



Thioacetamide-Induced Acute liver Failure and Prospect of Nano Antioxidant Based Therapy [*Herbal Approach*]

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Abstract

Acute liver failure (ALF) is a clinical condition with an unclear history of pathophysiology, *Punica granatum L peel* and *Pistacia atlantica* leaves are differentiated by the presence of a wide range of flavonoids and phenolic as antioxidant compounds. The purpose of this study was to investigate the hepatoprotective effects of *Punica granatum L. peel* and *Pistacia atlantica* leaves either in their normal and/or in their nano forms against thioacetamide induced acute liver failure in a rodent model. Male Wistar rats (n=60) were divided into six equal groups, the first group employed as a control; The second group administered a dose of 350 mg /Kg/ b.w of thioacetamide (TAA)-ip, from the third to the sixth group received TAA + [2mls / 100 g b.w/d] of aqueous extracts of *Punica granatum L* and *Pistacia atlantica* either in their normal and/or Nano forms consecutively for (14 days). In the TAA challenged group, there was a substantial increase in liver enzymes, lipid profiles, LPO (p= 0.05), and NO, as well as a substantial drop in GSH and SOD, which was followed by an increase in inflammatory cytokine (IL6, TNF-, and AFP), as well as a considerable increase in HSP70 level and DNA degradation. However, ip nano treatment of both extracts resulted in considerable and subsequent improvement of most of the compromised indicators. In conclusion: The current findings demonstrated the great performance of both plant nano extracts, as well as their hepatoprotective effects and possible therapeutic usefulness in the treatment of TAA-induced acute liver failure in experimental animals.

Keywords: Acute liver failure, Thioacetamide, HPLC, Nano herbal Extracts, TNF- α , IL6.

Introduction

Acute liver failure (ALF) is characterized by a rapid and remarkable impairment of hepatocyte function in patients with normal liver function [1]. Hepatic encephalopathy and a major cause of death by ALF, even if intensive care is given [2-6]. Liver transplantation is shown to be the most effective therapy for ALF, but this therapeutic choice is constrained by the lack of donor organs. The use of bio artificial livers is one of the other approaches to treatment, to overcome the time gap for transplantation or regeneration of the liver. [7, 4, 8, 9]. The effectiveness of new treatment methods for ALF has not yet been fully recognized [5, 9] and even the knowledge of the ALF pathophysiology is still inadequate [2, 6]. So there is a necessity to search for the discovery and implementation of efficacious safe nutraceuticals rather than chemically synthesized as well as an optimum experimental ALF model

with features similar to human disorder.

Thioacetamide (TAA) is a class of chemicals that is mostly recommended to generate the disease model in the very shortest duration [10, 11]. TAA has long been known as a hepatocyte toxic agent. The TAA biotransformation cycle into thioacetamide sulfoxide (TAAS) mainly takes place through P450 (CYP) cytochrome pathway, (CYP2E enzyme is mainly involved here) [12]. The binding of these metabolites to liver macromolecules dramatically inspire a moiety of reactive oxygen species, in turn, induces acute centrilobular necrosis of the liver [13]. These results in cirrhosis and primary cancer of the liver [14]. TAA will overpower the body's antioxidant defense mechanisms and spread rapidly to neighboring tissues to destroy cell vital ingredients that ultimately enter DNA [15]. There is currently

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widespread interest in the use of natural products especially the dietary polyphenols, which are considered safe and less side-effect compared to the synthesis of chemical drugs, and the best hepatoprotective agents due to their synergistic action [16, 17]. Dietary polyphenols, especially those in vegetables, fruits and legumes, have been reported to be capable of achieving a balance between natural antioxidants and free radicals by enhancing the role of natural antioxidant enzymes such as glutathione reductase (GR superoxide dismutase (SOD), glutathione peroxidase (GPx), and glutathione S transferase (GST) by specifically capturing free radicals [18]. Sweet fruit belongs to the *Punicaceae* family and is known worldwide as pomegranate / *Punica granatum L* because of its health value from all its components [19,20,23]. Although anti-inflammatory properties and resistance to exponential elevated glucose levels in diabetic rat models have been reported for pomegranate flowers and juices [21,22,23] much more attention has been paid recently to the pomegranate husk/peels it has been confirmed to be of great benefit to a broad spectrum of hepatic inflammation and other liver-related diseases [24, 25].

This conclusion assigned according to earlier studies has shown that pomegranate peels have a higher content of phenolic compounds than fruit pulp including a group of flavonoids (catechins, anthocyanins and other complex flavonoids) and hydrolyzing tannins (punicalin, pedunculagin, punicalagin, gallic and ellagic acid)[21, 26].

Pomegranate has shown evidence concerning a preventive cancer-chemo property [27]. Reducing the oxidative stress mediators that extensively contribute to its phenolic compounds and serve as a powerful tool for free radical scavenging properties makes it unique [28].

Pistachio is a type of nut produced by approximately 20 species of shrub such as *Pistacia terebinthus*, *Pistacia vera*, *Pistacia khinjuk*, *Pistacia lentiscus*, and *Pistacia atlantica* belonging to the Cashew family (*Anacardiaceae*) [29]. This Genus *Pistacia* is characterized by the presence of a wide range of diverse compounds such as flavonoids, triterpenes, sterols and phenolic compounds. The phenolic compounds are abundant in different parts of this genus [30, 31]. *Pistacia atlantica* has been shown to have antibacterial properties and is used in eczema, throat infections, kidney stones, asthma and stomach aches as an astringent, antipyretic, anti-inflammatory, antiviral, and stimulating agent [32]. Previous studies of Photochemistry and chromatographic fingerprints of this plant indicated that it contains phenolic compounds (such as gallic acid, quinic acid,

tetragalloylquinic acid,)and triterpenoids, pinene, terpinolene [33]. Although the pistachio is common, it was not sufficiently investigated as a safely eaten nut [34].

The use of nanoparticle science is completely exhibits improved novel properties compared with larger particles of the bulk materials due to the variations in the particle size, morphology and distribution. Nowadays, noble metals like silver, gold and platinum are commonly used in green chemistry without using toxic chemicals via a method involve the use of environmentally friendly processes as a simple and viable alternative to synthetic chemicals and physical processes [35]. Several researchers reported the biosynthesis and possible applications of metal nanoparticles by plant leaf extracts [36]. This work is the first to clarify the potential role of pomegranate peel and pistachio leaves extracts in their natural and nano forms to alleviate TAA-induced hepatic failure in the rat model. This research further explores a potential mechanism of action for both forms that could have therapeutic effects.

2. Materials and Methods.

2.1. Chemicals

All Chemicals, Silver nitrate [CAS #: 7761-88-8] & Purity 99.9% and thioacetamide [CAS# 62-55-5] & purity 98.5% were procured from Sigma-Aldrich (USA). The different solvents and chemicals used in the present study were of high analytical grade and purchased from a local vendor.

2.2. Authentication & preparation of the plant extracts

The pomegranate fruits were purchased from the local market at Cairo city, Egypt, 2018, and *Pistacia atlantica* leaves were collected from Beniwalid, Libya 2017. The plants materials were identified and authenticated by a plant taxonomist at the National Research Centre Herbarium (Cairo, Egypt) according to Veisi et al.[37]. Where a voucher specimen has been deposited for future reference. The pomegranates washed, cut into small pieces and left to dry in air, and the complete dryness occurred in an oven at 50°C. The dried peels were grounded to a fine powder. The pomegranate peel (PO) extract was obtained by addition 100 gm of pomegranate peel powder with 1000 ml boiled distilled water [Erlenmeyer flask] and boiled for 10 minutes with the help of Whatman filter paper (NO.3), the boiled materials were filtered to get aqueous fruit peel extract, which was used as such for metal nanoparticles synthesis. Finally stored at -4°C for further experiments. About 100 g of dried powder

of *Pistacia atlantica* leaves (PS) transferred into a 1000 mL Erlenmeyer flask with 500 mL of distilled water along with boiling for 30 min. The aqueous extract was filtered and stored at 4 °C [33].

High-performance liquid chromatography [HPLC]

HPLC analysis was carried out using an Agilent 1260 series. The separation was carried out using a Kromasil C18 column (4.6 mm x 250 mm i.d, 5 µm). The mobile phase consisted of water (A) and 0.05% trifluoroacetic acid in acetonitrile (B) at a flow rate 1 ml/min. The mobile phase was programmed consecutively in a linear gradient as follows: 0 min (82% A); 0–5 min (80% A); 5–8 min (60% A); 8–12 min (60% A); 12–15 min (85% A) and 15–16 min (82% A). The multi-wavelength detector was monitored at 280 nm. The injection volume was 10 µl for each of the sample solutions. The column temperature was maintained at 35 °C [Chart 1a, b], while *Pistacia atlantica* was evaluated by HPLC-MS system (Agilent Technologies 1100 series, Palo Alto, CA, USA). Chromatographic separation was carried out using a C18. The mobile phase consisted of eluent A (water containing 0.01% formic acid) and eluent B (acetonitrile). The separation was performed under gradient conditions from 5% B to 15% in 20 min, 20–40 min from 15 to 30%, and finally 55% B at 50 min. The flow rate was 0.1 mL/min. The injection volume was 10 µL. The diode array detector was set at 270 and 320 nm. [Chart 1 c].

2.3. Synthesis of nanoparticles

To synthesis silver nanoparticle (1MLAgNPs), silver nitrate (0.017 g) was added in 100 ml of double distilled water to prepare a 1mM silver nitrate solution. Then, 1 ml of aqueous extract of both extracts was added to 50 ml of silver nitrate (1 mM) solution to obtain silver nanoparticles. This setup was incubated in a dark chamber to minimize photo-activation of silver nitrate at room temperature under static conditions for the nucleation of the silver nanoparticles. The reduction of Ag to Ag₂O was confirmed by the color change of the solution from colorless to brown. [38]. The suspensions were subjected to centrifugation for the pellets to settle, which were then dried using a vacuum drier.

2.5 Characterization of the silver nanoparticles to measure the maximum production and activity of AgNPs, UV-visible spectra were taken using a Shimadzu UV-visible spectrophotometer.

2.4. Characterization of synthesized silver nanoparticles

2.4.1. UV-Vis spectral analysis was done by using Shimadzu Visible spectrophotometer (UV-1800,

Japan). The periodic scans of the optical absorbance between 300 and 700 nm with a double-beam UV-Visible spectrophotometer (Carry 100 with tungsten halogen light sources) were performed to investigate the reduction of silver ions by leaf extract. The presented results were obtained at room temperature.

2.4.2. Field emission scanning electron microscopy (FESEM):

The morphology, particle dispersion, and chemical composition of the prepared Nano-structures were investigated by (FESEM) (Quanta 450) equipped with EDS at accelerating voltage 30 kV. Crosscut samples were prepared by fracturing the membranes in liquid nitrogen. A thin layer of gold was coated on all the samples before microscopic analysis.

2.4.3. The Zeta potential of the biosynthesized AgNPs was performed on the Malvern Zeta size Nano series compact scattering spectrometer (Malvern Instruments Ltd.; Malvern, UK).

2.4.4. Transmission electron microscopy (TEM)

The particle size and surface morphology were analyzed using JEOL JEM 1230 transmission electron microscope (JEOL Ltd., USA). The samples for TEM analysis were cut into slices of a nominal thickness of 100 nm using an ultra-microtome with a diamond knife at ambient temperature. The cut samples were supported on a copper mesh for this analysis.

2.5. Animals

A total of 60 Wistar rats aged 2 months weighing 180–200 g, were taken from the breeding laboratory of the National research center and kept in an animal care facility. Rats were kept in polypropylene cages at room temperature with a 12 h light–dark cycle and relative humidity of 60% ± 1 %. Animals were provided purified water ad libitum with standard laboratory rat chow. The study was approved by the animal ethics committee of Institutional Review Board of National Research Center, having approval number 19 023 and the ethical guidelines for use and care of laboratory animals in experiments were strictly followed. The experimental animal protocol was designed to minimize pain or discomfort to the animals. TAA (Sigma, Aldrich Chemical Co., USA) was dissolved in physiological saline and the appropriate selected dose was injected 350 mg/kg b.w i.p. in 1-ml volume [39].

2.5.1. Experimental protocol

The rats were divided into 6 groups consisting of 10 rats each. **Group 1** was kept as negative control that received drinking water over 15 d along the

experimental period ; group II (TAA positive intoxicated rats injected intraperitoneally (IP) with 350 mg /Kg b.w of freshly prepared TAA in a single injection [39]. To prevent dehydration, 5 percent glucose solution was added to drinking water. OECD guidelines (test 423: Acute oral toxicity -Acute toxic class method; 2002) were followed to study the acute toxicity of both extracts on animal model. Based on the toxicity study, a daily dose of 2mls /100g b.w, was administered intraperitoneally, as follow. The third and fourth group were subjected to the same mentioned single dose of TAA, then after 24hrs they forced with 2mls/100g b.w of normal & nano pomegranate extracts respectively for 14 days, The fifth & sixth groups were subjected to the same mentioned single dose of TAA, then after 24hrs they forced with 2mls /100g b.w of normal & nano pistachio extracts respectively for 14 days. Throughout the experiment, the survival rate and BW were recorded. On day 14, the animals were sacrificed by cervical decapitation 24 hours after the last dose. Blood samples were immediately collected into sterilized tubes and let stand for 10 minutes to clot. The serum was separated by centrifugation at 3000 rpm for 10 min and stored at -80° C for assessment of liver functions and other biochemical analysis. Liver tissues were dissected and divided into two parts, the first part was fixed in 10% formaldehyde solution for histopathological and immunohistochemical examinations and the second part (1 g) was homogenized (1:5 w/v) in ice-cold 0.1 M potassium phosphate buffer (pH 7.4). The homogenate was centrifuged at 4,000 rpm for 15 minutes and the supernatant collected and liquated in Eppendorf tubes and stored at -20°C for different biochemical determinations.

