



Research Article

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Hepatoprotective Effect of Alpha lipoic Acid versus Intoxication with Imidacloprid Widely Used in KSA in Albino Rats

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ABSTRACT

Imidacloprid is a tobacco related insecticide. It may cause toxicity to rodents and plants pests and other creatures that can pose problems for agriculture. Lipoic acid acts as a potent antioxidant scavenging free radicals. The study aimed to assess biochemical and antioxidant alterations associated with hepatotoxicity. Various biochemical parameters, antioxidant status and comet as well as histopathological examinations were employed to evaluate the protective effect of lipoic acid. Sixty male albino rats were divided into six groups of 10 rats/ group. One group as control, and the other five served as experimental groups passed the treatment for 30 days. The study revealed that exposure to imidacloprid caused a significant alteration in serum biochemical parameters, antioxidant enzymes indicating liver toxicity. Liver DNA damage was indicated as significant changes in comet assay; histopathological degeneration in liver tissue architecture confirmed the above findings. However, lipoic acid supplementation with imidacloprid to rats, manifested significant protective effects. Lipoic acid may have therapeutic potential for use in the prevention of liver injury.

Keywords: *Imidaclopride Toxicity, Alpha Lipoic Acid, Antioxidant Enzymes, Liver Enzymes.*

INTRODUCTION

Poisoning due to pesticides has been considered as important public health problem in all over the world [1]. Imidaclopride neonicotinoids are highly toxic insecticides used in crop protection. Insecticides within this class include clothianidine, imidacloprid, acetamiprid and thiacloprid [2]. Imidacloprid (1-(6-chloro-3-pyridinyl) methyl -N-nitro-2-imidazolidinimine) is a relatively stable chloronicotinic insecticide, classified as Category I due to its high leaching potential [3]. Its mode of action is the inhibition of nicotinic receptors of acetylcholinesterase [2]. Imidacloprid has been commonly used as neonicotinoid insecticides in Saudi Arabia kingdom which has been considered as moderately hazardous according to [4]. Clinical effects such as hypertension tachycardia, nausea and vomiting may occur, on the other hand, seizures, respiratory failure and even death have been reported. Chronic exposure to imidaclopride induce adverse effects included increased cholesterol levels in the blood, and some stress

to the liver (measured by elevated liver cytochrome p-450 levels [5]. Lipoic acid ALA is an organic sulfur compound acting as cofactor in enzyme complexes. Although, it is covalently bound in nature but cannot be obtained from dietary sources. Studies have generally been concerned with the association between lipoic acid administration and oxidative stress [6]. ALA plays a fundamental role in the metabolisms such as affecting cellular metabolic processes, altering redox status of cells, and interacting with thiols. It affects energy production and it is considered as essential cofactor for mitochondrial respiratory enzymes [7].

This study has been designed to evaluate the serum biochemical, tissue antioxidant, DNA alterations as well as histopathological examination induced by imidacloprid in the liver of rats, and assess the role of lipoic acid in case of imidacloprid-induced toxicity.

MATERIALS AND METHODS

Chemicals:

Pesticide used:

Commercial products of Imidacloprid (20% EC Confidor) with chemical name of 1-(6-chloro-3-pyridylmethyl)-N-nitroimidazolidin-2-ylideneamine, were purchased from the agrochemical market in KSA.

Antioxidant used:

α -Lipoic acid (thiotic acid) was obtained from Sigma Co. (USA).

Animals

Adult male albino rats weighing (120-150) and being 3 months old were allotted from animal house of King Fahad Medical Research Center, Jeddah, King Abdulaziz University. The experimental animals were treated according to the guidelines protocol of Science College Committee, King Abdulaziz University. The animals were kept in polypropyl cages, fed ad libitum, and adjusted for two weeks before starting the experiment. The rats were housed at $23\pm 2^\circ\text{C}$ and 12 h dark/light cycles. All animals were treated according to the standard procedures [8].

Study design:

The rats were divided into 6 groups (10 rats/ each) and were treated as follows:

G1: non treated animals served as control group.

