

Influence of Pomegranate (*Punica granatum* L.) on Dimethoate Induced Hepatotoxicity in Rats

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Abstract—Pomegranate (*Punica granatum* L.) is an ancient fruit of great medical interest and rich source of antioxidants. Pesticides as dimethoate play a crucial role in the occurrence many diseases in plants, animal and human. Therefore the ability of Pomegranate (*Punica granatum* L.) to alleviate hepatotoxicity induced by organophosphate pesticide dimethoate was investigated. Albino male rats were divided randomly into 4 groups and kept at 7 animals per group in an environmentally controlled condition for 6 weeks. The first group was served as a control group (basal diet), the second group fed on basal diet supplemented with 5% freeze dried pomegranate seeds, the third group fed on 20 ppm dimethoate contaminated diet and the last group fed on dimethoate contaminated diet supplemented with 5% freeze dried pomegranate seeds. The results revealed that administration of dimethoate caused high significant increased in liver functions: alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) activities as well as lipid peroxide (malonaldehyde, MDA); on the other hand high significant decreased on glutathione (GSH), glutathione peroxidase (GPx), albumin and total protein were observed. However addition of 5% freeze dried pomegranate seeds significantly improved all previously mentioned parameters. These results indicate the dimethoate induced hepatotoxicity and highlight the protective effect of pomegranate seeds as a potential protective agent against dimethoate induced hepatotoxicity. This may be attributed to the powerful antioxidants (polyphenols, total phenols, and total flavonoids) which present in high levels in pomegranate as well as improving the immunity by activation of antioxidant enzymes GSH and GPx.

Keywords—Pomegranate, organophosphate pesticides, dimethoate, liver toxicity, free radicals, antioxidants.

I. INTRODUCTION

DIMETHOATE (O, O-diethyl S- methylcarbamoylmethyl phosphorodithioate), is a broad use systemic organophosphate insecticide/acaricide in agriculture against a wide range of insects, mites and fungal diseases of fruits, vegetables, and field crops as both systemic and contact pesticide; as well as indoor to control houseflies [1]. The use of dimethoate is very important in affecting many diseases in plants and animals as well as human [2]. It has been reported the toxicity of systemic pesticides dimethoate results in deleterious effects on many organs and systems in human and other mammals particularly the nervous system [3], [4],

immune system [5], reproductive system and sexual hormones [6], liver [7], [8], Kidney [9], pancreas [4], brain [9]. Organophosphosphate pesticides (OPI) dimethoate also included inhibition of both mammal's brain and plasma cholinesterase activity [10] and lactate dehydrogenase [11].

Peroxidation of oxidative stress following acute exposure to dimethoate has been reported also [12]-[15] and it has been demonstrated unequivocally that lipid peroxidation is one of the molecular mechanisms involved in OPI-induced toxicity [15]-[17]. Dimethoate can cause oxidative stress by the generation of free radicals and induce hepatic lipid peroxidation in mice [14] and rats [18], [12], [13], [15]. The cellular antioxidant status determines the susceptibility to oxidative damage and is usually altered in response to oxidative stress. The cellular antioxidant action is reinforced by the presence of dietary antioxidants. Accordingly, interest has recently grown in the role and usage of natural antioxidants as strategy to prevent oxidative damage in various health disorders with oxidative stress [15]. 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) [19]-[21].

Pomegranate (*Punica granatum* L.), a fruit native of the Middle East, has gained widespread popularity as a functional food and nutraceutical source is gaining tremendous attention due to its powerful antioxidant properties [22]. The health effects of the whole fruit, as well as its juices and extracts, have been studied in relation to a variety of diseases. *Punica granatum* (pomegranata) is used as food or as medication in folk medicine for antiviral, anthelmintic, antifungal, antibacterial and antimicrobial activity [23]. Studies have shown that pomegranate products prevent and/or reduced chemically cardiovascular disease and diabetes [24], induced tumors in skin [25], breast [26], lung [27], and colon [28] *in vivo* and *ex vivo* study. Recent studies showed pomegranate fruit and flower extracts to exhibit free-radicals scavenging properties with simultaneous potent hepatoprotection against chemically induced liver damage in rodents [29], [30].

The market has steadily grown, which is presumably due to the increasing consumer awareness of the potential health benefits attributed to pomegranates and phytochemicals [31]. The super fruits pomegranate is gaining tremendous importance of its potent antioxidants properties due to its high polyphenolic content, specifically anthocyanins, hydrolysable tannins (ellagitannins and gallotannins) and condensed tannins (proanthocyanidins). Polyphenolics, possess strong free radicals scavenging and antioxidant properties. Anthocyanins and the unique fatty acid profile of the seed oil may also play a

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role in pomegranate's health effects [32], [22]. In view of these facts the purpose of our study was to evaluate the protective effect of pomegranate against dimethoate-induced hepatotoxicity in rats.

