Identification of Novel Polyphenolic Secondary Metabolites from *Pistacia Atlantica* Desf. and Demonstration of their Cytotoxicity and CCl₄ induced Hepatotoxicity in Rat

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Abstract

AS of late a great deal of studies have been led to distinguish regular mixes for counteractive action of the advancement and repeat of malignant growths. The present investigation went for investigating the auxiliary metabolites substance of *Pistacia atlantica* Desf. (Anacardiaceae) leaves extracts and surveying their cytotoxic activity towards some malignant growth cell lines. In addition, the defensive impact of aqueous methanol and ethyl acetate extracts towards the CCl₄ induced hepatotoxicity in rodents was explored. Novel compounds isolation was done utilizing customary chromatographic systems. The structures of the novel components were clarified dependent on the UV, NMR spectral data information alongside their mass-spectrometric investigations.

The ethyl acetate extract of *P. atlantica* leaves contains a complicated mixture of phenolic acids and gallotannines, were elucidated for the first time from this plant, including polyphenolic acid; ellagic acid (1); 3,3’-dimethoxyellagic acid (2) and gallotannines, namely: 1,2,3,4,6-penta-O-galloyl-ß-C4-glucopyranose, (3); 1,6-digalloylglucopyranose (4); 1,3-digalloylglucopyranose (5); 2,3-digalloyl-glucopyranose,nilotcin (6) ;2,3,6-trigalloylglucopyranose (7) and 2,3-di-O-galloyl-4,6-O-hexahydroxydiphenoyl-(a/b)-ß-C4-glucopyranose, (8).

The identification of isolated compounds by conventional methods, spectroscopic analysis, including 1D-NMR, 2D-NMR, ESIMS and HRESI mass as well. The search for novel, potentially biologically active extract becomes much more efficient after identification of all compounds in that mixture.

In vitro cytotoxic activity of methanol and ethyl acetate extracts of *Pistacia atlantica* and resulted new compounds on four human cancer cell lines to be specific; Colorectal adenocarcinoma cell line (Caco-2 cell line), Prostate carcinoma cell line (PC3 cell line), hepatocellular carcinoma (HEPG2), Caucasian bosom adenocarcinoma (MCF-7). SRB assay was used to measure the potential cytotoxicity.

The ethyl acetate extract showed a higher cytotoxicity to Caco-2 cell line with IC₅₀ = 3.38 µg/ml and PC3 cell line with IC₅₀=14.3 µg/ml. Furthermore, the methanol extract was least cytotoxic to normal cell lines. The strong cytotoxic potential was observed in pure compound pentagalloyl glucopyranose (3) to all three cancer cell lines (HEPG2, Caco-2, MCF-7), IC₅₀ of HEPG2 value = 4.5 µg/ml the IC₅₀ for Caco-2 was 11 µg/ml and MCF7, IC₅₀=13.5 µg/ml as well, in comparison with pure compounds (4,7,8). The growth inhibition of 50% (IC₅₀) for each extract was calculated from the optical density of treated and untreated cells. Moreover, methanol and ethyl acetate extracts of *Pistacia atlantica* resulted in an attractive candidates for amelioration of hepatotoxicity induced by CCL₄ in rats through scavenging free radicals, improved liver functions, and normalizing the liver histopathological architecture.

Keywords: *Pistacia Atlantica* Desf., (Anacardiaceae), Novel polyphenolic compounds, in vitro cytotoxic activity, CCL₄, Hepatoprotective activity.

Introduction

*Pistacia* as a genus of flowering plants, family Anacardiaceae, have around twenty species, five of them are more popular such as: *P. vera, P. atlantica,* *P. terebinthus, P. khinjuk,* and *P. lentiscus.* Which are native at all of Africa, and southern Europe, warm and semi desert area across Asia and United States. *Pistacia atlantica* L is a species of *Pistacia* tree

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known by the English common name is (Mastic) tree. In Arabic, it is called (Butum). *Pistacia atlantica* is an evergreen shrub or small tree growing to 1–8-meter-tall [1]. In vitro antimicrobial activity of *Pistacia lentiscus* L. extracts. In Algeria, the *Pistacia atlantica* can be reach 25 m in height. [2]. Fatty acids and sterols of *Pistacia atlantica* fruit oil.