G2: were treated orally with α -Lipoic acid (60 mg/kg body weight) daily for 30 days [9]

G3: were treated orally with imidacloprid pesticide (1/40 LD50 =11.5mg/Kg) of imidacloprid, [10] daily for 30 days.

G4: were treated orally with imidacloprid pesticide (1/10 LD50=45 mg/Kg) of imidacloprid, [10] daily for 30 days.

G5: were treated orally with (1/40 LD50 of imidacloprid and co-administered with α -Lipoic acid (60 mg/Kg) daily for 4 weeks.

G6: were treated orally with (1/10 LD50 imidacloprid and orally co-administered with α -Lipoic acid (60 mg/Kg), daily for 30 days.

Sampling

At the end of the experimental period (30 days), all animals were sacrificed, the blood samples were collected from hepatic portal vein. After blood clotting, serum was separated by centrifugation at 3000 r.p.m. for 10 min at 4°C , and was kept for biochemical analysis. Livers were removed, washed with saline solution, dried and weighed, then kept for determination of DNA damage in liver cells by comet assay, biochemical estimations and enzyme activities.

Tissue preparation

Liver was removed from rats at the end of the experiment (28days) under ether anesthesia, and washed with cold saline buffer. Washed tissues were immediately stored at -80°C . For determination of enzymatic activities, the tissues were homogenized in ice cold 50mM sodium phosphate buffer (pH: 7) containing 0.1mM ethylenediaminetetraacetic acid (EDTA) yielding 10% (W/V) homogenate. The homogenates were centrifuged at 12,000g for 30 min at 4°C . The supernatant was used for the investigation of enzymes' activities.

Biochemical assay

Biomarkers of the oxidative stress were measured in liver homogenate. Lipid peroxidation was estimated as the concentration of thiobarbituric acid reactive products (malondialdehyde), protein carbonyl content, superoxide dismutase (SOD), catalase (CAT), and glutathione-s-transferase (GST) were measured by commercial diagnostic kits. AST and ALT Total protein, alkaline phosphatase (ALP) markers were determined using the commercial diagnostic kits.

Histopathological examination of liver tissues:

Liver tissue specimens were fixed in 10% formaline saline, then trimmed off, washed and dehydrated in ascending grades of alcohol. The dehydrated specimens were then cleared in xylene, embedded in paraffin blocks and sectioned at 4-6 μm thick. The obtained tissue sections were deparaffinized using xylol, and stained using hematoxylin and eosin (H&E) for histopathological examination through the electric light microscope [11]. The frequency and severity of lesions in the livers were assessed semi-quantitatively [12] by using a scale where, in grade 0: No apparent injury, grade I: Swelling of hepatocytes, grade II: Ballooning of hepatocytes, grade III: Lipid droplets in hepatocytes and grade IV: Necrosis of hepatocytes.

Comet assay

The comet assay is a technique that used for measuring and analyzing DNA breakage in cells. Unwinding of DNA under alkaline conditions, was used in this study. The markers of this assay were tail length measured from the middle of the head to the end of the tail, tail moment and the relative DNA content in the tail [12].

Extraction of RNA

RNA was extracted according to the method of [13] using RNeasy Mini Kit instructions (Catalogue no.74104)

cDNA Synthesis

Total RNA was digested with Deoxyribonuclease I (RNase-free DNase I, Fermentase, ThermoScientific - USA) to avoid genomic contamination. Subsequently, 1 μl DNase I-treated RNA was reversely transcribed in 20 μl reaction's mixture using (RevertAid Reverse Transcriptase ThermoFisher, (Catalog number: EP0441).