2.6. Biochemical analysis

The liver function indices, Serum alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP), total lipid profile (TP), total cholesterol (TC), triglyceride (TG) and total bilirubin were estimated using biodiagnostic kits (Biogamma, Stanbio, West Germany). The oxidative stress markers, lipid peroxidation (LPO), glutathione (GSH), superoxide dismutase (SOD) and nitric oxide (NO), were evaluated using the methods of Buege and Aust [40], Moron et al [41] Nishikimi et.al[42],Moshage,et.al[43],Bradford[44]respectively. Liver total protein contents were estimated by the method of Masoom Karimi et al [45]. The DNA fragmentation index was evaluated using the comet assay technique as described by Slaoui and Fiette [46]. The proinflammatory cytokines TNF- α , IL-6, Alpha fetoprotein (AFP) and HSP 70 were assayed using commercially available ELISA kits (R&D, Minneapolis, MN).

2.7. Histopathological studies

A portion of the liver was preserved in 10% buffer formalin for at least 24 hr. and then processed and embedded in paraffin according to the standard protocol. Sections were cut into 5 μ m thick portions, transferred onto glass slides, and stained with haematoxylin and eosin (HE), observed microscopically for any histopathological and immunohistochemical changes as well [47].

2.7.1. Histopathological and immunohistochemical examinations

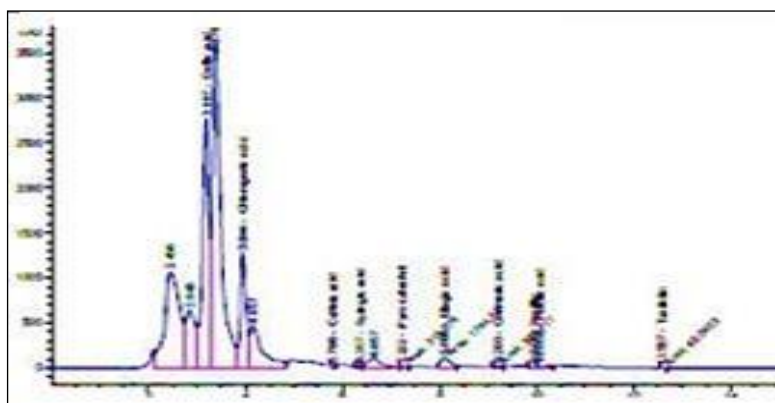
Five μ m thick sections of paraffinized liver stained with hematoxylin and eosin were examined histopathologically under Zeiss microscope (Carl Zeiss Microscopy GmbH 07745 Jena, Germany) with \times 200 magnification powers. For immunehistochemical examinations of IL6, TNF- α were performed on liver sections cuts from the paraffin blocks with a commercially available Anti-mouse IL6, TNF α antibodies (Santa Cruz Biotechnology, CA, USA) at the optimal working dilution of 1:100. Briefly, slides were sectioned at 4 μ m onto positively charged slides (Superfrost plus, Menzel-Glaser, Germany) and the slides were stained on an automated platform (DakoAutostainer Link 48). Heat-induced antigen retrieval was used for 30 min at 97°C in the high-PH EnVision™ FLEX Target Retrieval Solution and the primary antibody was used at a dilution of 1 in 100.The percent of positively stained brown cytoplasm (IL6, TNF α) were examined in 10 microscopic fields (under Zeiss light microscopy at \times 400).

2.8. Statistical analysis

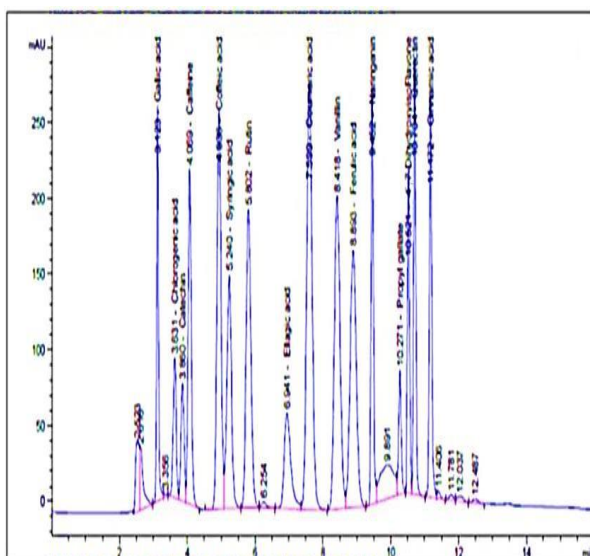
All data were expressed as mean \pm SD of ten rats in each group using the Costat computer program where an unshared letter is significant at $p < 0.05$. The correlations between all markers and inflammatory cytokines were analyzed using Pearson's correlation analysis test. The ROC-Curve method was done by SPSS version 16 and used to generate the Cut-off value. The area under the curve, Sensitivity, Specificity at $P < 0.05$ was set to be level of the significance [48].

3. Results:

Results of HPLC for pomegranate showed Gallic acid, chlorogenic acid and ellagic acid at concentrations of 160.89, 763.22 and 97.60 were the major components of the extract (Table 1-A, Chart 1a,b) while Sixteen polyphenol compounds were detected in pistachio, consisting of three major polyphenol groups, namely gallate, flavonoid and ellagic acid derivatives (Table 1-B, Chart 1c).



Chart[1a]:High-performance liquid chromatography (HPLC) of polyphenolic compounds detected in water extracts of pomegranate peel water extract.



Chart[1b]: High-performance liquid chromatography (HPLC) of Standard polyphenolic compounds

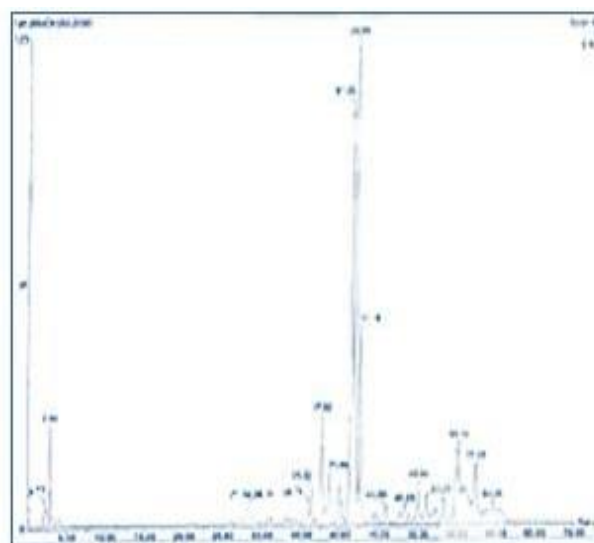


Chart [1c] High-performance liquid chromatography HPLC-MASS analysis of polyphenolic compounds in aqueous extract of *Pistacia atlantica* Desf.

Table 1: A. High-performance liquid chromatography of polyphenolic compounds detected in aqueous extracts of *Punica granatum*

CONTENTS	RTIME [MIN]	CONC. ($\mu\text{G}/\text{ML} = \mu\text{G}/0.1\text{ G}$)
Gallic acid	3.214	2160.89
Chlorogenic acid	3.925	763.22
Catechin	4.377	0.00
Methyl gallate	5.589	0.00
Coffeic acid	5.881	8.05
Syringic acid	6.425	8.22
Pyro catechol	7.130	32.00
Rutin	7.410	0.00
Ellagic acid	8.079	97.60
Coumaric acid	9.075	6.98
Vanillin	9.873	9.55
Ferulic acid	10.076	18.78
Naringenin	10.233	0.00
Taxifolin	12.449	4.67
Cinnamic acid	14.336	0.00
Kaempferol	14.731	0.00

B. High-performance liquid chromatography of polyphenolic compounds detected in aqueous extracts of *Pistacia atlantica*

Compounds	Mwt	RT
1. gallic acid	170	2.2
2. galloyl glucoside,	332	2.94
3. digalloyl glucosides	484	27.24
4. trigalloyl glucoside,	636	35.78
5. tetragalloyl glucosides	788	36.32
6. pentagalloyl glucoside	940	37.52
7. ,2''-O-galloyl-queracetin-3-O-galactoside	610	41.26
8. queracetin-3-O-rhamnogalactoside	610	41.79
9. queracetin-3-O-galactoside	464	41.79
10. ellagic acid diglucoside	626	42.46
11. luteolin-4'-O-glucoside	448	45.80
12. 2''-O-galloyl-luteolin-4'-O-glucoside	600	48.20
13. queracetin-3-O-glucuronide	478	49.94
14. kaempferol-3-O-glucoside	448	51.00
15. eriodictyol	288	55.14
16. luteolin	286	57.14
17. apigenin,	270	57.28
		59.55

3.1. Characterization of silver nanoparticles by UV-VIS absorption spectroscopy:

The current results revealed that the incubation of 50 ml of 1 mM AgNO₃ with 1 ml of Pomegranate & pistachio extract for 24 h at the room temperature led to the synthesis of AgNPs as indicated by the development of the brown color (Fig.1, Right). The absorption spectra of aqueous peel extracts obtained from Pomegranate plants, aqueous leaf extracts obtained from Pistachio were compared with the absorption spectra of silver nanoparticles prepared using these extracts to reveal the formation of silver phyto-nanoparticles. Further, UV-visible spectrophotometric analysis showing the maximum absorption of nanoparticles of both PO and PS at a wavelength of 425 nm (Fig.1, left).

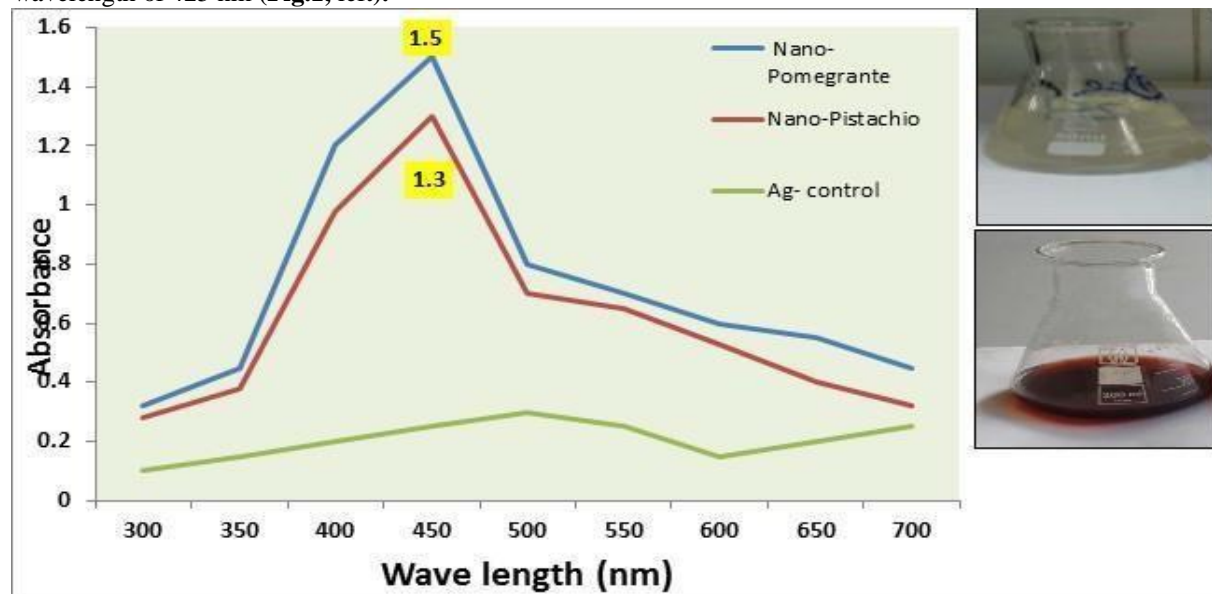


Fig .1: The visual observation of color changes of PO and PS nano extracts (Right) & UV-visible spectrophotometric analysis of nanoparticles of both extracts at maximum absorption wavelength of 425 nm (left).

3.2. Electron Microscope scanning:

Scanning electron microscope analysis showed that the synthesized AgNPs are polydispersed with particle

size range from 19-65 nm for Pistachio leaves **Fig. 2.** and 12-18 nm for Pomegranate peel **Fig. 3.**

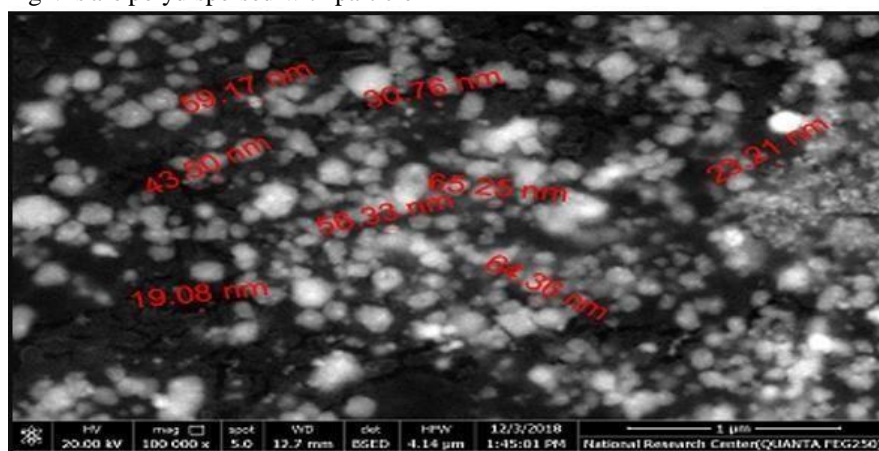


Fig. 2. SEM Pistachio nanoparticle micrograph with a size range from 19-65 nm.