Different parts of this plant have been traditionally used for the treatment of peptic ulcer and also as mouth freshener [3]. The liver is responsible for metabolism and detoxification of the most of components that enter the body [4]. (CCl₄), Carbon tetrachloride is a very lethal synthetic specialist, the most popular medication used to initiate liver harm tentatively. Histopathological segmenting of the liver tissues demonstrated that, CCl₄ prompted fibrosis, cirrhosis and hepatocarcinoma [5]. The lethal impact of CCl₄ is credited to trichloromethyl radical delivered during oxidative pressure [6]. The quantity of penetrated neutrophils, macrophages, Kupffer cells, lymphocytes and characteristic executioner cells are fundamentally expanded after liver damage incited by hepatotoxins, for example, CCl₄. It initiated enactment of liver inhabitant macrophages [7] as well as chemoattraction of extrahepatic cells (for example neutrophils and lymphocytes). The actuated macrophages are discharged and added to liver fibrosis, irritation and damage [8]. When the liver wound up harmed, its effective treatment with well known compound medications is restricted [9].

In this way, intrigue concerned the utilization of elective prescriptions for the treatment of hepatic ailment has been emerged, treatment of kidney, liver, heart, and respiratory framework issue, and the gum tar of *P. lentiscus*, *P. khinjuk*, *P. atlantica*, and *P. terebinthus* for their injury recuperating movement, and treatment of mind and gastrointestinal issue [10]. Logical discoveries likewise uncovered the wide pharmacological exercises from different pieces of these species, different sorts of phytochemical constituents like phenolic mixes, unsaturated fats, terpenoids and sterols have additionally been secluded and recognized from various pieces of Pistacia species [11]. Scarcely any investigations of real classes of the auxiliary metabolites were depicted, in *P. lentiscus* and *P. atlantica*: gallic corrosive and its subsidiaries with glucose and flavonol glycosides (quercetin glycosides) was likewise noted in the aeronautical pieces of *P. lentiscus*, *P. atlantica*, *Pistacia vera*, *Pistacia chinensis* and *Pistacia khinjuk*, [13]. The wealth of the flavonoids glycosides was likewise noted in the aeronautical pieces of *P. lentiscus*, *P. atlantica*, *Pistacia vera*, *Pistacia chinensis* and *Pistacia khinjuk*, [13]. Plants assume a significant job in the improvement of against disease drugs.

In this way, it is basic to look for extra enemy of malignant growth medicates in the treatment and the board of diseases. The revelation of new plant inferred anticancer operators incorporate cytotoxicity screening of plant removes, bioactivity guided separation of dynamic mixes with anticancer properties. *Pistacia atlantica* Desf., having a place with the Anacardiaceae family is portrayed by the nearness of a progression of plant metabolites including, flavonoids, triterpenes, sterols and phenolic mixes. The phenolic mixes have been recognized in these species: 3-(8-pentadecenylphenol; 3,4,5-tri-O-galloyl quinic corrosive; 1,2,3,4,5-penta-O-galloyl-β-D-4-glucopyranose and gallic corrosive from *P. eyer*, *P. lentiscus*, *P. atlantica* natural products [14], and from *P. lentiscus* disengaged digallic corrosive, [15]; monogalloyl glucose [12].

This examination was directed to assess four novel polyphenolic content in vitro cytotoxic action to be specific as: 1,2,3,4,5-penta-O-galloyl-β-D-C₄₁-glucopyranose (3); 1,6-digalloylglucopyranose (4); 2,3,6-trigalloylgluco-pyranose (7); 2,3-digalloyl-4,6-hexahydroxy diphenoyl glucopyranose (8). [16] and decide four bioactive unadulterated mixes (3,4,7,8). Besides, the defensive impacts of methanolic and ethyl acetate extracts towards CCl₄ incited hepatotoxicity in rodents was examined through measuring liver capacities, lipid profiles and histopathology of liver tissues.