II. MATERIALS AND METHODS

A. Chemicals

Dimethoate (98%) was purchased from Chemical Service (West Chester, PA, USA). All chemicals used in this study were obtained from Sigma Company (St. Louis, USA). Commercial kits were purchased from BioMerieux Company (L'Etoile, France) and from Eagle Diagnostics (Dollas, TX, USA).

B. Animals

Three to four weeks old male albino rats were obtained from the Animal House Colony, Giza, Egypt. Rats were housed on a 12h light-12h dark schedule, and fed with water ad-libitum for 6 weeks. All rats were adapted to three days on the control diet of the beginning of the experiment.

C. Preparation of Pomegranate Seeds Supplemented Diet

Pomegranates (*Punica granatum* L.) were obtained from the local market. Pulverize and lyophilized using freeze dryer system (Dura-Dry Freeze Dryer, Model PAC-TC-V4; FTS system, Inc., Stone Ridge, NY, USA). The dried pomegranate seeds were stored in the freezer until used.

D. Chemical Analysis of Pomegranate Seeds

Moisture content, total protein, fat, crude fiber and ash were determined in pomegranate seeds according to [33]. Flavonoid as catechin was determined according to the method of [34]. Total phenols gallic acid was determined as described by [35]. Polyphenols was measured according to [33] using Folin Denis Reagent and calculated as tannic acid.

E. Experimental Design

The rats were divided into four groups of 7 animals each and treated for 6 weeks as follow: group I: rats fed on basal diet (control), group II: rats fed on basal diet supplemented with 5% freeze dried pomegranate seeds, group III: rats received 20ppm dimethoate along with basal diet and group IV: rats received 20ppm dimethoate contaminated diet supplemented with 5% freeze dried pomegranate seeds. Food consumption, general condition and any other symptoms were observed daily and body weights were recorded weekly.

F. Samples

At the end of the experiment rats were fasted overnight and anesthetized with diethyl ether. Blood samples were collected in clean dry centrifuge tubes from hepatic portal veins. All blood samples were centrifuged for 15 min at 3000 rpm to separate the serum. The clear serum was kept frozen at -20°C till analysis. The effect of dimethoate and pomegranate seeds on liver were evaluated by assayed serum transaminases, alanine aminotransferase (ALT), aspartate aminotransferase (AST) activities according to the method of [36], alkaline phosphatase (ALP) by the method of [37]. Colorimetric

determination of albumin using bromocresol green at pH 4.2 according to the method of [38], estimation of total protein according to the method described by [39]. Lipid peroxide (malonaldehyde, MDA) was determined according to the method of [40]. Glutathione (GSH) activity and Glutathione peroxidase (GPx) were measured by the method of [41].

G. Statistical Analysis

Data was subjected to analysis of variance (ANOVA) and computing using the SAS General Linear Model producer [42]. Values were statistically significant when $P \leq 0.05$.

III. RESULTS AND DISCUSSION

Liver is one of the major organs for detoxification of xenobiotics. A large number of xenobiotics caused oxidative stress by generation free radicals in biological systems. There is a considerable importance of the investigation into free radical-mediated damage to biological systems due to pesticide exposure. A study of some commonly used plant products of antioxidants against xenobiotic-induced oxidative stress therefore appeared to be of interest [43], [44]. In the present study, oral administration of dimethoate to rats caused oxidative stress and elevation of hepatospecific enzyme activities, as well as severe alterations in different liver parameters. Data in Fig. 1 showed that treatment with dimethoate significantly increased ($P < 0.05$) the activities of liver function enzymes AST, ALT and AIP in serum compared to control animals. In clinical diagnosis increased level of AST, ALT and AIP activities indicates affected liver [45]. The affected liver function of dimethoate is similar to those reported by [46], [11], [47]; they found that dimethoate-treated rats increased activities of AST, ALT and AIP. The increased transaminase activity was reversed to normal level following pomegranate seeds supplementation. Treatment of pomegranate seeds alone doing not because any significant change in enzyme activities in serum compared with control. Ashoush, et al. [48] found that supplemented diet with pomegranate peel powder (PPP), whey powder (WP) and their combination (PPWP) exhibited a significant reduction in the levels of ALT and AST as compared with liver injury control rats group.