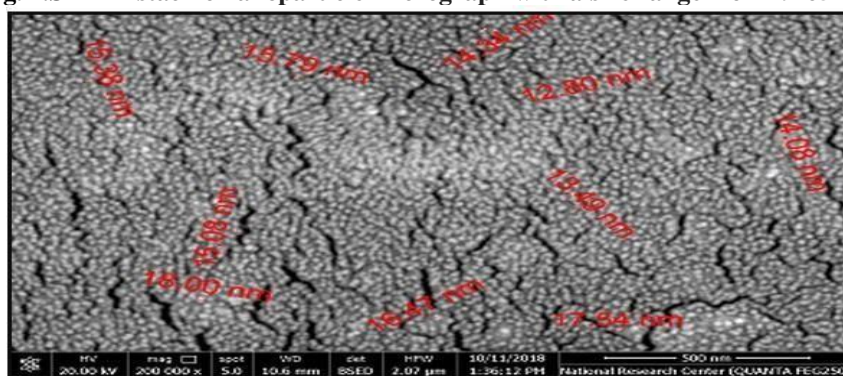


Fig. 3. SEM Pomegranate nanoparticle micrograph with a size range from 12-18 nm

3.3. Evaluation of physical stability of silver phytonanoparticles:

The physical stability of the silver nanostructures was evaluated in terms of ζ -potential. The surface charge of all the silver nanostructures of Pomegranate peel and Pistachio leaves were ranging from -10.6 to -13.3 mV, -17.6– 23.6 mV respectively.

3.4. Transmission electron microscopy (TEM)

TEM findings confirmed the spherical shape of the synthesized AgNPs with an average range of 4 to 42 nm, showing that the AgNPs pomegranate size is 17 nm **Fig.4**, whereas the AgNPs pistachio average size was 15 nm **Fig.5**.

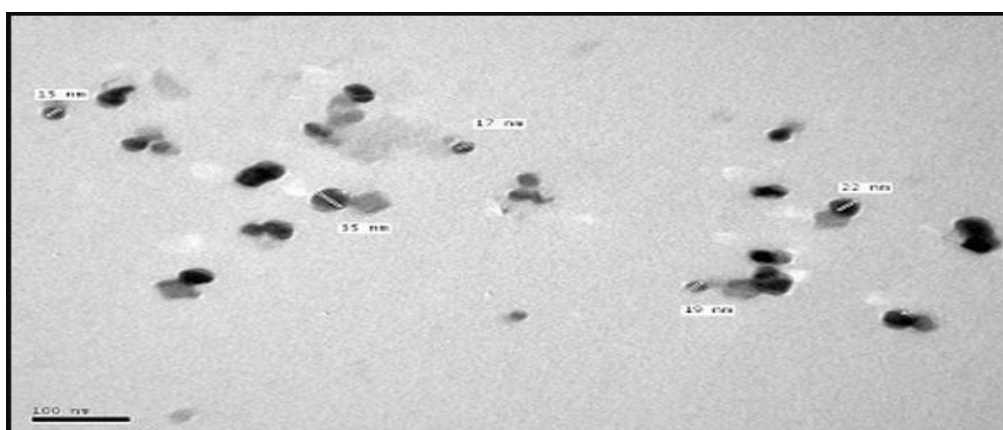


Fig. 4. TEM micrograph of Pomegranate silver nanoparticles, appear in spherical shape with mean size of 17 nm

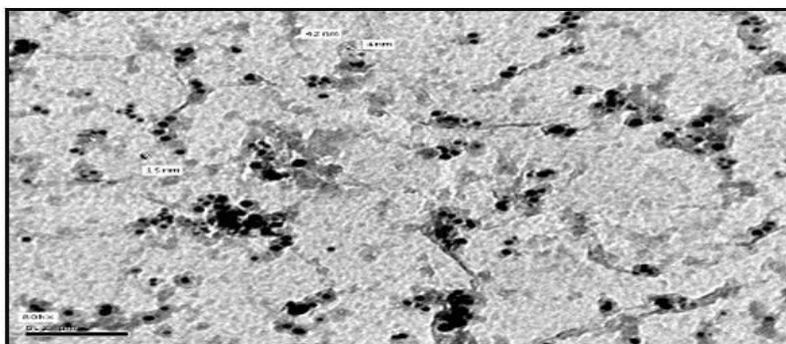


Fig. 5. TEM micrograph of Pistachio silver nanoparticles, appear in a- spherical shape with average meanzise of 15 nm.

3.5. Effect of pomegranate and pistachio in their normal and Nano forms on liver functions, lipid profile parameter:

Upon administration of TAA, there was a marked increase in serum activity, the liver function represented by (ALT, AST, and ALP) as well as bilirubin to approximately 2.5, 2.8, 2.15, and 1.93 fold, respectively, as well as a marked increase in the lipid profile represented by TP, TG, TC to approximately 2.6, 3, 2.2 fold, respectively, compared to the normal control group at $p < 0.0001$ (Table 2). Treatment of animals with pomegranate peel (PO) and pistachio leaves (PS) extract either in their normal or in nano forms (100 mg/kg) significantly decreased ALT, AST, and ALP levels. A notable increase in the level of bilirubin in

comparison with the challenged TAA group at $p < 0.0001$ (Table 2). Hence, the treatment showed very satisfying improvement levels as shown in Fig. 6. nano pistachio group showed the most highly improved % in liver function tests. On the other hand lipid profiles upon treatment with PO and PS in normal and nanoform a prominent significant reduction in (TP, TC, and TG) were recorded compared with TAA group at $p < 0.0001$ (Table 2). Therefore, we observed amelioration levels in these parameters as represented in Fig. 7. Whereas in total lipids, Pomegranate extract obtained the most percent improvement other than the remaining treatment classes, while Pistachio leaves extract showed a high percentage improvement in TC and TG parameters (Fig. 7) (Table 2).

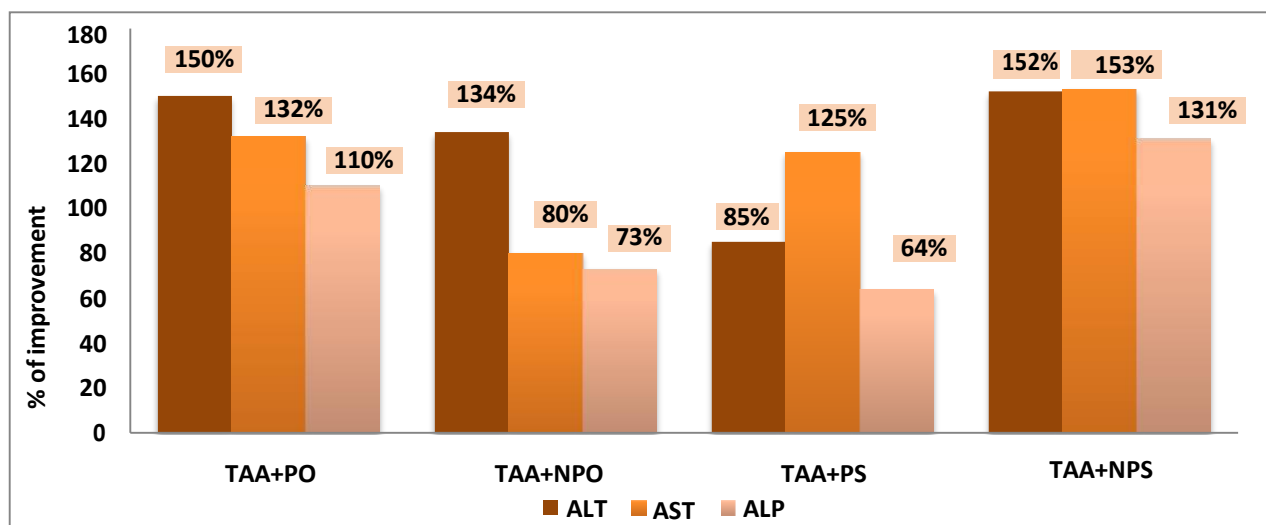


Figure (6) Therapeutic effects of both of Pomegranate and pistachio and its nanoparticles on serum ALT, AST and ALP in thioacetamide induced acute liver toxicity in rats. *Values are expressed as % of improvement related to normal control and thioacetamide groups.

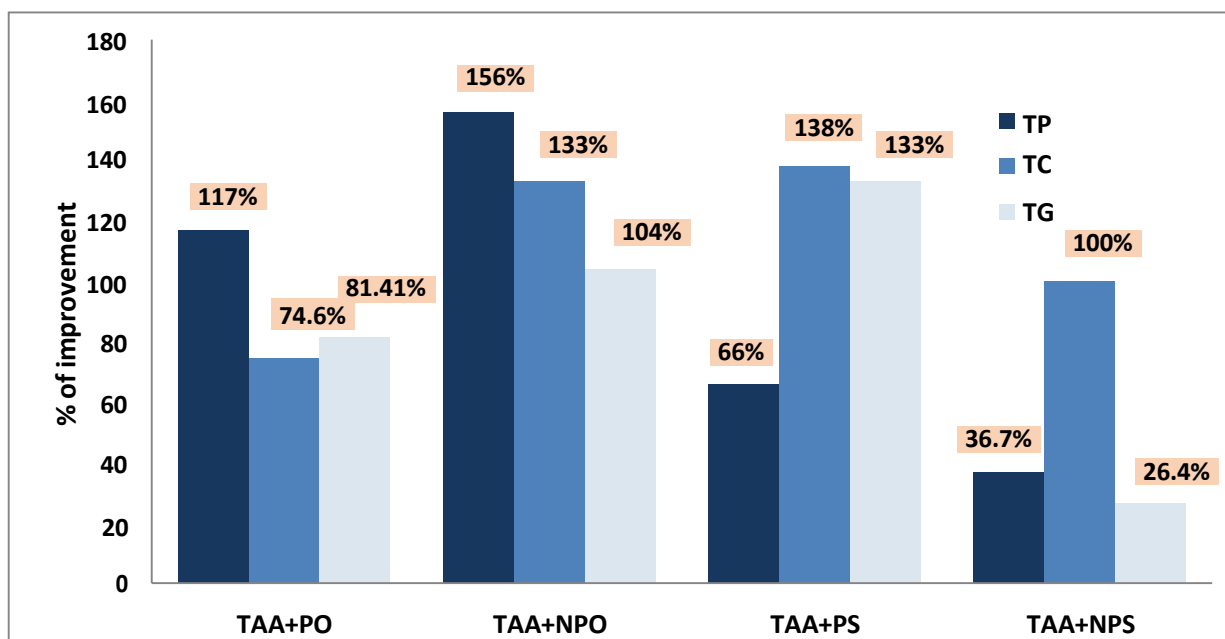


Figure (7) Therapeutic effects of both of Pomegranate and pistachio and its nanoparticles on serum Total lipid, Total cholesterol and Total glyceride in thioacetamide induced acute liver toxicity in rats. *Values are expressed as % of improvement related to normal control and thioacetamide groups.

Table (2): Therapeutic effects of both of *pomegranate*, *pistachio* and their nanoparticles on liver functions and lipid profile.

Groups Parameters	Control(-ve)	TAA (+ve)	TAA+PO	TAA+NPO	TAA+PS	TAA+NPS
ALT (U/ml)	48.6 ^c ±	121.4 ^a ±	48 ^c ±	55.8 ^c ±	79.6 ^b ±	47 ^c ±
AST (U/ml)	7.40 ±	9.04 ±	6.81 ±	6.72 ±	12.70 ±	5.41 ±
ALP (U/l)	54.4 ^d ±	157.2 ^a ±	85.25 ^c ±	113.8 ^b ±	89 ^c ±	74 ^c ±
Total Lipid (TP) (g/dl)	6.58 ±	10.70 ±	7.95 ±	9.67 ±	4.94 ±	17.70 ±
Total cholesterol (TC) (mg/dl)	108.48 ^{cd} ±	233.90 ^a ±	124.42 ^c ±	154.28 ^b ±	164.48 ^b ±	91.42 ^d ±
Total glyceride (TG) (mg/dl)	17.16 ±	15.72 ±	14.90 ±	16.58 ±	24.27 ±	5.60 ±
Total bilirubin (TB) (mg/dl)	0.402 ^c ±	0.909 ^a ±	0.439 ^c ±	0.58 ^b ±	0.609 ^b ±	0.610 ^b ±
	0.032 ±	0.06 ±	0.035 ±	0.057 ±	0.062 ±	0.066 ±
	40.20 ^c ±	90.02 ^a ±	63.49 ^b ±	36.22 ^d ±	34.52 ^d ±	36.05 ^d ±
	1.92 ±	2.12 ±	2.14 ±	2.18 ±	0.71 ±	2.70 ±
	96.32 ^e ±	297.1 ^a ±	146.08 ^d ±	196.6 ^c ±	278.2 ^a ±	250.8 ^b ±
	12.51 ±	18.9 ±	15.12 ±	22.45 ±	9.73 ±	8.75 ±
	1.172 ^d ±	2.26 ^b ±	1.83 ^c ±	2.57 ^b ±	3.44 ^a ±	2.57 ^b ±
	0.115 ±	0.22 ±	0.08 ±	0.22 ±	0.095 ±	0.33 ±

Each group has a mean ± SD of ten rats.

Statistical analysis was conducted using a one-way variance analysis (ANOVA) using the CoStat computer program with the lowest level (LSD) between $p < 0.05$ classes.

Groups with the same letters are not significantly different, whereas those with different letters vary considerably at $p < 0.05$.