**Materials and Methods**

**General**

NMR spectra were acquired in DMSO-d₆ on a JEOL ECA 500 MHz, NMR spectrometer, ¹H at 500 MHz and ¹³C 125MHz. Standard pulse sequence and parameters were used to obtain one-dimensional ¹H chemical shifts (δ) were measured in ppm, relative to TMS , ¹³C - NMR chemical shifts to DMSO-d₆ by adding 39.5 ppm and two dimensional COSY, HSQC and HMBC spectra. FTESI-MS spectra were measured on a Finnigan LTQ-FTMS (Thermo Electron, Bremen, Germany) (Department of Chemistry, Humboldt Universität zu Berlin). UV recording was made on a Shimadzu UV–Visible-1601 spectrophotometer. Paper chromatographic analysis was carried out on Whatman 1 and 3 MM using solvent systems:(1) H₂O; (2) 6% HOAc; (3) BAW (n-BuOH : HOAc : H₂O , 4:1:5, upper layer).

**Plant Material**

Fresh leaves of *Pistacia atlantica* Desf (Anacardiaceae) were collected on May 2016 from wadi sawajjin, Beni walid, Libya, the located at 180 kilometers North West of Tripoli. It was identified
According to [17]. A flowering voucher specimen is deposited in the herbarium of the Benghazi University, Libya.

Preparation of the Extract

The dried powdered *P. atlantica* leaves (2 Kg) was extracted by using Soxhlet, beginning with n-hexane, followed by ethyl acetate and finally with methanol with 2 L solvent every time. The three extracts were evaporated to dryness in a rotavapor at 40°C. The dried extracts weights were calculated to give n-hexane (35 g), ethyl acetate (59 g) and methanol (65g), respectively. The ethyl acetate and methanol extracts were preliminary investigated for their biological activity.

Isolation and Identification of Phenolics

The concentrated ethyl acetate extract (59 g) was chromatographed on a polyamide S6 and repeated Sephadex LH-20 column fractionation. The polyamide column and eluted by H2O/MeOH mixtures of decreasing polarities to yield eight subfractions (1-8), removal of the solvents were individually collected and purified. Compounds 1,2 (48mg .86 mg) was isolated pure from 2.50 g of fraction II. Compounds 3 (38 mg) and 4 (75 mg) were individually separated pure by fractionation of 2.6 g of fraction III over Sephadex LH-20 column using a H2O/MeOH mixture of decreasing polarity for elution Compounds 5,6 (45,75mg) were individually isolated pure from 2.3 g of fraction IV by fractionation on a Sephadex LH-20 column and 40% aqueous MeOH for elution, followed by preparative paper chromatography (prep. PC),using BAW system for final purification. Compound 7 (75 mg) was individually separated pure from 200 mg of fraction V by (prep. PC), using n-ButOH water saturated as solvent. Compound 8 (91 mg) was isolated pure from 3.5g of fraction VI by fractionation on a polyamide column using (methanol: toluene: H2 O) (60: 38: 2), followed by preparative PC, using BAW as solvent. Their chemical structures have been established by conventional methods of chemical and physical analysis and confirmed by 1H and 13CNMR spectroscopy.

Methods of Cytotoxic Assay

SRB assay was used to measure the potential cytotoxicity using the method of [18], cells were seeded in 96-multiwell plate (104 cell/well) for 24 h before treatment to allow attachment of the cell .

Different concentrations of the extract under test were added to the cell monolayer in triplicate wells. The monolayer cells were incubated with the extract for 48 h at 37°C and in atmosphere of 5% CO2, cells were then fixed, washed and stained with Sulforodamine B stain, excess stain was washed with acetic acid and attached stain was recovered with Tris EDTA buffer. Color intensity was measured in an ELISA reader, the relation between surviving fraction and extract concentration is plotted to get the survival curve of each tumor cell line after application of different concentrations of the extract.

