

# Effect of Punica and Silymarin on Hepatotoxicity Induced by Pesticides

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**Background and Study Aim:** Human beings are exposed to pesticides through consumption of contaminated food or exposure in the occupational environment. These compounds induce hepatotoxicity through generation of reactive oxygen species. There is much evidence indicating that natural substances from medicinal plants possess powerful antioxidant activities. The aim of the present study was to investigate the potential curative effects of punica and silymarin in rats exposed to fenitrothion.

**Materials and methods:** Animals were randomly allocated into one of the following groups (n= 10): (C) control group treated with oral distilled water, 3 ml/kg/day for 42 days, (F4) oral fenitrothion, 10 mg/kg/day for 28 days, (F6) oral fenitrothion, 10 mg/kg/day for 42 days, (Pun) fenitrothion, 10 mg/kg/day for 42 days and oral punica juice 3ml/kg/day for 14 days starting from day 29 of fenitrothion administration and (Sil) fenitrothion, 10 mg/kg/day for 42 days and oral silymarin, 100 mg/kg/day for 14 days starting from day 29 of

fenitrothion administration. Activities of hepatic enzymes including alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) were evaluated. Serum albumin and total bilirubin concentrations were measured. Catalase (CAT) activity, reduced glutathione (GSH) and malondialdehyde (MDA) content in liver were determined. Total phenolic and flavonoids content were assessed in plant samples.

**Results:** Exposure to fenitrothion caused a significant increase in AST, ALT and ALP activities, total bilirubin concentration and a significant decrease in serum albumin. The hepatic antioxidant capacity was significantly lowered in fenitrothion-treated rats as compared to the control group (p<0.05). Treatment with punica or silymarin significantly ameliorated these changes.

**Conclusion:** This study indicated the promising therapeutic potential of punica and silymarin against hepatotoxicity induced by pesticides. These effects could be attributed to their antioxidant properties.

## INTRODUCTION

In agriculture and in everyday life, to produce more and to get more profits, toxic chemicals, such as pesticides are used. But they are generating harmful consequences to animals, the environment and human health [1].

The liver plays a central role in the metabolism of substances and it can be considered as target organ of numerous chemicals used in the workplace. Many insecticides seem to be able to cause enzyme induction with the modification of hepatic metabolism of drugs and hormones, as well as significant changes

in the liver. Pesticides may induce oxidative stress leading to generation of free radicals and alternated antioxidant or oxygen free radical scavenging enzyme system [2].

Minimizing oxidative stress will promote the physical condition and prevent some degenerative diseases in which free radicals are involved. There is a widespread agreement that synthetic antioxidants need to be replaced with natural antioxidants because some synthetic antioxidants have shown potential health risks. Therefore, it is

of great importance to find new sources of safe and inexpensive antioxidants of natural origin in order to use them in modulating oxidative stress associated with chronic diseases [3].

*Punica granatum* (punicaceae) commonly known as pomegranate is rich in antioxidant of polyphenolic class which includes tannins, anthocynins and flavonoids. Pomegranate is the fruit of energy, vitality and medicinal value that have anthelmintic, immunostimulatory, hepatoprotective, anti-diarrhoeal and anti-cancer activities [4].

Silymarin, a polyphenolic flavonoid from the milk thistle plant (*Silybum marianum*), inhibits lipoprotein oxidation and acts as a free-radical scavenger. Its effectiveness against multiple disorders makes it a very promising drug of natural origin. It is beneficial too, because of its wide margin of safety, easy availability and low cost. Hence, this drug may have good potential towards the treatment of many diseases, both in human beings as well as animals [5].

The present study was designed to investigate the curative effect of punica as food supplement in a model of hepatotoxicity by the organophosphorous compound; fenitrothion in rats in comparison to silymarin.

## MATERIALS AND METHODS

### Materials:

Fenitrothion (Sumithion 50<sup>®</sup>, 50 mg/ml) was purchased from Kafr Elzayat Co. for Insecticide Ind., (Kafr Elzayat, Egypt). Fenitrothion suspension was freshly diluted in distilled water to 10 mg/ml and orally administered at a dose of 10 mg/kg [6].

*Punica granatum*: Ripe *Punica granatum* fruits, family Punicaceae were obtained from local source in Zagazig, Sharkiah, Egypt. Pomegranates were washed and manually peeled without separating the seeds. Juice was prepared using a commercial blender (Braun, ZK 200, Germany), filtered and immediately diluted with distilled water (1:3) and stored at -20°C for no longer than 2 months. Aliquots were defrosted immediately and orally injected at a dose of 3 ml/kg [7].