Real-Time RT-PCR (RT-PCR):

All PCR experiments were performed on the (Eppendorf, Realplex instrument, Germany) for the signal detection and analysis. Gene-specific primers were designed based on the gene sequences of *Rattus norvegicus* present on the NCBI homepage (<http://www.ncbi.nlm.nih.gov>). Highly purified salt-free primers for p21 and housekeeping genes were obtained from (Metabion, Germany). Expression was normalized to β -actin gene, which was used as an internal housekeeping control. The sequences of the primers were illustrated in Table (1). Real-time quantification was performed in the Stratagene MX3005P instrument using the Quantitect SYBR green PCR kit (Cat. No. 204141). The reactions were performed in a 25- μl volume mix containing 12.5 μl 2x SYBR Green PCR Master Mix, 1 μl primers, 2 μl cDNA, and 8.5 μl of RNase Free Water. Cycling conditions were as follows: 5 min at 94°C, 40 cycles of 15 s at 94°C, 30 s at 60 °C, and 30 s at 72 °C. Amplification curves and threshold cycle (Ct) values were determined by the stratagene MX3005P software. To estimate the variation of gene expression on the RNA of the different samples, the Ct of each sample was compared with that of the control group [14].

Primers used in SYBR Green real time PCR**Table 1.** The sequences of the primers

Gene	Primer sequence (5'-3')	Accession No.
β -actin	F-TCCTCCTGAGCGCAAGTACTCT	V01217
	R-GCTCAGTAACAGTCCGCCTAGAA	
P ₂₁	F-GCGGGACCGGGACATC	U24174
	R-CGCTTGGAGTGATAGAAATCTGTTAG	

Statistical Analysis

Results were expressed as mean \pm Standard deviation (S.D.) of the mean. Differences among means were tested by one-way analysis of variance using SPSS package version 19. Statistical significance was considered when $P < 0.05$.

RESULTS**Table 2.** Effect of supplementation with α - lipoic acid (60 mg/kg) on antioxidant enzymes in liver tissues of rats intoxicated with (1/10 LD50 and 1/40 LD50) Imidaclopride

Groups / Parameters	Catalase (CAT) (U/mg protein)	Super dismutase (SOD) (U/mg protein)	GST (mmol/min/mg protein)
Healthy Control	369.60 \pm 32.66	4.367 \pm 0.119	374.073 \pm 4.677
Healthy + α Lipoic acid	194.02 \pm 11.53 ^a	4.283 \pm 0.010	456.29 \pm 7.410 ^a
Imida (1/10LD50)	601.02 \pm 25.35 ^{a,b}	4.943 \pm 0.169 ^{a,b,c}	503.42 \pm 7.490 ^{a,b}

Imida (1/40LD50)	555.21 ± 46.98 ^{a,b}	4.269 ± 0.041	484.15 ± 3.395 ^{a,b}
Imida (1/10 LD50)+ α Lipoic acid	432.98± 36.37 ^{b,c,d}	4.573± 0.068	479.47 ± 6.4693 ^{a,b,c}
Imida (1/40 LD50)+ α Lipoic acid	422.88±40.746 ^{b,c,d}	4.348 ± 0.138	461.96 ± 11.191 ^{a,b,c,d}

All data are expressed as means ± SE for 10 rats.

a Significant difference versus control healthy group at p < 0.05.

b Significant difference versus +ve control group at p < 0.05.

c Significant difference versus Imida (1/10 LD50) group at p < 0.05.

d Significant difference versus Imida (1/40 LD50) group at p < 0.05.

e Significant difference versus Imida (1/10 LD50) + α Lipoic acid group at p < 0.05.

The results of this study showed significant reduction in catalase and GST activities in healthy group treated with α lipoic acid as compared to healthy control (Table 2). Rats treated with either high or low dose of Imida showed significant elevation

in catalase, SOD and GST activities comparing to healthy groups (P≤ 0.05). Animals treated with Imida & α lipoic acid showed a significant regression in the above mentioned groups as compared to intoxicated and control groups at P<0.05.