3.6. Effect of pomegranate and pistachios in their normal and nanoforms on oxidative stress:

GSH content, SOD activity, LPO and Nitric oxide (NO) levels were taken as markers of oxidative status. An obvious depletion was seen in the levels of GSH & SOD with a prominent increase in each of LPO and NO levels in the challenged TAA intoxicated group at $p < 0.0001$ (Table 3, Fig. 8). The treatment with Pomegranate and Pistachio (100 mg/kg) either in normal or nano forms effectively increased the levels of both GSH and SOD $p < 0.05$. While levels of LPO and NO showed a prominent reduction upon treatment with both extracts (Table 3, Fig. 8). While its nano form of pistachio revealed more powerful as it restored the NO value to 1.58 compared to 3.41 in the challenged TAA group. The TAA-intoxication

resulted in a remarkable elevation at ($p < 0.05$) in the measured inflammatory markers as 2 fold and 2.5 fold in IL6 and TNF α consecutively and about 6 fold increase in the levels of AFP as well as HSP $_{70}$ compared to the TAA intoxicated group (Table 3, Fig. 9). Treatment with either pomegranate peel and or pistachio normal form extracts markedly reduced AFP, TNF α , IL6 and HSP $_{70}$ level respectively with the advantage of pistachio either in normal or Nano forms as it revealed a higher restoration power than pomegranate with the inflammatory markers as seen in Table 3. So the results showed evidence for each of pomegranate and pistachio either in their normal or Nano forms showed different percentages of improvement as shown in Fig. 9.

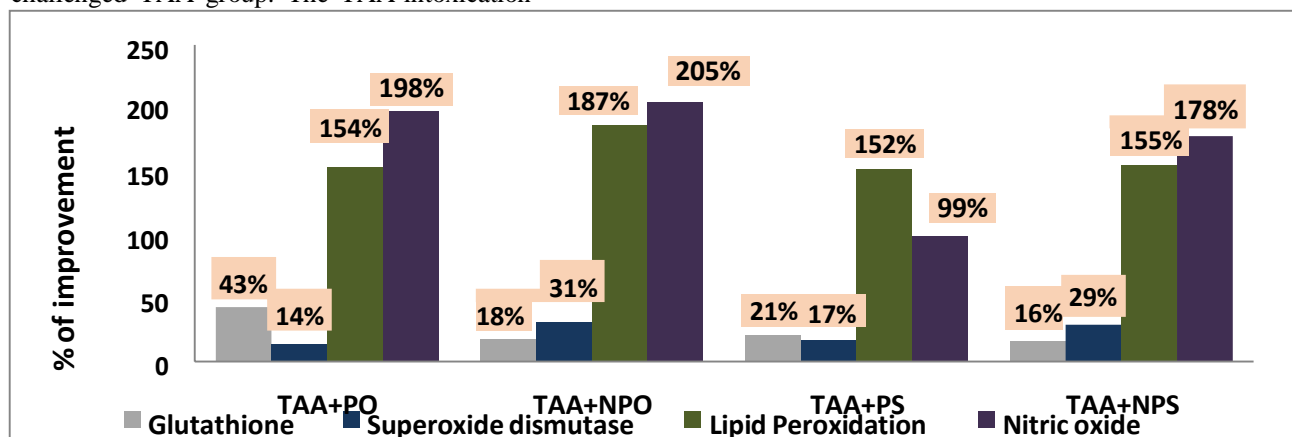


Figure (8) Therapeutic effects of both of Pomegranate and pistachio and its nanoparticles on oxidative stress markers in thioacetamide induced acute liver toxicity in rats. *Values are expressed as % of improvement related to normal control and thioacetamide groups.

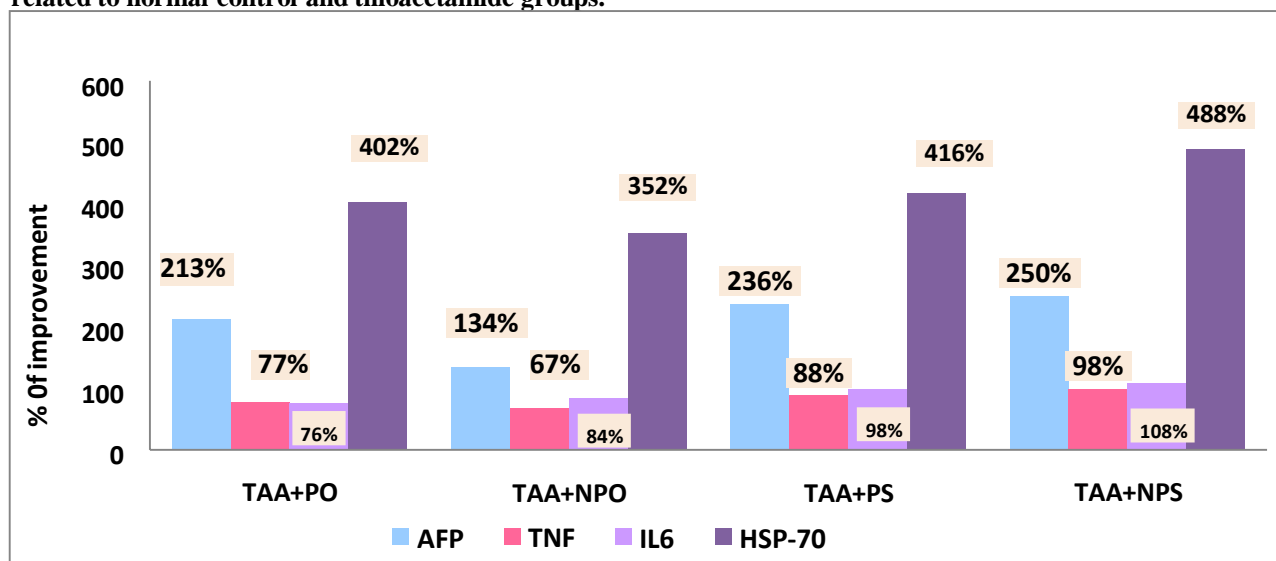


Figure (9) Therapeutic effects of both of Pomegranate and pistachio and its nanoparticles on inflammatory cytokines in thioacetamide induced acute liver toxicity in rats. *Values are expressed as % of improvement related to normal control and thioacetamide groups.

Table (3) Therapeutic effects of both of pomegranate, pistachio and their nanoparticles on oxidative stress markers and inflammatory cytokines.

Groups Parameters	Control (-ve)	TAA (+ve)	TAA+PO	TAA+NPO	TAA+PS	TAA+NPS
Glutathione (ug/mg protein)	48.50 ^a ± 2.62	21.80 ^d ± 3.02	42.20 ^b ± 2.40	30.43 ^c ± 1.68	31.98 ^c ± 3.06	29.97 ^c ± 4.13
Superoxide dismutase (Umol/mg protein)	0.123 ^a ± 0.008	0.048 ^d ± 0.004	0.065 ^c ± 0.007	0.087 ^b ± 0.009	0.069 ^c ± 0.006	0.083 ^b ± 0.006
Lipid Peroxidation (Umol /mg protein)	1.099 ^d ± 0.086	3.45 ^a ± 0.082	1.77 ^b ± 0.104	1.402 ^c ± 0.185	1.78 ^b ± 0.071	1.75 ^b ± 0.14
Nitric Oxide (mmol/g tissue)	1.034 ^d ± 0.18	3.41 ^a ± 0.31	1.37 ^{cd} ± 0.17	1.28 ^{cd} ± 0.12	2.39 ^b ± 0.14	1.58 ^c ± 0.21
AFP (ng/ml)	1.212 ^e ± 0.07	7.88 ^a ± 0.27	5.304 ^c ± 0.19	6.26 ^b ± 0.058	5.03 ^d ± 0.13	4.85 ^d ± 0.135
TNF (Pg/ml)	60.58 ^f ± 0.89	159.90 ^a ± 2.14	112.54 ^c ± 0.855	119.7 ^b ± 0.86	106.82 ^d ± 1.61	100.94 ^b ± 2.08
IL6 (Pg/ml)	93.87 ^f ± 2.39	236.77 ^a ± 9.95	165.95 ^b ± 2.71	158.36 ^c ± 1.89	145.67 ^d ± 3.60	135.47 ^e ± 2.39
HSP-70 (PG/mg)	111.75 ^f ± 4.90	791.69 ^a ± 6.65	344.24 ^c ± 4.95	400.34 ^b ± 7.40	329.39 ^d ± 6.56	249.78 ^e ± 4.74

The data are represented as average \pm SD of 10 rats in each group. Statistical analysis was conducted using a one-way variance analysis (ANOVA) using the CoStat computer program with the lowest level (LSD) between $p < 0.05$ classes. • Groups with the same letters are not significantly different, whereas those with different letters vary considerably at $p < 0.05$.

3.7. Effect of pomegranate and pistachio in their normal and nano forms on DNA degradation:

Comet assay is a sensitive and well-validated technique used to evaluate the fragmentation of DNA, a typical component of toxic DNA damage and apoptosis. Compared to the normal control group, TAA has substantially caused DNA damage. This DNA damage was demonstrated by a significant increase in the comet parameters presented as tail length with different three classes (Fig. 10, 11), Tail and tail moment DNA (percentage); the primary

Predictive parameter for DNA damage (Table 4). In the hepatotoxic community, there was a large broom-like tail and a small comet eye (Fig. 10, 11). The length of the tail increases as DNA damage increases as seen in Fig. 10, 11. Whereas, the administration of both pomegranate and pistachio in either their natural or nanoforms impaired the TAA effect by substantially reducing the length of the tail; with the dominance of the pomegranate and pistachio nano forms (Table 4, Fig. 12).

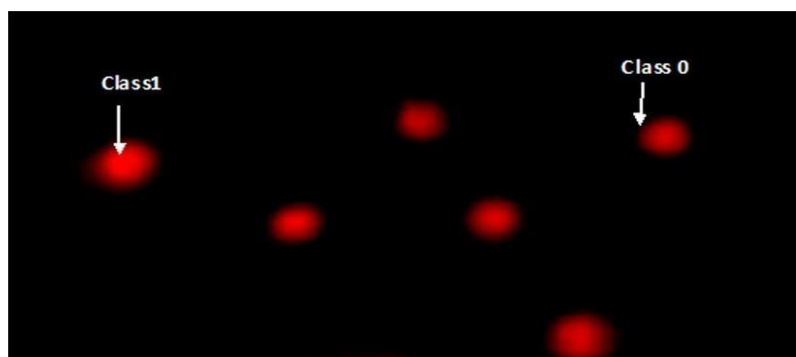


Fig. 10. Visual DNA damage score (class 0 and 1) using comet assay in liver tissues.

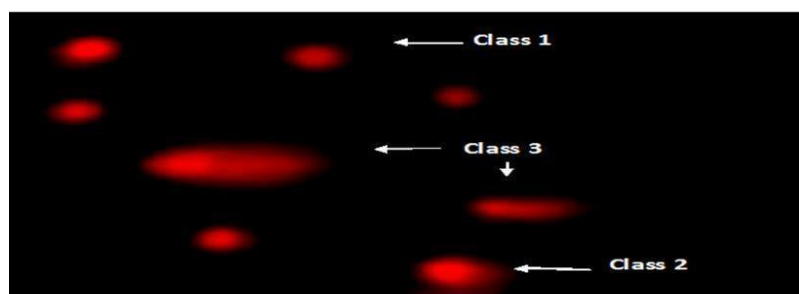


Fig. 11. Visual DNA damage score (classes 2 and 3) using comet assay in liver tissues.

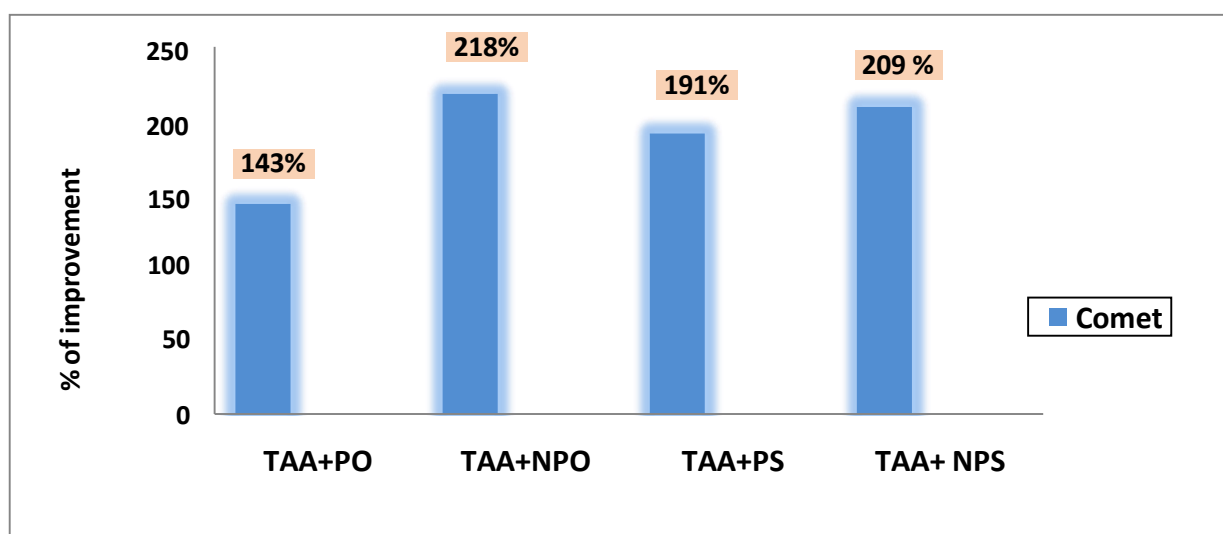


Fig.12: Therapeutic effects of both of Pomegranate and Pistachio and their nanoparticle forms on Comet assay. *Values are expressed as % of improvement related to normal control and thioacetamide groups.

Table (4): Therapeutic effects of both of pomegranate and pistachio and their nanoparticles on DNA damaged cells by comet assay.