Protective Effect of Pistacia extracts against CCl4 Induced Injury in Liver of Rats

**Animals.** Male Wistar albino rats (100- 120 gm) were selected for this study. They were obtained from the animal house, National Research Center, Egypt. All animals were kept in controlled environment of air and temperature with access of water and diet ad libitum.

**Ethics.** Anesthetic procedures and handling with animals complied with the ethical guidelines of Medical Ethical Committee of the National Research Centre in Egypt.

**Doses of Administration.** Administration regime was twice a week for six consecutive weeks. Five hundred microliters of CCl4 diluted 1: 9 (v/v) in olive oil were injected intraperitoneally (0.1 ml) . *Pistacia* ethyl acetate extract (ET) and methanol extract, (ME), (200 mg/kg body weight) were administered orally after intraperitoneal injection of CCl4.

**Experimental Design.** 24 male rats were used in this study. Animals were divided into 4 groups (6 rats each) as following:

- **Group-1:** Served as normal healthy control rats.
- **Group-2:** Rats were intraperitoneally injected with CCl4 alone.
- **Group-3:** Rats were intraperitoneally injected with CCl4 followed by oral administration of *Pistacia* ethyl acetate extract, ET (200 mg/kg body weight).
- **Group-4:** Rats were intraperitoneally injected with CCl4 followed by oral administration of *Pistacia* methanol extract, ME (200 mg/kg body weight).

**Hematological and Biochemical Studies: Sample Preparations**

Blood was collected from each animal by puncture of sublingual vein. Blood samples were divided into two parts. The first part was collected on EDTA for hematological analyses. The second part was collected into dry test tubes and then centrifuged at 3000 rpm in order to separate serum. The sera were kept at -20°C for further biochemical analysis. In order to collect the hepatic tissues, rats were immediately dissected. The liver was homogenized with 10% w/v ratio in ice-cold 50 mM Tris HCl buffer at pH 7.4 and then centrifuged at 10,000 rpm for 20 min at 4EC. The supernatant was collected and kept in deep freeze at -20°C for further analyses.

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**Estimation of Hematological Parameters**

The hematological parameters including red blood cell (RBC) count, white blood cell (WBC) count, platelet (PLT) count, hemoglobin (Hb) content and packed cell volume (PCV) were analyzed using Medonic M-Series analyzer (Clinical Diagnostics solutions Inc, Florida, USA).

**Estimation of Serum Biochemical Parameters**

In the serum of all the experimental groups, the levels of total lipids (TL), total cholesterol (TC), triglycerides (TG), low density lipoprotein cholesterol (LDL-C), high density lipoprotein cholesterol (HDL-C), total proteins (TP), albumin (A), globulin (G), aspartate aminotransferase (ASAT), alanine aminotransferase (ALAT), alkaline phosphatase (ALP), total bilirubin (TBil) and direct bilirubin (DBil) were measured colorimetrically using Biodiagnostics kits (Biodiagnostic company, Dokki, Cairo, Egypt).

**Lipid peroxide: The level of malondialdehyde (MDA) in the liver homogenate was assayed according to the technique described by [19]. The principle of this method depends on the reaction of the liberated MDA after lipid peroxidation (LPO) of the cell membranes with thiobarbituric acid in acidic medium.

Non-enzymatic and enzymatic antioxidant assay: The concentrations of non-enzymatic (glutathione, GSH) as well as enzymatic (catalase, CAT, superoxide dismutase, SOD, glutathione reductase, GR) antioxidants were estimated in the homogenate of the liver of control and treated rats. The method by which GSH content was measured was based on the reaction of 5,5′ Dithiobis-2-nitrobenzoic acid with GSH [20]. The CAT activity was estimated in accordance to the method described by (Aebi et al., 1984). The SOD activity assessment was based on the ability of SOD to inhibit the reduction reaction of nitro blue tetrazolium dye mediated by phenazine