Table 3. Effect of supplementation with alpha- lipoic acid (60 mg/kg) on some oxidant parameters in liver tissues of rats intoxicated with (1/10 LD50 and 1/40 LD50) Imidaclopride

Groups / Parameters	SH-Protein (μmol/dl)	MDA (mmol/ml)	Protein carbonyl(CP) (mmol/mg protein)
Healthy Control	14.94 ± 0.551	28.00 ± 0.677	142.06 ± 2.05
Healthy +α Lipoic acid	14.17 ± 0.087	28.23 ± 1.768	141.62 ± 2.06
Imida (1/10LD50)	10.04 ± 0.222 ^{a,b}	33.10 ± 0.481 ^{a,b}	182.34 ± 5.21 ^{a,b}
Imida (1/40LD50)	11.94 ± 0.309 ^{a,b}	35.90 ± 0.351 ^{a,b,c}	172.04 ± 2.17 ^{a,b}
Imida (1/10 LD50)+ α Lipoic acid	15.88 ± 0.425 ^{c,d}	34.73 ± 0.168 ^{a,b}	136.54 ± 4.73 ^{c,d}
Imida (1/40 LD50)+ α Lipoic acid	14.05 ± 0.242 ^{c,d}	27.43 ± 0.615 ^{c,d,e}	138.04 ± 7.89 ^{c,d}

All data are expressed as means ± SE for 10 rats.

a Significant difference versus control healthy group at p < 0.05.

b Significant difference versus +ve control group at p < 0.05.

c Significant difference versus Imida (1/10 LD50) group at p < 0.05.

d Significant difference versus Imida (1/40 LD50) group at p < 0.05.

e Significant difference versus Imida (1/10 LD50) + α Lipoic acid group at p < 0.05.

The depicted results in Table (3) declared that intoxication with high and low doses of Imida induced remarkable elevation in each of well-known oxidative markers MDA and PC significant versus control groups at p<0.05. Pronounced reduction in SH-protein was recorded significant versus control groups at p<0.05, too. Supplementation with alpha lipoic acid induced remarkable improvement in the SH-protein level in liver tissues versus non supplemented groups. The alleviation in MDA and PC level reported in alpha lipoic supplemented groups might be attributed to the elevation in SH protein.

Table 4. Effect of supplementation with alpha- lipoic acid (60 mg/kg) on liver biomarkers in serum of rats intoxicated with (1/10 LD50 and 1/40 LD50) Imidaclopride

Groups / Parameters	ALT (U/l)	AST (U/l)	ALP (U/l)	Total protein (g/dl)
Healthy Control	29.88 ± 0.757	50.50 ± 2.117	126.91 ± 8.964	7.96 ± 0.318
Healthy +α Lipoic acid	35.17 ± 1.387 ^a	50.99 ± 0.993	124.58 ± 14.047	7.937 ± 0.431
Imida (1/10LD50)	56.64 ± 1.67 ^{a,b}	65.29 ± 0.817 ^{a,b}	148.47 ± 9.681 ^{a,b}	6.18 ± 0.197
Imida (1/40LD50)	57.63 ± 1.77 ^{a,b}	65.34 ± 0.916 ^{a,b}	128.93 ± 13.26 ^c	4.71 ± 0.384
Imida (1/10 LD50)+ α Lipoic acid	39.74 ± 1.27 ^{a,b,c,d}	52.35 ± 0.761 ^{c,d}	124.77 ± 12.51 ^c	6.85 ± 0.110
Imida (1/40 LD50)+ α Lipoic acid	25.46 ± 0.519 ^{a,b,c,d,e}	54.58 ± 0.385 ^{a,b,c,d}	135.25 ± 4.126 ^{a,b}	5.99 ± 0.346

All data are expressed as means ± SE for 10 rats.

a Significant difference versus control healthy group at p < 0.05.

b Significant difference versus +ve control group at p < 0.05.

c Significant difference versus Imida (low dose) group at p < 0.05.

d Significant difference versus Imida (high dose) group at p < 0.05.

e Significant difference versus Imida (low dose) + α Lipoic acid group at p < 0.05.

Results in table (4) revealed a significant increase in serum ALT & AST level rats treated with high and low doses of Imida, while treatment with alpha-lipoic acid induced a significant regression in liver enzymes comparing to the untreated group. Also, it could be seen that there was a significant reduction in total protein in rat groups treated with Imida when compared to all the examined groups, meanwhile lipoic acid restrained protein content. Regarding ALP level, there was a significant increase in Imida (high dose), this was reduced after the treatment with lipoic acid. No significant difference was observed between healthy control group and low dose Imida group.