The aqueous extract of silymarin was provided as a kind gift from MEBACO, (Arab Company for Pharmaceutical and Medicinal plants, Cairo, Egypt). Silymarin suspension was prepared by suspending 100 mg of Silymarin extract in 1 ml

distilled water and orally injected at a dose of 100 mg/kg [8].

Voucher specimens (reference number VS-020713-02-Pun and VS-020713-03-Sil) were deposited in the herbarium of the department of Pharmacology, Faculty of Pharmacy, Zagazig University, Egypt.

### Animals

Male albino rats weighing 160 ±10 g were obtained from National Research Center, Cairo, Egypt and were housed in plastic cages, allowed free access to a standard diet and tap water. The rats were housed at 23 ± 2° C 12 hr dark/light cycle. All experimental procedures were approved by the Ethical Committee for Animal Handling at Zagazig University (ECAHZU) (NO: P7-3-2013).

Animals were randomly allocated into one of the following groups (n= 10): C (control group treated with oral distilled water, 3 ml/kg/day for 42 days), F4 (oral fenitrothion, 10 mg/kg/day for 28 days), F6 (oral fenitrothion, 10 mg/kg/day for 42 days), Pun (fenitrothion, 10 mg/kg/day for 42 days and oral punica juice 3ml/kg/day for 14 days starting from day 29 of fenitrothion administration) and Sil (fenitrothion, 10 mg/kg/day for 42days and oral silymarin, 100 mg/kg/day for 14 days starting from day 29 of fenitrothion administration).

At the end of the experiment, after overnight fasting, blood was collected from the retro-orbital plexus and centrifuged at 3500 rpm for 15 minutes with or without heparin and serum/plasma was collected and stored at -20°C. Animals were sacrificed by decapitation and liver were excised for preparation of tissue homogenates.

### Methods

Total phenolic and total flavonoid contents in the plant materials were determined by colourimetric methods [9-10].

Catalase (CAT) activity, reduced glutathione (GSH) and malondialdehyde (MDA) content in liver were determined colorimetrically [11-12- 13].

The following parameters were assayed in serum using kits supplied by Biodiagnostic Co (Cairo, Egypt); ALT, AST and ALP activities, total bilirubin, albumin concentrations.

### Statistical analysis:

Data are expressed as means ± SE. The statistical significance of the data was determined using one way analysis of variance (ANOVA) followed by Tukey's post hoc test using SPSS software

package version 10. The level of significance was taken as  $P < 0.05$ .

## RESULTS

### Determination of total flavonoid and total phenolic content of punica juice and silymarin extract.

The total flavonoids and total phenolics content of punica and silymarin were found to be ( $228.75 \pm 6.91$  and  $217.35 \pm 5.65$  mg catechin/100g sample) and ( $37.16 \pm 0.49$  and  $36.41 \pm 0.47$  mg gallic acid/100g sample) respectively as shown in (Table 1).

### Determination of free radical scavenging activity of punica juice and silymarin extract.

The remaining percent of DPPH (2,2-diphenyl-1-picrylhydrazyl) and  $H_2O_2$  which is indicative of the free radical scavenging activity of punica and silymarin were found to be (12.2% and 12.42%) and (14.45% and 15.08) respectively as shown in (Table 2).

### Effects on different liver functions

Administration of fenitrothion for 28 and 42 days (F4 and F6 respectively), caused a significant increase in serum ALT, AST, ALP activities and serum total bilirubin compared with control group, while, treatment with punica or silymarin

for 14 days (starting from day 29 along with fenitrothion) reversed these changes to near control values causing a significant decrease compared with F6 group (Table 3).

On the other hand, administration of fenitrothion for 28 and 42 days (F4 and F6 respectively), caused a significant decrease in albumin concentration compared with control group. Administration of punica or silymarin for 14 days (starting at day 29 days along with fenitrothion) caused a significant elevation in albumin concentration compared with F6 group (Table 3).

### Effect on oxidative stress biomarkers

Administration of fenitrothion for 28 and 42 days (F4 and F6) caused a significant decrease in catalase (CAT) activity and glutathione (GSH) content in liver compared with control group. On the other hand, administration of fenitrothion for 28 and 42 days (F4 and F6) caused a significant increase in malodialdehyde (MDA) content in liver compared with control group (Table 4).

Treatment with punica or silymarin for 14 days (starting from day 29 along with fenitrothion) caused a significant increase in liver catalase activity and glutathione (GSH) content while liver malodialdehyde (MDA) content were significantly reduced (Table 4).