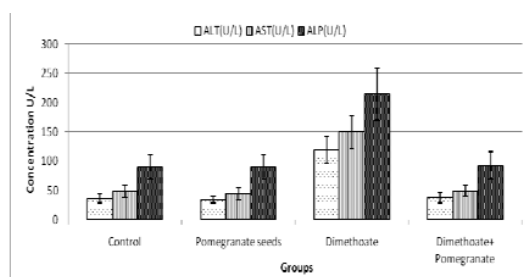


Fig. 1 Effect of pomegranate seeds on liver functions dimethoate treated rats

Data represented in Fig. 2 showed that dimethoate treated group high significantly decreased ($P < 0.05$) serum total protein (TP) and albumin levels in rats compared to control;

whereas groups treated with pomegranate seeds alone or pomegranate seeds supplemented to dimethoate treated group caused very significant improved ($P < 0.05$) in serum TP and albumin levels. The reduction in serum protein, particularly albumin, could be attributed to changes in protein and free amino acid metabolism and their synthesis in the liver. Takeo [49] emphasized that human albumin has a significant high affinity with binding with alkylating agents and xenobiotics. Also, the protein level suppression may be due to loss of protein either by reducing in protein synthesis or increased proteolytic activity or degradation [50]. In addition, the observed decrease in serum proteins could be attributed in part to the damaging effect of dimethoate on liver cells, as confirmed by the increase in activities of serum AST and ALT. This finding was confirmed by [46] that found that dimethoate decrease serum total protein and albumin.

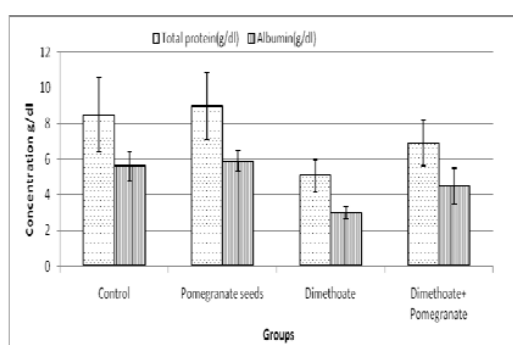


Fig. 2 Effect of pomegranate seeds on albumin and total protein of dimethoate treated rats

It has been demonstrated that lipid peroxidation is one of the molecular mechanisms involved in organophosphate pesticides-induced toxicity [15]-[17]. The results of Fig. 3 illustrated that rats fed on the 20ppm dimethoate contaminated diet showed a remarkable increased to the value of lipid peroxidation (malondialdehyde) compared with rats fed on basal diet (control). This compound is the breakdown product of the major chain reactions leading to oxidation of polyunsaturated fatty acid and thus serves as a marker for oxidative stress in the body [51]. Oxidative stress can be viewed as the disturbance in the oxidant-antioxidant balance in favor of the former. Previous studies indicate that dimethoate intoxication can cause oxidative stress by the generation of free radicals and induce hepatic lipid peroxidation in mice [14] and rats [18], [12], [13], [15].

However, the increased in serum lipid peroxidation due to dimethoate treatment very significantly reduced when diet supplemented with pomegranate seeds. Parmar and Kar [52] reported that administration of pomegranate peel extract in atherogenic diet-fed animals significantly reduced the tissue (hepatic, cardiac, and renal) and serum lipid peroxidation as compared to the respective values of atherogenic diet-fed animals.

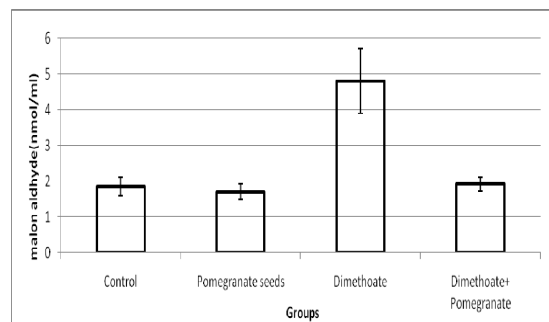


Fig. 3 Effect of pomegranate seeds on malonaldehyde (MDA) of dimethoate treated rats

The results of Fig. 4 illustrated that dimethoate treatment highly depleted glutathione content (GSH). Glutathione deficiency contributes to oxidative stress, which plays a key role in aging and the pathogenesis of many disease including, kwashiorkor, seizure, Alzheimer's disease, liver disease, HIV, AIDS, cancer, heart attack and diabetes [53]. The GSH depletions, especially utilize in acute hepatotoxicity necrosis, liver failure or death [45]. Dietary supplemented with pomegranate seeds to contaminated dimethoate diet significantly increased GSH content and consequently reduced to damage in liver. Glutathione plays important roles in antioxidant defense, nutrient metabolism and regulation of cellular events.