Table 5. Effect of supplementation with alpha- lipoic acid (60 mg/kg) on comet assay in liver tissues of rats intoxicated with (1/10 LD50 and 1/40 LD50) Imidaclopride

parameter	Healthy Control	Healthy + ve α Lipoic acid	Imida (1/10LD50)	Imida (1/40LD50)	Imida (1/10 LD50)+ α Lipoic acid	Imida (1/40 LD50)+ α Lipoic acid
Tail moment unite	0.85 \pm 0.071 a	1.16 \pm 0.043 a	1.61 \pm 0.079 a,b	1.61 \pm 0.094 a,b	1.58 \pm 0.146 a,b	1.25 \pm 0.082 a,c,d,e
Tail length (μ m)	4.30 \pm 0.17	4.46 \pm 0.14	5.86 \pm 0.09 a,b	5.18 \pm 0.14 a,b,c	4.88 \pm 0.20 a,c	4.62 \pm 0.23 c,d
DNA %	18.59 \pm 0.56	19.18 \pm 0.87	29.97 \pm 1.22 a,b	26.79 \pm 1.07 a,b,c	22.22 \pm 0.78 a,b,c,d	21.15 \pm 1.00 c,d

All data are expressed as means \pm SE for 10 rats.

a Significant difference versus control healthy group at $p < 0.05$.

b Significant difference versus +ve control group at $p < 0.05$.

c Significant difference versus Imida (low dose) group at $p < 0.05$.

d Significant difference versus Imida (high dose) group at $p < 0.05$.

e Significant difference versus Imida (low dose) + α Lipoic acid group at $p < 0.05$.

Table 6. Effect of supplementation with alpha- lipoic acid (60 mg/kg) on gene expression in liver tissues of rats intoxicated with (1/10 LD50 and 1/40 LD50) Imidaclopride

Gene	Healthy Control	Healthy + ve α Lipoic acid	Imida (1/10LD50)	Imida (1/40LD50)	Imida (1/10 LD50)+ α Lipoic acid	Imida (1/40 LD50)+ α Lipoic acid
P21	1	1.08 \pm 0.079	1.164 \pm 0.09	1.224 \pm 0.12	1.966 \pm 0.121	2.11 \pm 0.179

Regarding the comet assay results, it was clear that rats treated with either low or high dose of Imida showed significant increase in DNA content as well as tail moment and length comparing to the healthy groups. Treatment with α - lipoic acid for the toxicity group results showed a significant decrease in DNA content accompanied by the decreased tail moment and length (Table 5). Gene expression results revealed non-significant up-regulation in P21 gene concentration in α -lipoic acid treated group as compared to the healthy groups (Table 6).

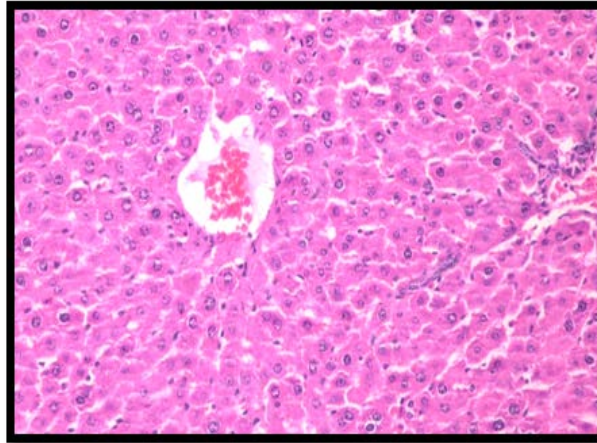
Histopathological findings:

Control group (A) and (B) showed normal histological structure of hepatic lobules and organization of hepatic cords (grade 0) {fig.1&2}. The histopathological examination of hepatic tissues of (group C) revealed massive fatty change of hepatocytes which characterized by signet ring cells appeared as cluster scatter all over the hepatic lobules, and centrilobular necrosis were also seen (grade IV) {fig.3} (Table 7).