Groups	cells No.		Comet class				Damaged DNA cells (mean ± SD)
	Analyzed	Total comets	0	1	2	3	
Negative control	500	33	467	25	8	0	6.61 ± 0.54 ^d
TAA	500	123	377	35	43	45	24.64 ± 1.14 ^a
TAA+PO	500	73	427	28	26	19	14.62 ± 0.89 ^b
TAA+NPO	500	51	449	24	17	10	10.20 ± 1.3 ^c
TAA+PS	500	61	439	21	23	17	12.22 ± 1.4 ^{bc}
TAA+NPS	500	54	446	16	22	16	10.81 ± 0.8 ^c

Class 0= no tail; 1= tail length < diameter of nucleus; 2= tail length between 1X and 2X the diameter of nucleus; and 3= tail length > 2X the diameter of nucleus. (*): No of cells analyzed were 100 peran animal.

3.8. Pearson’s correlations and ROC-Curve of the parameters in all groups.

Except for GSH and SOD activity, Table 5 shows positive correlations between liver functions, lipid profile, oxidative stress markers, and inflammatory

cytokines at various correlation levels. Table 6 shows the area under the curve AUC equal 1 and showed the same sensitivity and specificity values for virtually every biomarker.

Table (5) Pearson's correlations between liver function, lipid profile, oxidative markers and inflammatory cytokines.

R(Person Correlation)	AFP	TNF α	IL6	HSP70
ALT	0.621**	0.735**	0.773**	0.849**
AST	0.857**	0.905**	0.889**	0.928**
ALP	0.694**	0.778**	0.801**	0.863**
TG	0.733**	0.713**	0.643**	0.653**
TC	0.571**	0.714**	0.835**	0.816**
TP	0.763**	0.821**	0.805**	0.858**
TB	0.513**	0.398**	0.263	0.244
GSH	-0.804**	-0.786**	-0.684**	-0.734**
SOD	-0.854**	-0.885**	-0.873**	-0.830**
MDA	0.759**	0.862**	0.916**	0.924**
NO	0.680**	0.768**	0.804**	0.832**
AFP	1	0.977**	0.979**	0.886**
TNF α	0.977**	1	0.773**	0.849**
IL6	0.921**	0.979**	1	0.928**
HSP70	0.886**	0.961**	0.979**	1

* The Correlation is significant at the level of 0.05. ** The correlation is significant at the level of 0.01.

Table 6: Sensitivity & specificity of the measured parameters in all groups (ROC- curve)

Parameters	Groups	AUC	Cut –off value	Sensitivity %	Specificity %	P value	95% CI
AFP	TAA+ PO	1.000	6.555	100.0 %	100.0 %	0.009	1.000 - 1.000
	TAA + NPO	1.000	6.930	100.0 %	100.0 %	0.009	1.000 - 1.000
	TAA+PS	1.000	6.355	100.0 %	100.0 %	0.009	1.000 - 1.000
	TAA+ NPS	1.000	6.280	100.0 %	100.0 %	0.009	1.000 - 1.000
TNF α	TAA+PO	1.000	135.070	100.0 %	100.0 %	0.009	1.000 - 1.000
	TAA+NPO	1.000	138.770	100.0 %	100.0 %	0.009	1.000 - 1.000
	TAA+PS	1.000	132.885	100.0 %	100.0 %	0.009	1.000 - 1.000
	TAA+NPS	1.000	130.075	100.0 %	100.0 %	0.009	1.000 - 1.000
IL6	TAA+PO	1.000	196.930	100.0 %	100.0 %	0.009	1.000 - 1.000
	TAA+NPO	1.000	192.835	100.0 %	100.0 %	0.009	1.000 - 1.000
	TAA+PS	1.000	187.925	100.0 %	100.0 %	0.009	1.000 - 1.000
	TAA+NPS	1.000	182.290	100.0 %	100.0 %	0.009	1.000 - 1.000
HSP70	TAA+PO	1.000	567.575	100.0 %	100.0 %	0.009	1.000 - 1.000
	TAA+NPO	1.000	596.535	100.0 %	100.0 %	0.009	1.000 - 1.000
	TAA+PS	1.000	561.165	100.0 %	100.0 %	0.009	1.000 - 1.000
	TAA+NPS	1.000	520.700	100.0 %	100.0 %	0.009	1.000 - 1.000

3.9. Histopathologic examination

Histopathological analysis of the representative liver tissues of the control group showed normal morphology, hepatocyte structure and central vein (Fig. 13 A). TAA-intoxicated group Displayed Focal inflammatory cell infiltration in the hepatic parenchyma covering the central dilated vein Fig. 13 B, with a diffuse spread of Kupfer cells between hepatocytes. Pomegranate and /pistachio treatment 2mls/100g b.w either in normal or Nano forms have shown an observable healing effect presented as shown in (Fig. 13 C, D, E, F) with only some degenerative changes.

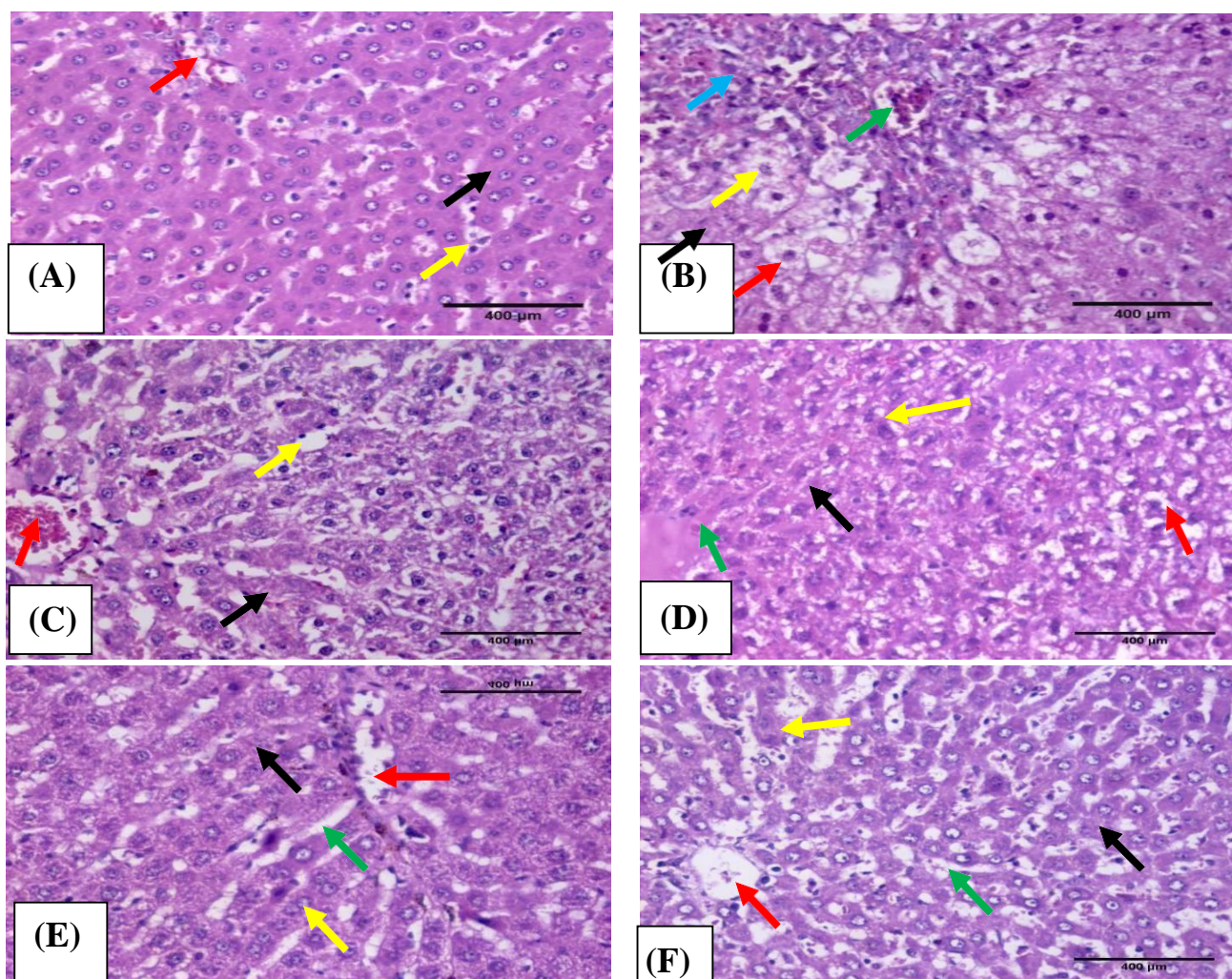


Fig. 13.A. The liver portion of the normal control group showed normal structure and morphology of the liver tissue, thin-plated hepatocytes (black arrow) and sinusoids (yellow arrow), central red vein. **B.** Liver section from animal with TTA group showed partial distortion of lobular hepatic architecture (black arrow), with moderate hydropic degeneration of hepatocyte (red arrow) and binucleated hepatocytes (yellow arrow), portal tract with vein congestion (green arrow) thick fibrous tissue in portal tract (blue arrow). **C.** Liver section of animal treated with pomegranate group displayed Hepatocytes arranged in thin plates (black arrow) and congested central vein (red arrow) with a preserved architecture, moderately dilated and congested sinusoids (yellow arrow). **D.** Liver section of animal treated Nano pomegranate group showed intact lobular hepatic architecture, hepatocyte with thin plates (black arrow) with moderate hydropic degeneration (red arrow) and binucleated hepatocytes (yellow arrow), central vein (green arrow). **E.** The liver section of the animal treated with Pistachio extract showed hepatic tissue intact lobular hepatic structure and architecture, hepatocytes arranged in thin plates with mild hydropic changes (black arrow) and central vein (red arrow), binucleated hepatocytes (yellow arrow), slightly dilated and congested sinusoids (green arrow). **F.** Liver section of animal treated with Nano Pistachio group showed Hepatic tissue with nearly normal structure and architecture, hepatocytes arranged in thin plates (black arrow) and congested central vein (red arrow) binucleated hepatocytes (yellow arrow), mildly dilated and congested sinusoids (green arrow) (H&E, x400).

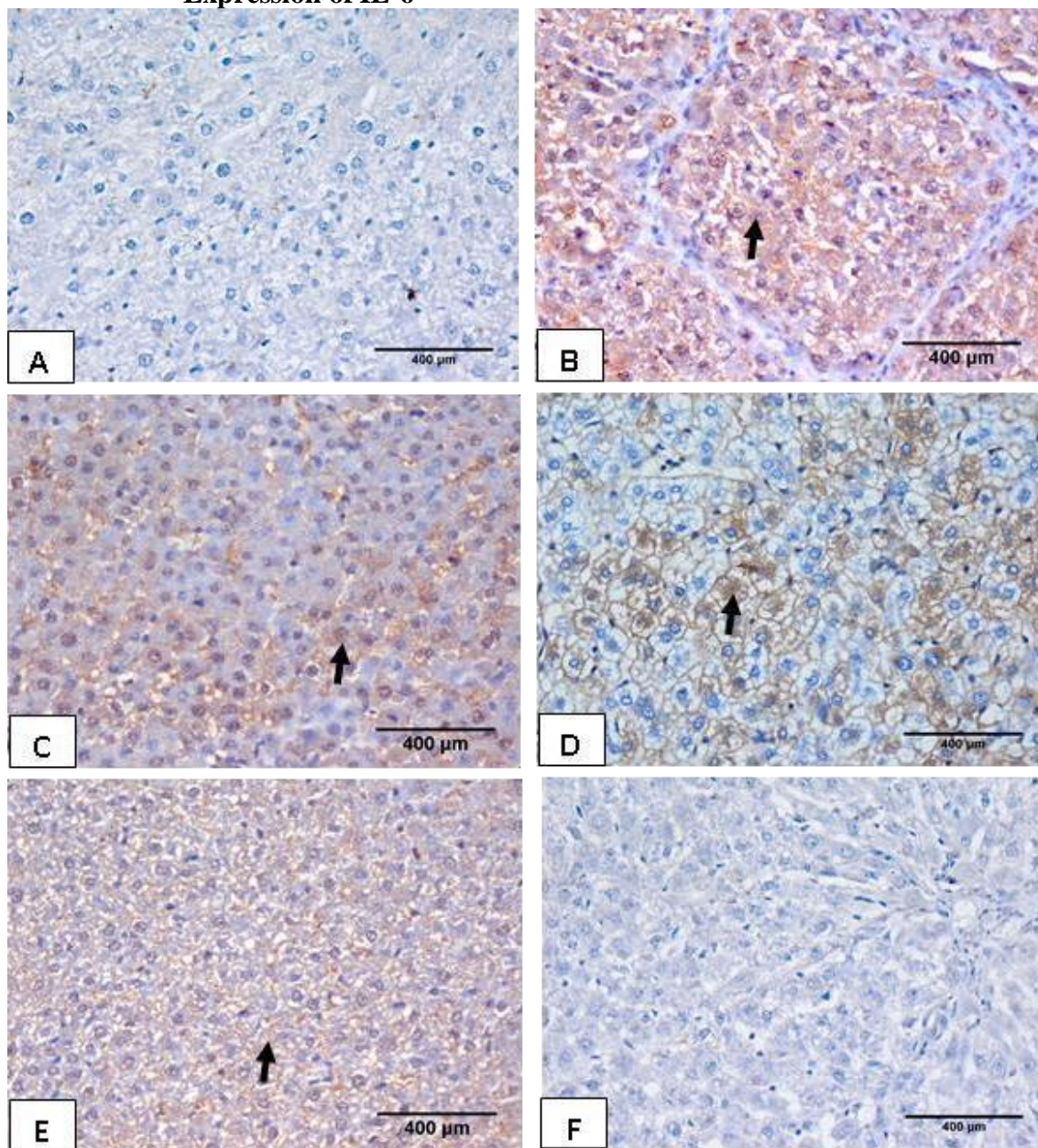
3.10. Effect of pomegranate / pistachio either in their normal and/or their Nano form on the inflammatory markers by immunohistochemistry. The anti-inflammatory effect of pomegranate / pistachio either in normal and/or Nano forms was further evaluated via immunohistochemical

expression of proinflammatory markers, IL-6 and TN- α in hepatic tissues (Fig. 14 A - L). TAA intoxication 350 mg/kg resulted in a substantial elevation in IL-6 and TNF- α expression compared to control group (Fig. 14, A, G) respectively that was located mostly in the cytoplasm of hepatocytes in portal tract and fibrous

septa. Treatment with pomegranate/and or pistachio either in their normal or nano forms improved and down-regulated the expression of both IL6 (Fig.14, B-F) and TNF- α (Fig.14, I - L) respectively, with the

notion has been shown that pomegranate / pistachio in its nano forms is more likely to inhibit TAA induced inflammatory response compared to the positive control (Fig.14.B -L).