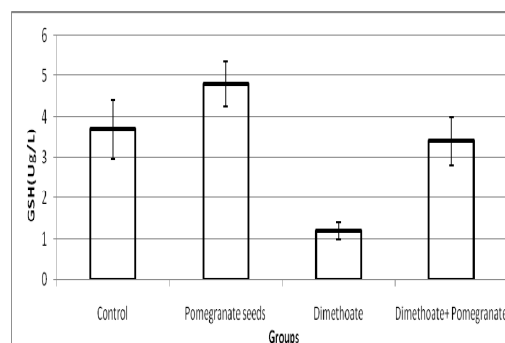


Fig. 4 Effect of pomegranate seeds on glutathione (GSH) of dimethoate treated rats

The results of Fig. 5 showed that dimethoate treatment very significantly decreased ($P < 0.05$) in endogenous antioxidants glutathione peroxidase (GPx) activity (used as marker of oxidative stress in liver) as compared to negative control. The decreased in GPx due to dimethoate high significantly reduced ($P < 0.05$) when diet supplemented with pomegranate seeds. It is noted that rats give the basal diet of pomegranate has antioxidant enzymes activities (GSH and GPx) lower than those don't give pomegranate seeds. This means that pomegranate seeds increased the immunity.

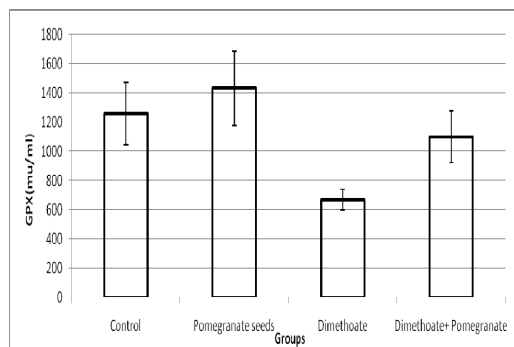


Fig. 5 Effect of pomegranate seeds on glutathione peroxidase (GPX) of dimethoate treated rats

Enzymes such as Glutathione peroxidase, superoxide dismutase and catalase are the main system that opposes oxidation by catalytically remove free radicals and alter reactive species which can also be scavenged [54]. It was found that *Punica granatum* L. (*Punicaceae*) peels extracts had the highest free radicals scavenging capacity of the tested medicinal plants which are being used traditionally for treatment of diabetes in Jordan [55].

Our results indicate that dietary supplementation of pomegranate seeds can protect against the oxidative stress and reduced consequently the risk of hepatotoxicity as well as improved liver functions in dimethoate-treated rats suggesting that pomegranate seeds have potent antioxidant properties. In addition detoxification activity of pomegranate seeds believed to be attributed to improve the immunity by activation antioxidant enzymes such as glutathione (GSH) and glutathione peroxidase (GPx) and preventing oxidative damage. The chemical composition of pomegranate seeds in the Table I revealed that it contains high nutritive value, fibers and a variety of biologically active compounds (polyphenols, total phenols and total flavonoids). Phenolics is known as potential chemopreventive agents and are essential for counteracting oxidation stress; possess strong free radicals scavenging and antioxidant properties [56], [57].

Antioxidant activity may be related to diverse phenolic compounds present in pomegranate including punicalagin isomers, ellagic acid derivatives and anthocyanins (delphinidin, cyanidin and pelargonidin 3-glucosides and 3, 5-diglucosides). These compounds are known for their properties in scavenging free radicals and inhibiting lipid oxidation [32], [58], [59].

The other mechanism by which pomegranate inhibited the toxicity of dimethoate may be attributed to the interaction of fibers with dimethoate and inhibit absorption of dimethoate from intestine. It has been suggested that dietary supplementation with pomegranate may prevent DNA damage [60], [61].

TABLE I
CHEMICAL COMPOSITION AND ANTIOXIDANTS OF POMEGRANATE SEEDS (ON THE BASIS OF DRY WEIGHT)

I Chemical composition	Fresh	Freeze dried
Moisture %	71.9±8.5	7.2±0.8
Protein %	4.8±1.0	9.1±1.1
Fat %	1.2±0.3	2.3±0.07
Fiber %	9.1±1.5	15.5±1.8
Ash %	0.8±0.1	3.8±0.5
Carbohydrate %	12.2±1.5	62.1±4.2
II Antioxidants (mg/100g)		
Total polyphenols	36.7±6	35.4±5.1
Total Flavonoids	30.4±4.2	29.6±3.9
Total Phenols	110.1±18.3	108±17.5

IV. CONCLUSION

In conclusions, this study revealed that pomegranate has multi-protective agents that can protect humans against the oxidative stress and reduce consequently the risk of chronic diseases and prevent disease progression.

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