On the other side, (group D) showed centrilobular degenerative changes appeared as swelling, and few intracellular fat droplets of hepatocytes (grade III) {fig.4}. Animals' group (group E) treated by protective, showed vacuolar degeneration of hepatocytes and eosinophilic regenerated hepatocytes (grade II) {fig.5}. On the other hand, (group F) treated with protective revealed mild swelling of hepatocytes in the peripheral zone (grade I)

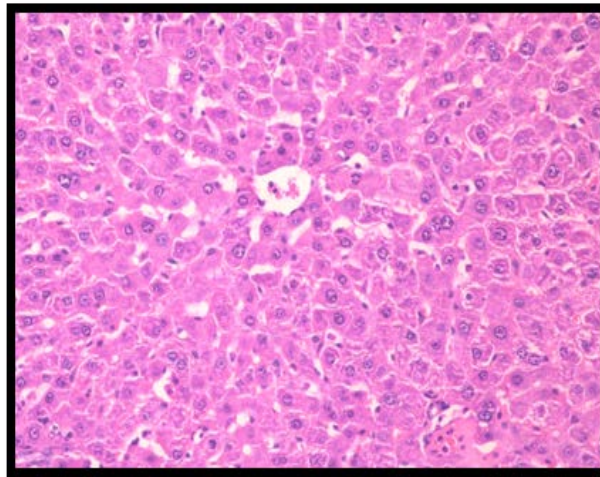
Table 7. Histological grading of different animal's groups:

Animal group	Grading of liver injury
A (control)	0
B (+ve alpha lipoic)	0
C Imida (1/10LD50)	IV
D Imida (1/40LD50)	III
E Imida (1/10 LD50)+ alpha Lipoic acid	II
F Imida (1/10 LD50)+ alpha Lipoic acid	I



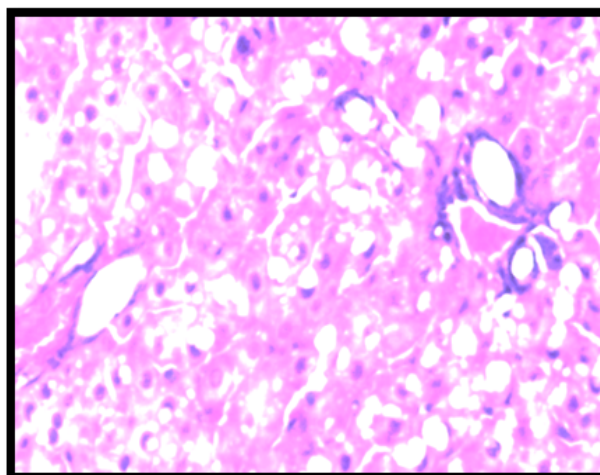
A-Control -ve group

Figure 1. The normal histological structure (grade 0) (H&EX200)



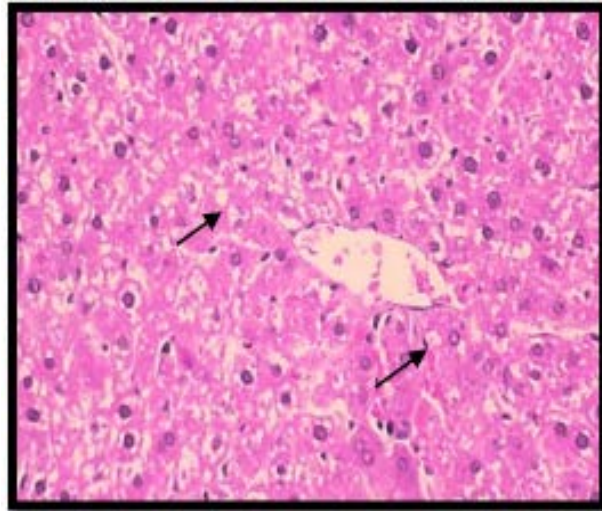
B- alpha lipoic acid

Figure 2. normal histological structure (grade 0) (H&EX200)