methosulphate [21]. The principle for measuring the GR activity was based on it The Comet Assay: Comet assay was performed referring to the protocol developed by [23], with minor modifications. Rats liver cells of each treatment were mixed with low melting point agarose (ratio of 1:10 v/v), then pipetted to precoated slides with normal melting point agarose. The slides were kept flat at 4°C for 30 min in dark environment. The third layer of low melting point agarose was then pipetted on slides, left to solidify at for 30 min 4°C. The slides were transferred to pre-chilled lysis solution, kept for 60 min at 4°C. After that, slides were immersed in freshly prepared alkaline unwinding solution at room temperature in the dark for 60 min. Slides were subjected to an electrophoresis run at 0.8 V/cm, 300 m Amps for 30 min. The slides were rinsed in neutralizing solution followed by immersion in 70% ethanol and then air dried. Ethidium bromide was used for slides stain then and visualized by using Zeiss epifluorescence microscope (λ<sub>max</sub>510–560 nm, barrier filter λ 590 nm) with a magnification of ×400. 100 cells per animal were scored then analyzed with DNA damage analysis software (Comet Score, TriTek corp., Sumeduck, VA22742).

**Statistical analysis:** Data were statistically analyzed by the aid of Statistical Package of the Social Sciences, SPSS version 23 (copyrighted by IBM SPSS software, USA). Two-way analysis of variance (ANOVA) were used to study the effect of the type of treatment on tested groups (control; Rats were intraperitoneally injected with CCl<sub>4</sub> alone; rats injected with CCl<sub>4</sub> followed by oral administration of *Pistacia* ethyl acetate extract or *Pistacia* methanol extract. Data were expressed as a mean ± standard error of mean (SEM).

**Histopathological Study:** Liver tissues were excised from sacrificed animals, individually weighed, and, from them, 5 μm thickness slices were cut, fixed in 10% paraformaldehyde, and embedded in paraffin wax blocks. Tissue sections of 5 μm thickness were stained with hematoxylin and eosin (H and E).

**Results and Discussion**

The isolation and identification of four novel bioactive gallotannine compounds (3,4,7,8) were elucidated from this plant for the first time. (Figure 1)

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Compound (3): 1,2,3,4,6-penta-O-galloyl-β-C1-glucopyranose. Pure compound was isolated as an off-white amorphous powder (86 mg), negative ESI-MS analysis, m/z 939.1146 [M-H]− (calc. for C41H31O26−939.1109). 1H-NMR (CD3OD), glucose moiety δppm 6.23 (d, 8.3 Hz, H-1), 5.89(t, 9.6 Hz, H-3), 5.61 (t, 9.6 Hz, H-4), 5.58 (dd, 9.6, 8.3 Hz, H-2), 4.40 (m, H-5), 4.51 (d, 12.2 Hz, H-6a), 4.37 (dd, 12.2, 4.2 Hz, H-6f). Galloyl moieties: δppm 7.10, 7.04, 6.97, 6.94, 6.89 (s, each 2H) α-C-NMR (CD3OD) glucose moiety: δppm 93.9 (C-1), 74.3 (C-5), 74.1 (C-3), 72.1 (C-2), 69.8 (C-4), 63.1 (C-6). Galloyl moieties: 167.9, 167.3, 167.0, 166.9, 166.2 (carbonyl group signals), 146.6, 146.5, 146.4, 146.4, 146.3 (C-3, C-5), 140.9, 140.5, 140.4, 140.2, 140.1 (C-4), 121.0, 120.3, 120.2, 120.1, 119.6 (C-1), 110.6, 110.4, 110.38, 110.35, 110.3 (C-2, C-6).  

