effects. *Toxicology and Applied Pharmacology, 270*(2), 164–173.

Lebaron, M. J., Gollapudi, B. B., Terry, C., Billington, R., & Rasoulpour, R. J. (2014). Human relevance framework for rodent liver tumors induced by the insecticide sulfoxaflor. *Critical Reviews in Toxicology, 44*(S2), 15–24. <https://doi.org/10.3109/10408444.2014.910751>

Mehri, F., Goodarzi, M. T., & Esfahani, M. (2020). The Possible Protective Effect of Resveratrol on Diazinon-Induced Oxidative Stress and Hepatic Injury. *Avicenna Journal of Medical Biochemistry, 8*(1), 44–48. <https://doi.org/10.34172/ajmb.2020.06>

Miller, L. C., & Tainter, M. (1944). Estimation of the ED50 and its error by means of logarithmic-probit graph paper. *Proceedings of the Society for Experimental Biology and Medicine, 57*(2), 261–264.

Said, N. I., Abd-Elrazek, A. M., & El-dash, H. A. (2021). The protective role of resveratrol against sulfoxaflor-induced toxicity in testis of adult male rats. *Environmental Toxicology, 36*(10), 2105–2115.

Shalaby, S. E. M., Farrag, A. R. H., & El-Saed, G. S. M. (2010). Toxicological potential of thiamethoxam insecticide on albino rats and its residues in some organs. *JASMR, 5*(2), 165–172.

Singh, N. P., McCoy, M. T., Tice, R. R., & Schneider, E. L. (1988). A simple technique for quantitation of low levels of DNA damage in individual cells. *Experimental Cell Research, 175*(1), 184–191.

Soujanya, S., Lakshman, M., Anand Kumar, A., & Reddy, A. (2012). Histopathological and ultrastructural changes induced by imidacloprid in brain and protective role of vitamin C in rats. *J Chem Pharma Res, 4*(9), 4307–4318.

Toor, H. K., Sangha, G. K., & Khera, K. S. (2013). Imidacloprid induced histological and biochemical alterations in liver of female albino rats. *Pesticide Biochemistry and Physiology, 105*(1), 1–4. <https://doi.org/10.1016/j.pestbp.2012.10.001>

Toś-Luty, S., Obuchowska-Przebirowska, D., Latuszyńska, J., Tokarska-Rodak, M., & Haratym-Maj, A. (2003). Dermal and oral toxicity of malathion in rats. *Ann Agric Environ Med, 10*, 101–106.

Uguralp, S., Mizrak, B., & Karabulut, A. B. (2005). Resveratrol reduces ischemia reperfusion injury after experimental testicular torsion. *European Journal of Pediatric Surgery, 15*(02), 114–119.

Vohra, P., & Khera, K. S. (2015). Alterations in key enzymes and micromorphology of vital organs during exposure of imidacloprid in albino rats. *Int J, 3*, 134–144.

Wadsworth, T. L., & Koop, D. R. (1999). Effects of the wine polyphenolics quercetin and resveratrol on pro-inflammatory cytokine expression in RAW 264.7 macrophages. *Biochemical Pharmacology, 57*(8), 941–949.

- Walle, T., Hsieh, F., DeLegge, M. H., Oatis, J. E., & Walle, U. K. (2004). High absorption but very low bioavailability of oral resveratrol in humans. *Drug Metabolism and Disposition*, 32(12), 1377–1382.
- Wang, X., Anadón, A., Wu, Q., Qiao, F., Ares, I., Martínez-Larrañaga, M.-R., Yuan, Z., & Martínez, M.-A. (2018). Mechanism of neonicotinoid toxicity: impact on oxidative stress and metabolism. *Annual Review of Pharmacology and Toxicology*, 58, 471–507.
- Wang, Y., Peng, S., Zhou, Q., Wang, M., Han, G., Yan, C., Yang, H., & Wang, G. (2006). Role of mitochondria in cellular oxidative injuries induced by butenolide. *CHINESE JOURNAL OF PHARMACOLOGY AND TOXICOLOGY*, 20(6), 484.
- Whitehorn, P. R., O'connor, S., Wackers, F. L., & Goulson, D. (2012). Neonicotinoid pesticide reduces bumble bee colony growth and queen production. *Science*, 336(6079), 351–352.
- Zama, D., Meraihi, Z., Tebibel, S., Benayssa, W., Benayache, F., Benayache, S., & Vlietinck, A. J. (2007). Chlorpyrifos-induced oxidative stress and tissue damage in the liver, kidney, brain and fetus in pregnant rats: The protective role of the butanolic extract of *Paronychia argentea* L. *Indian Journal of Pharmacology*, 39(3), 145.

How to cite this article

Said, A. A., Said, N. I. and Kamel, H. M. (2023). The effect of resveratrol against sulfoxaflo- induced hepatotoxicity in adult male rats. *Science Archives*, Vol. 4(1), 24-33. <https://doi.org/10.47587/SA.2023.4105>

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