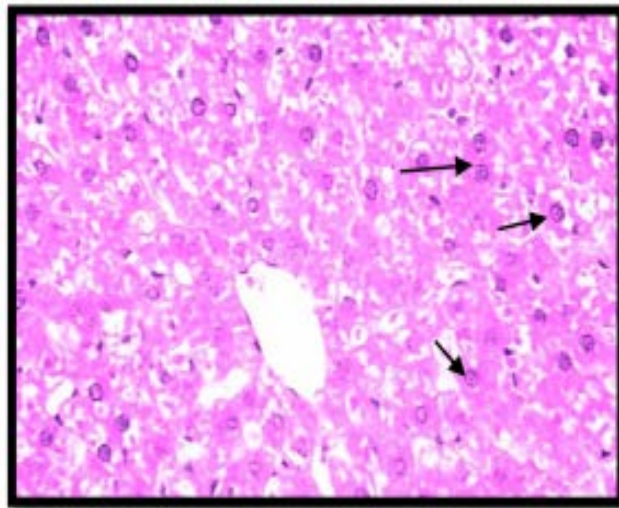
C- 1/10 imedaclopride

Figure 3. The massive vacuolation of hepatocytes and centrilobular necrosis (grade IV) (H&EX200)



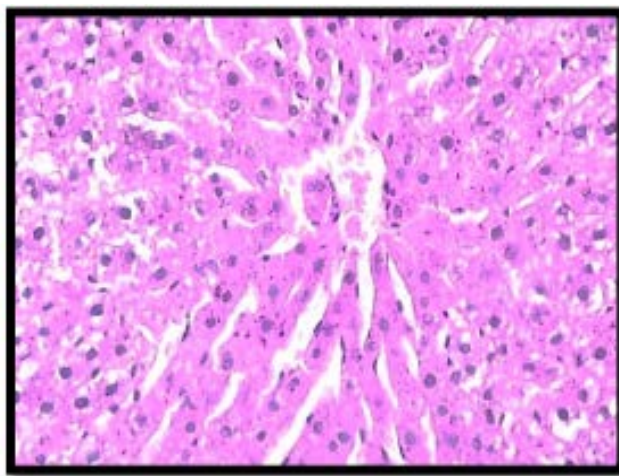
D- 1/40 imedaclopride

Figure 4. The moderate vacuolation of hepatocytes with few intracellular fat droplets arrows (grade III) (H&EX200)



E- 1/10 imedaclopride + alpha lipoic acid

Figure 5. The moderate vacuolation with presence of regenerated hepatocytes arrows (grade II) (H&EX200)



F- 1/40 imedaclopride+ alpha lipoic acid

Figure 6. Themild vacuolation of peripheral zone hepatocytes (grade I) (H&EX200)

DISCUSSION

Natural products represented a rich source of small chemical molecules that possess antiproliferation and anticancer properties. It is necessary to evaluate the antioxidant functions of any natural product in biological systems before using them as drugs [15]. Lipoic acid scavenges hydroxyl radicals, hypochlorous acid, and singlet oxygen. Imidacloprid is an insecticide which has low toxicity via dermal exposure, and moderate toxicity if ingested; but upon inhalation, its toxicity is variable [16]. Oral exposure to high dose of imidacloprid causes significant toxic effects announced as significant elevation in CAT, MDA, PC, GST and SOD with no change in GSH level [17], which showed that, SOD and CAT enzyme activities were increased in acute phase damage, because SOD and CAT are enzymes used as detoxifying agents, and they are the most powerful enzymes against ROS products [2]. A significant reduction was observed in GSH concentration in hepatic tissue after imidacloprid treatment, this might be due to GSH utilization as an antioxidant in terminating the free radical reaction causing GSH exhaustion during oxidative stress. Previous research suggested that the imida can cause oxidative stress and inflammation in organs like liver and brain in rats [18].

ROS attacks polyunsaturated fatty acids within the membrane lipids, causing lipid peroxidation which leads to the disruption of cell structure and function [19]. GSH level was very high in group treated with high dose of imida and lipoic acid, a possible explanation of the significant increase in GSH levels observed in this group could be the activation of nuclear factor-erythroid-2-related factor 2 (Nrf2). Nrf2 is a key transcription factor that mediates the expression of antioxidant and detoxification genes regulated by the antioxidant response elements (ARE) [20]. LA may also elevate GSH levels through boosting cysteine via extracellular cystine reduction, or via facilitation of cysteine uptake. An increased MDA level in liver tissues suggested enhanced lipid peroxidation and downstream tissue damage, and the failure of antioxidant defense mechanisms [21].