Compound (4): 6-di-O-galloyl-β-C1-glucopyranose. Pure material was not a non-crystalline creamy white amorphous Powder (75 mg). It exhibited an negative ESI-MS [M-H] at m/z = 483 in its 1H- NMR Spectral Data (DMSO-d6) δppm β-glucose moiety 5.52 (1H, d, J = 8 Hz, H-1), 3.2-3.7 (sugar protons overlapped with water protons, H-2, H-3, H-4), 3.50 (1H, d, J = 12 Hz, H-6d), 4.21 (1H, dd, J = 12 Hz and J = 4.5 Hz, H-6f). Galloyl moieties in β-anomer 6.89(2H, s) and 6.95(2H, s). 13C-NMR Spectral Data (DMSO-d6) δppm glucose moiety 94.9 (C-1), 75.2 (C-2), 76.2 (C-3), 69.8 (C-4), 73(C-5), 63.7 (C-6). Galloyl moieties in β-anomer 118.9, 119.7 (C-1′, C-1′′), 109.1, 109.5 (C-2′, C-2′′, C-3′, C-3′′, 6′), 146.0, 146.1 (C-3′′, 5′, C-3′, 5′′), 139.0, 139.5 (C-4′, C-4′′), 165.1, 166.2 (C-7′, C-7′′).  

Compound (7): 2, 3, 6-tri-O-galloyl-(α/β)-C1-glucopyranose, isolated as an off-white amorphous powder (75 mg). UV spectral data at 275 nm which is glucopyranose, nilocitin (6) are described before. The known compounds ellagic acid (1); 3,3′,4,6,7-penta-O-galloyl-β-C1-glucopyranose isolated pure as off-white amorphous powder (91 mg), was found to possess chromatographic characters, dark blue response towards FeCl3 spray reagent, UV at λmax 275 nm and comparable for galloylated hexahydroxydiphenylol glucoses. The [M−H] at m/z proved a molecular weight is 786. 1H-NMR Spectral Data (DMSO-d6) δppm α-glucose moiety, 5.38 (1H, d, J = 2.5 Hz, H-1), 5.01 (1H, dd, J = 8 and 2.5, H-2), 5.69 (1H, t, J = 8 Hz, H-3), 4.89 (1H, m, H-4), 4.57 (1H, m, H-5), 5.12 (m, H-6a) and 3.81 (d, J = 12.5 Hz, H-6f) β-glucose moiety, 4.94 (1H, d, J = 8 Hz, H-1), 5.03 (1H, t, J = 8 Hz, H-2), 5.53 (1H, t, J = 8 Hz, H-3), 4.34 (1H, t, J = 8 Hz, H-4), 4.34 (1H, m, H-5), 5.12 (m, H-6a) & 3.74 (d, J = 12.5, H-6f). Galloyl moieties in α- and β-anomers: 6.89, 6.88, 6.82, 6.77 (s, H-2 and H-6) Hexahydroxydiphenylol moiety in α- and β-anomers: 6.40, 6.36, 6.26, 6.22 (s, H-3 and H-3′). 13C-NMR Spectral Data (DMSO-d6) δppm α-glucose moiety: 90.22 (C-1), 70.22 (C-2), 70.56 (C-3), 70.43 (C-4), 69.96 (C-5), 62.92 (C-6), β-glucose moiety 95.06 (C-1), 73.05 (C-2), 71.63 (C-3), 71.63 (C-4), 66.25 (C-5), 62.92 (C-1), Galloyl moieties in α- and β-anomers: 119.24, 119.01, 118.86 (C-1), 109.35, 109.27, 109.23 (C-2&C-6), 145.94, 145.84, 145.71, 145.68 (C-3&C-5), 139.44, 139.21 (C-4), 165.86, 165.74, 165.60, 164.99 (C=O), Hexahydroxydiphenylol moiety in α- and β-anomers: 116.07 (C-1&6C-1′, 124.79, 124.21 (C-2&C-2′), 105.61, 105.8 (C-3&C-3′), 144.37 (C-4&4′, 136.11, 135.95 (C-5&C-5′), 144.37 (C-6&C-6′), 168.13, 168.10, 167.45, 167.39 (C-7′&C-7′′). The known compounds ellagic acid (1); 3,3′-dimethoxyxellagicacid (2) [24], and gallotannines, 1,3-digalloyl glucopyranose (5); 2,3-digalloyl glucopyranose, nilocitin (6) are described before from this plant. [16]  