Expression of IL-6



Expression of TNF- α

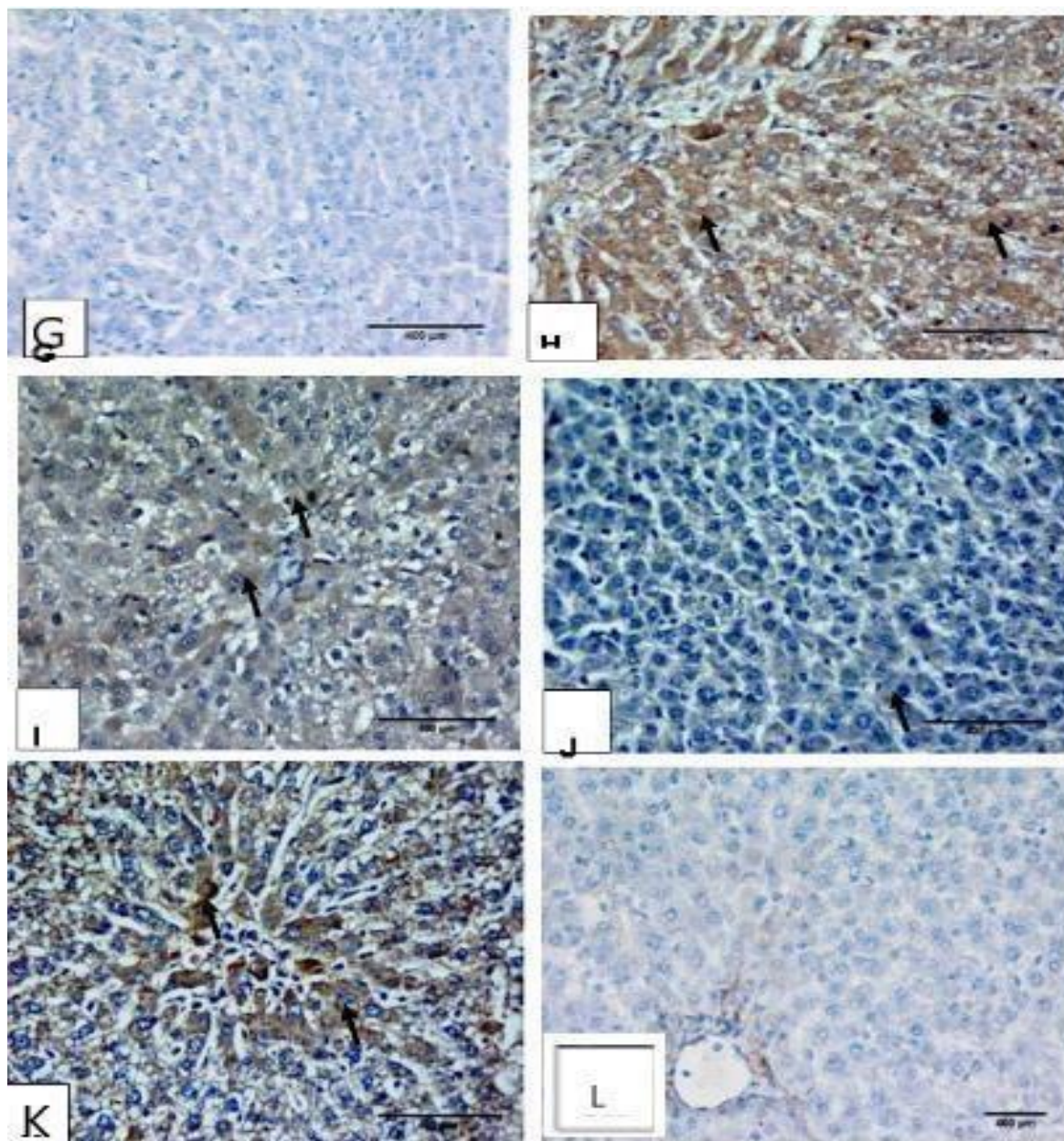


Fig. 14: **A.** liver section from normal control group showed negative expression of IL6, **B.** liver section from positive control group showed moderate expression of IL6 as brownish cytoplasmic stain of hepatocytes in about 60% (black arrow), **C.** liver section from animal treated with pomegranate group showed moderate expression of IL6 as brownish cytoplasmic stain of hepatocytes in about 40% (black arrow), **D.** liver section from animal treated Nano pomegranate group showed moderate expression of IL6 as brownish cytoplasmic stain of hepatocytes in about 10% (black arrow), **E.** liver section from animal treated Pistachio group showed mild expression of IL6 as brownish cytoplasmic stain of hepatocytes in about 25% (black arrow). **F.** liver section from animal treated Nano Pistachio group showed negative expression of IL6 (DAB, IHC, x400). **G.** liver section from normal control group showed negative expression of TNF α , **H.** liver section from positive control group showed marked expression of TNF α as brownish cytoplasmic stain of hepatocytes in about 70% (black arrow), **I.** liver section from animal treated with pomegranate group showed moderate expression of TNF α as brownish cytoplasmic stain of hepatocytes in about 40% (black arrow), **J.** liver section from animal treated Nano pomegranate group showed moderate expression of TNF α as brownish cytoplasmic stain of hepatocytes in about 20% (black arrow), **K.** liver section from animal treated Pistachio group showed moderate expression of TNF α as brownish cytoplasmic stain of hepatocytes in about 30% (black arrow), **L.** liver section from animal treated Nano Pistachio group showed negative expression of TNF α (DAB, IHC, x400).

4. Discussion:

Oxidative stress appears to play a major role in the outbreak and progression of a wide array of diseases, including the liver [49]. TAA has been one of the most widely reported to exhibit the damaging effect on liver, in sort of acute liver failure, liver fibrosis, cirrhosis and liver carcinogenicity and it was preferred over carbon tetrachloride (CCl₄)-induced liver toxicity model due to several features, such as higher reproducibility, less mortality, more reliability across different species, a longer latency period of damage induction with the human resemblance. Owing to its multiple effects, TAA was used to induce acute liver failure in rats model in the present study [50]. Emerging research evidence indicates that natural compounds/plants have shown beneficial effects of antioxidants in the defense of the liver against TAA-induced injury [51] and can improve immune function [52] either in normal or in nonfarm [53].

The results of the present study demonstrated the efficacy of the nanoparticles synthesized from the natural product as an excellent capping and reducing agent capable of producing pomegranate and pistachio nanoparticles (*P. Granatum L*) (*pistashia atlantic*) aqueous nano-extracts with average spherical size ranging from 7–17 nm. The present study showed that TAA-intoxication in a single dose 350 mg/kg/bw had superabundant hepatotoxic symptoms represented via the elevation in liver enzymes (AST, ALT and ALP), lipid profiles (TP, TC and TG) as well as total bilirubin (Table 2). This observation was close to Fontana et al [54] and Al shawsh et al [55] findings as they demonstrated that TAA has been shown to interfere with the continuous movement of RNA from the nucleus to the cytoplasm, may cause membrane injury resulting in elevation and release of serum markers of the liver [56]. Such leakage of the enzymes into the blood was accompanied in our study with partial distortion of lobular hepatic architecture, with moderate hydropic degeneration of hepatocyte and binucleated hepatocytes, portal tract with vein congestion thick fibrous tissue in the portal tract in addition to inflammatory infiltration of the liver which revealed severe liver injury (Table 2, Fig.13B). Interestingly, our findings demonstrated an obvious amelioration and improvement in all parameters when treated with pomegranate peel extract and /or pistachio either in normal or nano forms as indicated by reduction or normalization of the levels of aminotransferases, alkaline phosphatase, lipid profile, total bilirubin (Table 2, Fig. 6, 7). These improvements extend to liver architecture (Fig.13 C-F). These observations are most likely attributed to the presence of hydrolysable polyphenols, specifically ellagitannins, which were considered to be the most active antioxidants among

the tannins contained therein. These compounds (elagic acid, punicalagin, punicalin and gallic acid) have been shown to have increased antioxidant and pleiotropic effects and are believed to act in synergy [57]. Our findings were found to be similar to studies of Husain et al [58], Bachoual et al [59] and seem to be compatible with Esmailzadeh et al [60] as they demonstrated that pomegranate juice significantly reduces the total cholesterol level. The results also go in consistent with Al-Shaabi et al [61] as they mentioned that the administration of Pomegranate peel extract ameliorate liver enzymes, control lipogenesis, and improve the histologic image of the liver. And in agreement with Sheridan et al [62]; Kay et al [63] as they demonstrated that pistachio has a beneficial effect on the improvement of aminotransferase levels and blood lipid profile. This can be attributed to the existence of ellagitannins hydrolysable polyphenols as high antioxidant compounds, similar to that in pomegranate. In addition to other antioxidants that can effectively play a key role in stopping oxidative reactions in chains such as fatty acids, flavonoids, and phenolic compounds [32, 33] tocopherols, carotenes, lutein, selenium, and phytoestrogens [64].

In another observation, both nanoforms of pomegranate and pistachio treated groups showed significantly increased levels of bilirubin. The increased levels further described nanoforms of pistachio as protective polyphenols. These findings appear to be consistent with previous data related to Bilirubin IX α as it recognized as a potent antioxidant by Vitek et al. [65] while bilirubin is toxic in children, it is considered for health promotion in adults. At the same time, according to some clinical literature, moderately elevated serum bilirubin levels were generally correlated with decreased incidence of chronic disease [66].

A pronounced increment in lipid peroxidation and NO levels were recorded in hepatic homogenate. These results were concomitant with a pronounced, significant reduction in hepatic-GSH and SOD levels. The results can be delegated to the thionio-sulfur compound of TAA as a hepatotoxic which induce hepatic damage and act as an initiator for a series of severe inflammatory changes via generation of ROS and down regulator of the antioxidant defense mechanism. [67] Moreover In liver disease L-arginine-NO pathway is activated, and lead to the formation of nitric oxide, the matter which can explain the significant increase in nitric oxide level upon intoxication by TAA [68] (Table 3).

Interestingly, the great significant reduction effect on the lipid peroxidation level as well as nitric oxide synchronized with increment in glutathione and SOD levels were taken by the nano forms of each of pomegranate & pistachio extracts respectively as they proved to be more superior compared to their normal

form (**Table 3, Fig. 8**). The improvement registered in the measured redox parameters maybe in agreement with what previously reported that Stimulation of the Nrf2 /ARE pathway is necessary for antioxidant defense enzyme induction and intracellular GSH modulation in response to stress [69] (**Table 3**). These nano form results can be interpreted upon their benign nature and the presence of essential functional groups make AgNPs very stable and prevent nanoparticles from being anchored. Besides, the existence of the bioactive molecules on the surface of AgNPs may increase its efficacy and bioavailability. It can also enhance its solubility, improve plasma half-life, preventing degradation in the intestinal environment, and increasing permeation in the small intestine which may confer strong antioxidative activity [70].

Our findings are further consistent with the previous study that pomegranate peel extract had the highest free radical scavenging capacity among the medicinal plants tested and not only juice and oilseed [71, 72] and another study for Alturfan et al [73] demonstrated that pistachio consumption can provide antioxidant protection.

In the current study and following treatment with TAA high plasma levels of AFP, TNF- α , IL6, and HSP70 were observed in serum as well as in tissue (**Table 3, Fig. 9**). These results may meet the fact that alteration in the redox status would stimulate the entire inflammatory cascade including the activation of a series of transcription factors [74, 75]. AFP is regarded as a biomarker of proliferating liver stem cells under conditions of liver injury [76]. Our data showed a marked increase in AFP's post-TAA liver content by about 6 fold compared to [1.2ng/ml] in normal group (**Table 3**), nano pistachio was in front of its normal and other pomegranate forms to attenuate and improve the AFP over-production and protect its normal transcription levels in liver, This may explain the mechanism by which nano pistachio worked to prevent its abnormal expression by binding to the enhancer region of the AFP gene. It also may encourage the fetal hepatocyte to start to regenerate. This observation is under Hessin et al [77] who demonstrated that Pistachio can modulate the alpha-fetoprotein transcriptional levels.

TNF- α is a community of cytotoxic pro-inflammatory cytokines, believed to play a vital role in liver damage initiation [78]. Increased circulating TNF- α activates cell surface TNF- α receptors, resulting in activation of the stress-related protein kinases JNK and IKK β , leading to increased development of additional inflammatory cytokines such as IL6. TNF reduction is considered a treatment to reduce damage to the liver [79]. Surprisingly attenuation of the TAA-induced up-regulation of the cytokines TNF α , IL6 together was achieved by pomegranate and pistachio either in normal or in nanoforms. These data seem to be parallel with

Coursodon Boyle [80] who reported that the increased levels of proinflammatory cytokine IL 6 in rat ileum model necrotizing enterocolitis have been normalized after POM seed oil treatment and also go per the reports of Grace et al [81] and Paterniti et al [82], who were the first to reveal that Pistachio extract significantly reduced the non-mitochondrial oxidative burst in macrophages associated with inflammatory response. While the other attributed the decrease in TNF- α and Interleukins production to the presence of polyphenols in pistachios which possess antioxidant and anti-inflammatory properties. These data were demonstrated immunohistochemically in (**Fig.14**).