Different studies showed that lipoic fights oxidative stress by scavenging free radicals in a reusable form by regenerating them [6]. The results of this study showed that lipoic acid treatment caused significant regression in antioxidant enzymes comparing to toxicity group.

LA was taken up by different cells and tissues, then reduced in mitochondria to form dihydrolipoic acid (DHLA). Both, LA and DHLA are amphipathic molecules act as antioxidants both in lipophilic and hydrophilic environments [22]. The LA/DHLA redox couple can scavenge a variety of free oxygen radicals including superoxide and peroxy radicals. LA can raise intracellular levels of GSH by being a transcriptional inducer to genes controlling GSH synthesis via Nrf2/ARE signaling pathway besides its capability to raise cysteine uptake [23]. The toxic potential of imida increased the biochemical markers of liver injury, namely ALT, AST and ALP with high dose exposure of imida when compared with the low dose treated animals. On the other side, the improvement of liver function was observed in those groups of lipoic-treated rats given daily oral doses of lipoic acid. The results of the current study were inconsistent with the earlier reports [17]. Ultimately, in this study, the liver of rats exposed to high dose imida treatment evidenced congestion and fatty degeneration. The hepatotoxic effects noted here were in agreement with the findings of [24] when the liver cell plasma membrane was damaged, and a variety of enzymes normally located in the cytosol were released into the bloodstream.

Evident from the increased ALT and AST activities, decreased total protein reduced glutathione concentration in the liver. Histologically, the liver showed marked dilation, congestion of central vein, portal vein and sinusoidal spaces, vacuolation/fatty change and degenerated hepatocytes. Ultra-thin sections of the liver revealed swollen nuclei, varied size and shape of mitochondria, disrupted chromatin and rough endoplasmic reticulum. These findings ran parallel with the present histological examination.

The quantitation of these enzymes in serum was thus a useful marker of the extent and type of hepatocellular damage. Increases of ALT and AST levels in peripheral blood indicated the presence of an inflammatory state in liver cells, and chronic hepatic inflammation might lead to hepatic fibrosis. In the present study, Imida led to the increased serum ALT and AST levels; however, the supplementation with ALA significantly normalized ALT and AST levels possibly indicating an enhancement of liver cell regeneration [25].

Moreover, total protein levels were significantly decreased in the group that was treated with high dose of Imida as compared with the control group. These findings were in agreement with a study by [26]. The reduction in total protein might be due to hepatotoxicity induced by imidacloprid. It is well known that proteins are susceptible to damage by ROS, and oxidative modification of proteins may lead to the structural alternation and functional inactivation of many enzyme proteins [27].

Different studies indicated that organophosphate-induced ROS causes tissue damage and has important role in liver pathophysiology by inhibition enzymes that involves in DNA synthesis and pathology of membrane polyunsaturated fatty acids [4].

In present investigation, treatment of rats with imida increased DNA damage in rat liver. Imidacloprid induced DNA damage indicating agent-induced genotoxicity. [28] Observed that apoptosis and fragmentation of liver DNA were elevated in rats treated with imidacloprid by two different doses of 2 and 8 mg/kg. Toxicity might cause DNA damage by secondary mechanisms. Imidacloprid caused unorganized DNA synthesis in primary liver cells [29]. Free radicals due to oxidative stress could induce DNA damage, so the destructive effects of Imidacloprid on genomic content might be due to releasing free radicals, and the misbalance between free radical production and body antioxidant capacity [30].

In summary, it can be concluded that both low and high doses of administration of Imidacloprid increased the generation of ROS which has been important in the cascade of events leading to liver toxicity. The administration of inhibited lipoic acid reduced liver toxicity via its antioxidant potentials. Hence, it promised protection of liver against the biochemical abnormalities caused by imida administration.

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