Evaluation of in vitro cytotoxicity: 

The ethyl acetate extract showed a higher cytotoxicity to Caco-2 cell line with IC50 = 3.38 µg/ml and PC3 cell line with IC50 = 14.3 µg/ml. Furthermore, the methanol extract was least cytotoxic to normal cell lines. (Figure 2.3). The strong cytotoxic potential was observed in pure compound (3). 1,2,3,4-pentagalloyl glucopyranose to all three cancer cell lines (HEPG2, Caco-2, MCF-7). IC50 of HEPG2 value = 4.5 µg/ml. The IC50 for Caco-2 was 11 µg/ml and MCF7; IC50 = 13.5 µg/ml as well, in comparison with pure compounds (4,7,8). (Figure 4)
The growth inhibition of 50% (IC$_{50}$) for each extract was calculated from the optical density of treated and untreated cells when compared with a chemotherapeutic anticancer drug Doxorubicin, where’s was less cytotoxic to cell line PC$_3$ may be due to the increasing the drug dose decreased the surviving fraction of cancer cells.

**Effect on Hematological Parameters:**

Data results of hematological parameters (Table 1) revealed that the type of treatment is significantly affected all the studied blood parameters except for the PLT count that there is no any significant differences among all the studied groups.

Rats of CCl$_4$, administered group showed a notable decline in the RBC and WBC counts, Hb content and PCV, in comparison to controls. As compared to the rats of CCl$_4$-treated group, the rats administered Pistacia ethyl acetate and methanolic extracts after CCl$_4$ administration exhibited significant elevations in the RBC, WBC counts, Hb content and PCV. This data is in accordance with [25] (Meral and Kanter et al., 2003), who reported that rats treated with CCl$_4$ for 45 days significantly decreased the red blood cell count (RBC), white blood cell count (WBC), packed cell volume (PCV), and Hb levels while other plant like Nigella sativa treatment significantly increased the reduced RBC, WBC, PCV, and Hb levels.

**Effect on Serum Biochemical Parameters:**

The lipid profile of the experimental animals as affected by the administration of CCl$_4$ alone, Pistacia ethyl acetate and methanolic extracts plus CCl$_4$ are shown in (Table 2). The serum levels of TL, TC, TG, LDL-C and HDL-C of the rats were markedly influenced by the type of treatment. In comparison to control group, all the studied lipid profile parameters of CCl$_4$ treated group were significantly elevated except the levels of HDL-C that were notably reduced.

On the other hand, rats treated Pistacia ethyl acetate and methanolic extracts plus CCl$_4$ exhibited a marked reduction in the levels of TL, TC, TG and LDL-C, as compared with th CCl$_4$ treated group.

<table>
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<th>Parameters</th>
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<th>Pist Et + CCl$_4$</th>
<th>Pist Me + CCl$_4$</th>
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<td>Red blood cell count (×10$^12$ L$^{-1}$)</td>
<td>5.99±0.31</td>
<td>6.71±0.62</td>
<td>6.91±0.51</td>
<td>6.06±0.4</td>
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<tr>
<td>White blood cell count (×10$^9$ L$^{-1}$)</td>
<td>5.9±0.82</td>
<td>15.5±1.91</td>
<td>14.7±0.87</td>
<td>14.4±0.74</td>
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<td>Platelet count (×10$^9$ L$^{-1}$)</td>
<td>471±43.3</td>
<td>783±41.6</td>
<td>483±33.7</td>
<td>435±34.84</td>
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<tr>
<td>Hemoglobin content (g d L$^{-1}$)</td>
<td>12.6±0.38</td>
<td>9.3±0.32</td>
<td>12.3±0.85</td>
<td>11.6±0.92</td>
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<tr>
<td>Packed cell volume (%)</td>
<td>34.2±1.23</td>
<td>39.1±1.91</td>
<td>36.7±1.78</td>
<td>36.1±2.46</td>
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</table>

Data are represented as mean ± standard error.