On the other hand, Heat-shock proteins (HSPs) are unique proteins that are synthesized rapidly in cells in response to different kinds of stress, and acting as intracellular protein repair [83]. A marked increase in HSP70 level was recorded following the single injection of TAA to be online with Kuramochi et al [84] who demonstrated that mRNA expression of HSP 70 tended to be increased with an injection of TAA. However, in our study down regulation of HSP70 expression level was achieved by each extracts (Pomo and Pistachio) by different percentages (**Table 3, Fig.9**), but again pistachio extract in its nano form was the most powerful as an anti-inflammatory compared to pomegranate. This is may be interpreted upon the powerful flavonoids beside other phenolic compounds benzoic acid, and hydroxycinnamic acid derivatives, that have antioxidant properties exist in the ---pistachio to induce strong HSP70 immunoreactivity, prevent protein denaturation and succeeded to overcome TAA induced liver damage [85, 86]. These data are parallel to what previously reported by Ibrahim et al. [87] who demonstrated that POM could prevent protein denaturation, as a way to combat hepatic oxidative stress caused by CCl₄ and also when he attributed the suppression of heat shock protein 70 and inducible nitric oxide synthase 3-nitrotyrosine to pomegranate emulsions [88].

Moreover, the current data demonstrated an excessive amount of ROS after surpassing various endogenous anti-oxidative defensive mechanisms oxidized the DNA biomolecules (**Fig10, 11**). Our finding was in agreement with Afifi et al [89], Zargar et al [15] who found a rise in hepatocyte DNA fragmentation following injection of TAA which may be attributed to the increase in cell death via both apoptosis and necrosis [90]. However, pomegranate and /or pistachio intervention either in normal or nano forms as antioxidants were significantly improved and increased DNA resistance to oxidant damage with the superiority of the nano forms of both plant extracts (**Table 4, Fig.12**). The current results may be consistent with that study of Ahmed et al [91] as they revealed that pomegranate extract with its strong

antioxidants, anti-inflammatory, anti-apoptotic and ATP-replenishing was able to protect rats from DNA damage. The same data was confirmed by Majidinia et al [92] as he proved polyphenols rich green extracts which are the main constituent of Pistachio leaves and Pomegranate peel as the main effector in decreasing the ROS level and DNA fragmentation. Eventually,

Conclusion:

Our findings indicated the potential and beneficial role of either pomegranate or pistachio in their normal forms in general and/or in their nano particles in particular, improving the deleterious effects of TAA oxidative stress-mediated damage and reinforcing the antioxidant defense mechanism through its rich

the authors verified the findings by positive correlations between the entire markers measured (**Table 5**) Specificity & sensitivity for all markers were further determined by Roc curve and the region under the curve was shown to be equal to 1 (**Table 6**) which concluded that the measured parameters are good markers in case of acute liver failure.

antioxidant and anti-inflammatory properties material. Moreover, it also reflects the potential of plant extracts to integrate biomolecules that serve as both a reduction and stabilization agents for producing stable and shape-controlled AgNPs with effective medicinal benefits that could contribute to the development of new drugs for acute liver disease.

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Conflicts of interest:

The authors declare no potential conflicts of interest.

Reference:-

1. T.Wang, R.Men, M.Hu, X.Fan, X.Yang, X.Huang, L. Yang, Protective effects of *Punicagranatum* (pomegranate) peel extract on concanavalin A-induced autoimmune hepatitis in mice. *Biomed .Pharmacother* (2018) 100, 213-220.
2. Y. L.Wu , X. C.Feng, L. H.Lian, Y. Z.Jiang, J. X. Nan, The protective effects of total saponins from *Ornithogalumsaundersiae* (Liliaceae) on acute hepatic failure induced by lipopolysaccharide and d-galactosamine in mice. *J. Ethnopharmacol* 132 (2010) (2), 450-455.
3. W. M.Lee, R. T.Stravitz, A.M .Larson, Introduction to the revised American Association for the Study of Liver Diseases Position Paper on acute liver failure. *Hepatology* 55 (2012) (3), 965-967.
4. G.Tritto, N. A.Davies, R. Jalan, Liver replacement therapy. In *Seminars in respiratory and critical care medicine* 33 (2012) 01(70-79). Theme Medical Publishers.
5. K. A.Wlodzimirow, S.Eslami, A.Abu-Hanna, M. Nieuwoudt, R. A. F.M. Chamuleau, Systematic review: acute liver failure—one disease, more than 40 definitions. *Aliment.Pharmacol.Ther.* 35 (2012)(11), 1245-1256.
6. W.Bernal, J. Wendon, Acute liver failure. *N. Engl. J. Med.* 369 (2013)(26), 2525-2534.
7. A.G.Bonavita, K.Quaresma, V.Cotta-de-Almeida, M. A.Pinto, R. M.Saraiva, L. A. Alves, Hepatocyte xenotransplantation for treating liver disease. *Xenotransplantation* 17 (2010) 3, 181-187.
8. Y.Yu, J. E.Fisher, J.B.Lillegard, B.Rodysill, B.Amiot, S. L. Nyberg, Cell therapies for liver diseases. *Liver Transplant.* 18 (2012)(1), 9-21.
9. R. Bañares, M. V. Catalina, J. V.aquero, Liver support systems: will they ever reach prime time. *Curr.Gastroenterol.Rep* 15(2013) (3), 312.
10. R.F.Butterworth, M.D.Norenberg, V.Felipo, P.Ferenci, J.Albrecht, A.T.Blei, ISHEN Commission on Experimental Models of HEJ. Experimental models of hepatic encephalopathy: ISHEN guidelines. *Liver International.* 29 (2009) (6):783-788.
11. M.J.Tuñón, M.Alvarez, J.M.Culebras, J. González-Gallego, An overview of animal models for investigating the pathogenesis and therapeutic strategies in acute hepatic failure. *World J. Gastroenterol.* 15 (2009) (25):3086.
12. Y.M.Koen, D.Sarma, H.Hajovsky, N.A. Galeva, T.D.Williams, J.L.Staudinger, R.P. Hanzlik, Protein targets of thioacetamide metabolites in rat hepatocytes. *Chem. Res. Toxicol.* 26 (2013)(4):564-574.
13. D.Cheng, W.Xu, L.Yuan, X. Zhang, Investigation of drug-induced hepatotoxicity and its remediation pathway with reaction-based fluorescent probes. *Anal. Chem.* 89 (2017)(14):7693-7700.
14. L.F.Quintanilha, T.Takami, Y.Hirose, K.Fujisawa, Y.Murata, N.Yamamoto, R.C.Goldenberg, S.Terai, I. Sakaida, Canine mesenchymal stem cells show antioxidant properties against thioacetamide induced liver injury in vitro and in vivo. *Hepatology Research* 44 (2014)(10):E206-217.
15. S.Zargar, T.A.Wani, A.A.Alamro, M.A. Ganaie, Amelioration of thioacetamide-induced liver toxicity in Wistar rats by rutin. *Int. J.Immunopathol Pharmacol.* 30 (2017) (3):207-214.
16. S.A.Hassan, M. El sherbiny, A.N. Hashim, The remarkable role of *Rosemary & Tamarixaphylla* against radiation based therapy. *Int J Pharma Bio Sci* (2017) (4): (B) 292-301
17. K.Okaiyeto, U.U. Nwodo, L.V. Mabinya, A.I. Okoh, A review on some medicinal plants with hepatoprotective effects. *Pharmacogn Rev* 12 (2018)(24):186.
18. S.A. Hassan, A.M. Hagrassi, O. Hammam, A.M. Soliman, E .Ezzeldin, W.M. Aziz. Brassica juncea L.(Mustard) Extract Silver NanoParticles and Knocking off Oxidative Stress, ProInflammatory Cytokine and Reverse DNA Genotoxicity. *Biomolecules.* 2020 Dec; 10(12):1650.
19. M.G.Miguel, M.A.Neves, M.D. Antunes, Pomegranate (*Punicagranatum* L.): A medicinal plant with myriad biological properties- A short review. *J. Med. Plant Res.* 4 (2010)(25):2836-2847.
20. A.Fadavi, M.Barzegar, M.H.Azizi, M. Bayat, Physicochemical composition of ten pomegranate cultivars (*Punicagranatum* L.) grown in Iran. *FOOD SCI TECHNOL INT.* 11 (2005) (2):113-119.
21. E.P .Lansky, R.A .Newman, *Punicagranatum* (pomegranate) and its potential for prevention and treatment of inflammation and cancer. *J. Ethnopharmacol.* 109 (2007)(2):177-206.
22. I. Celik, A. Temur, I. Isik , Hepatoprotective role and antioxidant capacity of pomegranate (*Punicagranatum*) flowers infusion against

- trichloroacetic acid-exposed in rats. *Food Chem Toxicol* 147 (2009)1:145-149.
23. S.Banihani, S.Swedan, Z. Alguraan ,Pomegranate and type2 diabetes. *Nutr. Res. Rev.* (2013) (5) :341-348.
 24. T. Ismail, P. Sestili, S. Akhtar,Pomegranate peel and fruit extracts: a review of potential anti-inflammatory and anti-infective effects. *J. Ethnopharmacol.*143(2012) (2),397-405.
 25. X.We, S.Li, T.Li, L.Liu, Y.Liu, H.Wang,M. Zhao,Pomegranate peel extract ameliorates liver fibrosis induced by carbon tetrachloride in rats through suppressing p38MAPK/Nrf2 pathway. *J. Funct. Foods*(2019)103712.
 26. N.Z.Shaban, M.A.El-Kersh, F.H.El-Rashidy, N.H. Habashy,Protective role of Punicagranatum (pomegranate) peel and seed oil extracts on diethylnitrosamine and phenobarbital-induced hepatic injury in male rats. *Food Chem.*141(2013)(3):1587- 1596.
 27. P.Sharma, S.McClees, F. Afaq,Pomegranate for prevention and treatment of cancer: an update. *Molecules*22 (2017)(1):177.
 28. V.Sorrenti, C.L.Randazzo, C.Caggia, G.Ballistreri, F.V.Romeo, S.Fabroni, N.Timpanaro, M.Raffaele, L. Vanella,Beneficial Effects of Pomegranate Peel Extract and Probioticson Pre-adipocyte Differentiation. *Front. Microbiol*(2019)10, 660.
 29. M.Bozorgi, Z.Memariani, M.Mobli, M.H. SalehiSurmaghi, M.R.Shams-Ardekani, R. Rahimi,Five Pistacia species (P. vera, P. atlantica, P. terebinthus, P. khinjuk, and P. lentiscus): a review of their traditional uses, phytochemistry, and pharmacology. *. Sci. World J.*(2013)2013.
 30. W.Bhouri, S.Derbel, I.Skandrani, J.Boubaker, I.Bouhleb, M.B.Sghaier, S.Kilani, A.M.Mariotte, M.G.Dijoux-Franca, K. Ghedira, L. Chekir-Ghedira,Study of genotoxic, antigenotoxic and antioxidant activities of the digallic acid isolated from Pistacialentiscusfruits. *Toxicol In Vitro.*24 (2010)(2):509-515.
 31. M.Ammari, H.Othman, A.Hajri, M.Sakly, H. Abdelmelek,Pistacialentiscus oil attenuates memory dysfunction and decreases levels of biomarkers of oxidative stress induced by lipopolysaccharide in rats. *Brain Res. Bull.* (2018)140:140-147.
 32. V.Samavati, M. Adeli,Isolation and characterization of hydrophobic compounds from carbohydrate matrix of Pistaciaatlantica. *Carbohydr.Polym.*(2014)101:890-896.
 33. Z.B.Ahmed, M.Yousfi, J.Viaene, B.Dejaegher, K.Demeyer, D.Mangelings, Vander Y. Heyden,Antioxidant activities of Pistaciaatlantica extracts modeled as a function ofchromatographic fingerprints in order to identify antioxidant markers. *MICROCHEM J.*128 (2016) 208-217.
 34. M.Ghasemynasabparizi, A.Ahmadi, S.M. Mazloomi,A review on pistachio: Its composition and benefits regarding the prevention or treatment of diseases. *J OCCUP HEALTH.*4 (2015) 1:57-69.
 35. V.Pareek, A.Bhargava, R.Gupta, N.Jain, J. Panwar,Synthesis and applications of noble metal nanoparticles: a review. *Adv. Sci. Eng. Med.* 9 (2017) 7:527-544.
 36. C.L.Keat, A.Aziz, A.M.Eid, N.A. Elmarzugi,Biosynthesis of nanoparticles and silver nanoparticles. *Bioresour Bioprocess.* (2015)(1):47.
 37. H.Veisi, A.R.Faraji, S. Hemmati, A. Gil, Green synthesis of palladium nanoparticles using Pistaciaatlantica kurdica gum and their catalytic performance in Mizoroki–Heck and Suzuki–Miyaura coupling reactions in aqueous solutions. *Appl Organomet Chem.*(2015)(8):517-23.
 38. S.Shankar, X.Teng, G.Li, J.W. Rhim, Preparation, characterization, and antimicrobial activity of gelatin/ ZnO nanocomposite films. *Food Hydrocoll.* .45(2015) 264-2671.
 39. E.Koblihová, I.Mrazova, Z.Vernerova, M. Ryska,Acute liver failure induced by thioacetamide: selection of optimal dosage in Wistar and Lewis rats. *Physiol Res*1.(2014)63(4).
 40. J.A.Buege, S.D. Aust,Microsomal lipid peroxidation.In *Methods in enzymology* (1978) (Vol. 52, pp. 302-310).Academic Press.
 41. M.S. Moron, J.W.Depierre, B. Mannervik, Levels of glutathione, glutathione reductase and glutathione S-transferase activities in rat lung and liver. *Biochimica et BiophysicaActa (BBA)-General Subjects.*.5(1979)82(1):67-78.
 42. M Nishikimi, NA Rao, K. Yagi,The occurrence of superoxide anion in the reaction of reduced phenazinemethosulfate and molecular oxygen. *BIOCHEMBIOPH RES CO.*46 (1972) (2): 849-854.
 43. H.Moshage, B.Kok, J.R.Huizenga, P.L. Jansen,Nitrite and nitrate determinations in plasma: a critical evaluation. *CLIN CHEM.*41 (1995)(6):892-896.
 44. M.M. Bradford,A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem.*72 (1976) (1-2), 248-254.
 45. M.MasoomiKarimi, M.JafariSani, A.Zaree Mahmud abadi, S.R. KhatibiEffect of acute toxicity of cadmium in mice kidney cells. *IJT.*6 (2012) (18):691-698.
 46. M. Slaoui, L. FietteHistopathology procedures: from tissue sampling to histopathologica evaluation. In *Drug safety Evaluation* (2011) (pp. 69-82). Humana Press.
 47. M.H.Zweig, G. Campbell Receiver-operating characteristic (ROC) plots: a fundamental