**Table (1):** Total flavonoid and phenolic content of punica juice and silymarin extract

	Total flavonoids (mg catechin/ 100g sample)	Total phenolics (mg gallic acid/ 100g sample)
<b>Punica</b>	$228.75 \pm 6.91$	$37.16 \pm 0.49$
<b>Silymarin</b>	$217.35 \pm 5.65$	$36.41 \pm 0.47$

**Table (2):** Free radical scavenging activity of punica juice and silymarin extract determined by the percent remaining of DPPH and  $H_2O_2$

	% remaining of $H_2O_2$	% remaining of DPPH
<b>Punica</b>	12.2%	14.45%
<b>Silymarin</b>	12.42%	15.08%

Table (3): Effects of fenitrothion (10 mg/kg) alone or in combination with punica (3ml/kg) or Silymarin (100 mg/kg) on different liver functions. Data are presented as mean  $\pm$  SE. (n = 10)

Parameter	Control	F4	F6	Pun	Sil
Serum ALT (U/L)	41.25 $\pm$ 0.36	54.00 $\pm$ 0.86*	55.17 $\pm$ 0.42* <sup>§</sup>	45.0 $\pm$ 0.29* <sup>##</sup>	42.17 $\pm$ 0.60* <sup>##</sup>
Serum AST (U/L)	62.67 $\pm$ 0.88	146.00 $\pm$ 4.37*	147.83 $\pm$ 2.06* <sup>§</sup>	114.33 $\pm$ 0.65* <sup>##</sup>	71.75 $\pm$ 2.57* <sup>##</sup>
Serum ALP (U/L)	107.59 $\pm$ 1.61	257.63 $\pm$ 6.94*	236.69 $\pm$ 6.91* <sup>§</sup>	118.84 $\pm$ 7.54* <sup>##</sup>	130.78 $\pm$ 1.89* <sup>##</sup>
Serum total bilirubin (mg/dl)	0.32 $\pm$ 0.01	0.89 $\pm$ 0.01*	0.93 $\pm$ 0.01* <sup>§</sup>	0.59 $\pm$ 0.02* <sup>##</sup>	0.54 $\pm$ 0.02* <sup>##</sup>
Serum albumin (gm/dl)	4.80 $\pm$ 0.15	3.21 $\pm$ 0.02*	3.28 $\pm$ 0.04*	4.27 $\pm$ 0.01* <sup>##</sup>	4.29 $\pm$ 0.06* <sup>##</sup>

\* Significantly different from control group

<sup>§</sup> Significantly different from F4 group

<sup>#</sup> Significantly different from F6 group

<sup>€</sup> Significantly different from Sil group at p< 0.05 using ANOVA followed by Tukey's Post Hoc test.

Table (4): Effects of fenitrothion (10 mg/kg) alone or in combination with punica (3ml/kg) or silymarin (100 mg/kg) on liver oxidant state catalase glutathione and malondaldehyde. Data are presented as mean  $\pm$  SE. (n = 10).

Level	Control	F4	F6	Pun	Sil
Catalase activity ( $\mu$ mole/min/mg wet tissue)	0.60 $\pm$ 0.01	0.31 $\pm$ 0.004* <sup>#</sup>	0.25 $\pm$ 0.002* <sup>§</sup>	0.62 $\pm$ 0.0* <sup>##</sup>	0.61 $\pm$ 0.01* <sup>##</sup>
GSH content (mg/gm wet tissue)	24.49 $\pm$ 0.18	15.33 $\pm$ 0.14* <sup>#</sup>	12.09 $\pm$ 0.07* <sup>§</sup>	25.26 $\pm$ 0.21* <sup>§</sup>	25.06 $\pm$ 0.29* <sup>##</sup>
MDA content ( $\mu$ mole/gm wet tissue)	56.16 $\pm$ 1.41	95.44 $\pm$ 1.83* <sup>#</sup>	95.94 $\pm$ 3.33* <sup>§</sup>	66.33 $\pm$ 0.77* <sup>##</sup>	64.66 $\pm$ 1.10* <sup>##</sup>

\* Significantly different from control group,

<sup>§</sup> Significantly different from fenitrothion 4 weeks (F4) group

<sup>#</sup> Significantly different from fenitrothion 6 weeks (F6) group

<sup>€</sup> Significantly different from silymarin (Sil) group at p< 0.05 using ANOVA followed by LSD and Tukey' Post Hoc test.

## DISCUSSION

Analysis of punica juice and silymarin extract showed the presence of flavonoids that may contribute to their antioxidant activity. These results are in accordance with previous studies showing the presence of flavonoids in punica [14] and silymarin [15].