- evaluation tool in clinical medicine. *Clin chem.* 39 (1993) (4):561-577.
48. H.Jaeschke, M.R.McGill, A. Rama chandran Oxidant stress, mitochondria, and cell death mechanisms in drug-induced liver injury: lessons learned from acetaminophen hepatotoxicity. *Drug metabolism reviews.* 44 (2012)(1):88-106.
49. M.C. Wallace, K. Hamesch, M. Lunova, Y. Kim, R. Weiskirchen, P. Strnad S.L., Friedman. Standard operating procedures in experimental liver research: thioacetamide model in mice and rats, *Lab. Anim.* (2015); 49 21e29.
50. P.N. Ansil, A .Nitha, S.P. Prabha, et al. Protective effect of *Amorphophallus campanulatus* (Roxb.) Blume. tuber against thioacetamide induced oxidative stress in rats. *Asian Pac J of Trop.Med* (2011); 4: 870–877
51. S.Li, H.Y.Tan, N.Wang, F.Cheung, M.Hong, Y. Feng, The potential and action mechanism of polyphenols in the treatment of liver diseases. *Oxid. Med. Cell. Longev.* 2018; 2018.
52. C.Ricordi, M.Garcia-Contreras, S. Farnetti, Diet and inflammation: possible effects on immunity, chronic diseases, and life span. *J Am Coll Nutr.* 34 (2015)(sup1):10-13.
53. B.Şahin, E.Demir, A.Aygün, H.Gündüz, F. Şen, Investigation of the effect of pomegranate extract and monodisperse silver nanoparticle combination on MCF-7 cell line. *J. Biotechnol.* (2017) 260:79-83.
54. L.Fontana, E.Moreira, M.I.Torres, M.I. Fernández, A.Rios, F.S.De Medina, A. Gil Serum amino acid changes in rats with thioacetamide-induced liver cirrhosis. *Toxicol.* 106 (1996)(1-3) : 197-206.
55. M.A.Alshawsh, M.A.Abdulla, S.Ismail, Z.A. Amin, Hepatoprotective effects of *Orthosiphonstamineus* extract on thioacetamide-induced liver cirrhosis in rats. *Evid.Based Complementary Altern.Med.* (2011)2011.
56. S.Binduja, P.K.Visen, R.Dayal, D.P.Agarwal, G.K. Patnaik, Protective action of ursolic acid against chemical induced hepato-toxicity in rats. *Indian J Pharmacol.* 28 (1996)(4):232.
57. Z.Amri, F.Zaouay, H.Lazreg-Aref, H.Soltana, A.Mneri, M.Mars, M. Hammami, Phytochemical content, fatty acids composition and antioxidant potential of different pomegranate parts: comparison between edible and non-edible varieties grown in Tunisia. *Int. J. Biol. Macromol.* (2017) 104:274-280.
58. H.Husain, U.Latief, R. Ahmad, Pomegranate action in curbing the incidence of liver injury triggered by Diethylnitrosamine by declining oxidative stress via Nrf2 and NFκB regulation. *Sci. Rep.* 8 (2018) (1):8606.
59. R.Bachoual, W.Talmoudi, T.Boussetta, F.Braut, J. El-Benna An aqueous pomegranate peel extract inhibits neutrophil myeloperoxidase in vitro and attenuates lung inflammation in mice. *Food Chem. Toxicol.* 49 (2011) 6:1224-1228.
60. A. Esmailzadeh, F.Tahbaz, I.Gaieni, H.Alavi-Majd, L. Azadbakht, Cholesterol-lowering effect of concentrated pomegranate juice consumption in type II diabetic patients with hyperlipidemia. *Int J Vitam Nutr Res.* 76 (2006)3:147-151.
61. S.N.Al-Shaabi, M.I.Waly, L.Al-Subhi, M.H. Tageldin, N.M.Al-Balushi, M.S. Rahman, Ameliorative effects of pomegranate peel extract against dietary-induced nonalcoholic fatty liver in rats. *Prev Nutr Food Sci.* 21 (2016)(1):14.
62. M.J.Sheridan, J.N.Cooper, M.Erario, C.E. Cheifetz, Pistachio nut consumption and serum lipid levels. *J Am Coll Nutr.* 26 (2007)(2):141-148.
63. C.D.Kay, S.K.Gebauer, S.G.West, P.M. Kris-Etherton, Pistachios increase serum antioxidants and lower serum oxidized-LDL in hypercholesterolemic adults. *Nutr. J.* 140 (2010) (6):1093-1098.
64. X.Zhao, H.Sun, A.Hou, Q.Zhao, T.Wei, W. Xin, Antioxidant properties of two gallotannins isolated from the leaves of *Pistacia weinmannifolia*. *Biochim. Biophys. Acta, (BBA)-General Subjects.* 1725 (2005)(1):103-110.
65. L.Vitek, M.Jirsa Jr, M.Brodanová, M.Kaláb, Z.Mareček, V.Danzig, L.Novotný, P. Kotal, Gilbert syndrome and ischemic heart disease: a protective effect of elevated bilirubin levels. *Atherosclerosis.* 160 (2002)(2):449-456.
66. A.R.Hamoud, L.Weaver, D.E.Stec, T.D. Hinds Jr, Bilirubin in the liver-gut signaling Axis. *Trends Endocrinol Metab.* 29 (2018) (3): 140-150.
67. F.Li, F.Lang, Y.Wang, C.Zhai, C.Zhang, L.Zhang, E. Hao, Cyanidin ameliorates endotoxin-induced myocardial toxicity by modulating inflammation and oxidative stress through mitochondria and other factors. *Food Chem Toxicol.* (2018) 120:104-111.
68. Y.Iwakiri, M.Y. Kim, Nitric oxide in liver diseases. *Trends pharmacol.Sci.* 36 (2015) (8): 524-536.
69. X.Liu, K.Chen, L.Zhu, H.Liu, T.Ma, Q.Xu, T. Xie, Soyasaponin Ab protects against oxidative stress in HepG2 cells via Nrf2/HO-1/NQO1 signaling pathways. *J. Funct. Foods.* (2018a) 45:110-117.
70. A.K.Keshari, R.Srivastava, P.Singh, V.B. Yadav, G. Nath, Antioxidant and antibacterial activity of silver nanoparticles synthesized by *Cestrum nocturnum*. *J. Ayurveda Integr.Med.* (2018).
71. A.Ammar, M.Turki, O.Hammouda, H. Chtourou, K.Trabelsi, M.Bouaziz, O. Abdelkarim, A.Hoekelmann, F.Ayadi, N.Souissi, S. Bailey, Effects of pomegranate juice supplementation on oxidative stress biomarkers following weightlifting exercise. *Nutr.J.* 9 (2017) (8):819.

72. H.Mollazadeh, H.R.Sadeghnia, A.Hoseini, M.Farzadnia, M.T. Boroushaki, Effects of pomegranate seed oil on oxidative stress markers, serum biochemical parameters and pathological findings in kidney and heart of streptozotocin-induced diabetic rats. *Renal failure*.38 (2016) (8):1256-1266.
73. A.A.Alturfan, E.E.Alturfan, E. Uslu,Pistachio Consumption has Beneficial Effects in Diabetic Rat Model. *FÜ SağBil Tıp Derg*.23 (2009)131-135.
74. T.Hussain, B.Tan, Y.Yin, F.Blachier, M.C.Tossou, N. Rahu,Oxidative stress and inflammation: what polyphenols can do for us.*Oxid. Med. Cell. Longev*.(2016) 2016.
75. J.A.Del Campo, P.Gallego, L.Grande,Role of inflammatory response in liver diseases: Therapeutic strategies. *World J hepatol*.10 (2018) (1):1.
76. X.Wang, C.Shen, J.Yang, X.Yang, S.Qin, H.Zeng, X.Wu, S.Tang, W. ZengAlpha- Fetoprotein as a Predictive Marker for Patients with Hepatitis B-Related Acute-on-Chronic Liver Failure. *Can. J.Gastroenterol*.(2018) 2018.
77. A.F.Hessin, R.R.Hegazy, A.A.Hassan, N.Z.Yassin ,SA. Kenawy,Resveratrol prevents liver fibrosis via two possible pathways: Modulation of alpha fetoprotein transcriptional levels and normalization of protein kinase C responses. *Indian J. Pharmacol*.49 (2017) (4):282.
78. S.Patial, J.Luo, K.J.Porter, J.L.Benovic,N. Parameswaran,G-protein-coupled-receptor kinases mediate TNF α -induced NF- κ Bsignalling via direct interaction with and phosphorylation of I κ B α . *BIOCHEM J*. 425 (2010) (1):169-180.
79. F.Li, F.Lang, Y.Wang, C.Zhai, C. Zhang, L.Zhang ,E. Hao,Cyanidin ameliorates endotoxin-induced myocardial toxicity by modulating inflammation and oxidative stress through mitochondria and other factors. *Food Chem Toxicol*.(2018)120:104-111.
80. C.F.Coursodon-Boyiddle, C.L.Snarrenberg, C.K.Adkins-Rieck, J.Bassaganya-Riera, R.Hontecillas, P.Lawrence, J.T.Brenna, Z.E.Jouni, B. Dvorak,Pomegranate seed oilreduces intestinal damage in a rat model of necrotizing enterocolitis. *AM J. ofPhysiol-Gastr. L*.303 (2012)(6):G744-751.
81. M.H.Grace, D.Esposito, M.A.Timmers, J.Xiong, G.Yousef, S.Komarnytsky, M.A. Lila,Chemical composition, antioxidant and anti-inflammatory properties of pistachio hullexttracts. *Food chem*. (2016)210:85-95.
82. I.Paterniti, D.Impellizzeri, M.Cordaro, R.Siracusa, C.Bisignano, E.Gugliandolo, A.Carughi, E.Esposito, G.Mandalari, S. Cuzzocrea,Theanti-inflammatory and antioxidant potential of pistachios (*Pistaciavera L.*) in vitro and in vivo. *Nutrients*.9 (2017) (8):915.
83. K.Nishizawa,M. Nishizawa,Extracellular Heat Shock Proteins as a Natural Protector of Cells and Organisms: A Review of Recent Studies. *Ann. Biomed. Res*. 1 (2018) (2):109
84. M.Kuramochi, T.Izawa, M.Pervin, A.Bondoc, M.Kuwamura, J.LaMarre, J. Yamate,Attenuation of thioacetamide-induced hepatocellular injury by short-term repeated injections associated with down-regulation of metabolic enzymes and relationship withMHC class II-presenting cells. *Exp. Toxicol. Pathol*. 69 (2017)(8):589-597.
85. F.Mahjoub, K.A.Rezayat, M.Yousefi, M.Mohebbi, R. Salari,PistaciaatlanticaDesf. A review of its traditional uses, phytochemicals and pharmacology. *J. Life Med*. 11 (2018)(3):180.
86. R.Hu, Y.He,M.A.Arowolo, S. Wu, J.He,Polyphenols as Potential Attenuators of Heat Stress in Poultry Production. *Antioxidants*.(2019)(3):67.
87. Z.S.Ibrahim, M.A.Nassan, M.M. Soliman, Ameliorative effects of pomegranate on carbon tetrachloride hepatotoxicity in rats: A molecular and histopathological study. *MOL MED REP*. 13 (2016) (4):3653-3660.
88. A.Bishayee, R.J.Thoppil, A.S.Darvesh, V.Ohanyan, J.G.Mezzaros, D. Bhatia,Pomegranatephytoconstituents blunt the inflammatory cascade in a chemically induced rodent model ofhepatocellular carcinogenesis. *J. Nutr. Biochem*24 (2013) (1):178-187.
89. N.A. Afifi, A.Ramadan, W.El- Eraky, A.A.A. Salama, A.A.El-Fadaly ,A. Hassan, Quercetin protects against thioacetamide induced hepatotoxicity in rats through decreasedoxidative stress biomarkers, the inflam-matory cytokines; (TNF-), (NF-B) and DNAfragmentation. *Der.PharmaChem*. 9 (2016)848-855.
90. L.H. Chen, C.Y. Hsu, C.F. Weng , Involvement of P53 and Bax/Bad triggering apoptosis in thioacetamide-induced hepatic epithelial cells. *World J.Gastroenterol*.12 (2006) (32):5175.
91. M. A. Ahmed, E. M. El Morsy, A. A. Ahmed, Pomegranate extract protects against cerebral ischemia/reperfusion injury and preserves brain DNA integrity in rats. *Life sci*.110, (2014) 61-69.
92. M.Majidinia, A.Bishayee, B. i.Yousef, Polyphenols: Major regulators of key components of DNA damage response in cancer. *DNA repair*. (2019)102679.