Apart from being important dietary components, many therapeutic benefits of flavonoids are known in animal systems. Flavonoids have antioxidant, anti-proliferative, antitumor, anti-inflammatory, and pro-apoptotic activities [16].

The antioxidant activity of pomegranate aril juice, attributed to a great extent to total phenols and anthocyanins content [17]. Similarly, silymarin possesses antioxidant, immunomodulatory, anticancer, antiinflammatory, antihepatotoxic activities [18].

In the present study, results of DPPH and H<sub>2</sub>O<sub>2</sub> reducing power indicated the high anti-oxidant

activity [19] of punica juice and silymarin extract. These observations are in accordance with those reported for punica [20] and silymarin [18].

Organophosphorus pesticides are widely used in the world and causing toxic effects on nontarget organisms especially mammalian. Due to the role of liver in detoxification of environmental xenobiotics, it is at great risk of injury and induces hepatotoxicity [21].

Hepatotoxicity by pesticides may occur in many ways, such as changes in the activities of liver enzymes, serum albumin and bilirubin concentrations which account for many indices of liver function [22].

The current study has shown that fenitrothion caused a significant increase in ALT, AST and ALP activities compared with control group. Pesticides may damage liver cells and liver transaminases may be used to monitor liver damage after exposure. These results coincide

with previous studies that showed a significant increase in liver enzymes in rats and humans exposed to organophosphorus insecticides (fenitrothion and chlorpyrifos) [23].

Furthermore, it was found that serum bile acids were the most sensitive markers for detecting liver injury, suggesting that serum bile acids could be a valuable biomarker of hepatotoxicity caused by toxics. In the present study, fenitrothion treated rats showed significant increase in the level of bilirubin which is a normal metabolic product of haemoglobin in red blood cells [24].

These results are in accordance with those obtained previously which indicated that the mechanism for pesticides hepatotoxicity is impairment of bile acid transport, causing cholestasis and subsequent hepatocellular apoptosis or necrosis [22].

The present study showed a decrease in albumin concentration following fenitrothion administration as previously stated [25]. The albumin level suppression may be due to loss of protein either by reducing in protein synthesis or increased proteolytic activity or degradation [26].

There is a considerable importance of the investigation of free radical-mediated damage to biological systems due to pesticide exposure [27-28], the current study has shown that administration of fenitrothion resulted in an evident state of oxidative stress in the liver as evidenced by the decrease in catalase activity, glutathione content and increase in malodialdehyde content.

Previous studies indicate that fenitrothion intoxication can cause generation of free radicals and induce hepatic lipid peroxidation in rats [29]. Organophosphorus pesticide may induce oxidative stress which can be viewed as the disturbance in the oxidant-antioxidant balance in favor of the former [30].

Natural antioxidants from fruits and vegetables are reported to provide substantial protection that slows down the process of oxidative damage caused by reactive oxygen species (ROS) [31].

In the current study, coadministration of punica juice or silymarin extract with fenitrothion restored the altered liver functions as ALT and AST activities. These results are in accordance with those previously obtained for punica [32] and for silymarin [33]. This alleviation in ALT and AST activities may be due to stabilizing

membrane integrity that prevent the leakage of intracellular enzymes [34-35].

Similarly, treatment with punica juice or silymarin extract with fenitrothion, reversed the altered ALP activity and bilirubin concentration to near normal values. These results are in accordance with those obtained previously for punica [36] and for silymarin [37].

The amelioration in ALP activity and bilirubin concentration might be attributed to the ability of polyphenols and flavonoids present in punica [32] and silymarin [33] to treat the impairment of metabolism and excretion of bilirubin.

Interestingly, administration of punica juice and silymarin extract corrected the disturbed serum albumin content. These results match those obtained previously for punica and silymarin [38]. The elevation of serum albumin might be attributed to the anabolic effect of flavonoids [39] or stimulation of RNA synthesis [40] and increased activity of mixed-function oxidation system [41].

Antioxidants play an important role in ameliorating the damaging effects of oxidative stress on cells [42]. In the current study, oral administration of punica juice or silymarin extract normalized the oxidative stress biomarkers; CAT, GSH and MDA in liver that were altered by fenitrothion. The antioxidant activity of punica and silymarin may be attributed to their content flavonoids which have been found to reduce xenobiotic-induced hepatotoxicity in animals and counteract the damaging effects of oxidative stress, cooperating with natural systems like glutathione and other endogenous protective enzymes [43].

## CONCLUSION

Our findings demonstrated that punica and silymarin may be helpful in reducing the hepatotoxic adverse effects of fenitrothion by maintaining optimum cellular biochemical hemostasis.

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