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Table 2: Effect of oral administration of CCl₄ alone or with different Pistacia extracts, on the concentrations of serum total lipid (TL), total cholesterol (TC), triglycerides (TG), low density lipo-protein cholesterol (LDL-C) and high-density lipoprotein cholesterol (HDL-C) of male albino rats

<table>
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<th>Parameters</th>
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<th>Pist. Me + CCl₄</th>
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<tr>
<td>TL (mgdL⁻¹)</td>
<td>512.04±43.06</td>
<td>658.8±50.38</td>
<td>488.40±38.07</td>
<td>440.80±31.76</td>
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<tr>
<td>TC (mgdL⁻¹)</td>
<td>118.20±2.97</td>
<td>228.8±20.31</td>
<td>122.40±13.68</td>
<td>103.80±5.44</td>
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<tr>
<td>TG (mgdL⁻¹)</td>
<td>104.40±7.34</td>
<td>164.80±4.59</td>
<td>106.00±9.39</td>
<td>101.40±8.33</td>
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<tr>
<td>LDL-C (mgdL⁻¹)</td>
<td>61.20±9.87</td>
<td>159.02±16.76</td>
<td>55.60±8.03</td>
<td>43.80±4.49</td>
</tr>
<tr>
<td>HDL-C (mgdL⁻¹)</td>
<td>36.60±6.40</td>
<td>27.06±3.95</td>
<td>40.00±5.52</td>
<td>39.80±4.73</td>
</tr>
</tbody>
</table>

Data are represented as mean±standard error.

Table 3: Effect of oral administration of CCl₄ alone or with different Pistacia extracts, on the concentrations of serum total protein (TP), albumin (A), globulin (G) and A/G ratio of male albino rats

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>CCl₄</th>
<th>Pist. Et + CCl₄</th>
<th>Pist. Me + CCl₄</th>
</tr>
</thead>
<tbody>
<tr>
<td>TP (g dL⁻¹)</td>
<td>6.68±0.22</td>
<td>6.52±0.30</td>
<td>6.24±0.05</td>
<td>6.19±0.08</td>
</tr>
<tr>
<td>A (g dL⁻¹)</td>
<td>4.42±0.13</td>
<td>3.42±0.15</td>
<td>4.12±0.09</td>
<td>4.36±0.07</td>
</tr>
<tr>
<td>G (g dL⁻¹)</td>
<td>2.46±0.24</td>
<td>3.70±0.18</td>
<td>2.62±0.19</td>
<td>2.59±0.11</td>
</tr>
<tr>
<td>A/G ratio</td>
<td>1.72±0.16</td>
<td>0.85±0.09</td>
<td>1.38±0.16</td>
<td>1.36±0.13</td>
</tr>
</tbody>
</table>

Data are represented as mean±standard error.

Glutathione reductase (GR) of male albino rats

The present study has established that CCl₄ treatment could have affected the lipid metabolism of liver (triglyceride and cholesterol levels). This is evidenced from the present observations in which CCl₄ caused a significant (p < 0.05) increase in the levels of lipid parameters. In this connection, [26]

In this manner, the A/G proportion of this gathering was surprisingly diminished. Then again, the rodents of Pistacia ethyl acetate derivation and methanolic extracts in addition to CCl₄ treated gatherings showed a stamped increment in the levels of albumin whites and A/G proportion however a checked decline in the degrees of globulin, when contrasted with the CCl₄ treated gathering.

In this examination the huge (p<0.05) decline in serum albumin of rats treated with CCl₄ when contrasted with control may shows poor liver capacities or weakened union, either essential as in liver cells harm or auxiliary to decreased protein consumption and diminished retention of amino acids brought about by a mal ingestion disorders or lack of healthy sustenance, or misfortune protein in pee, because of nephritic disorder and unending glomerulonephritis [29].

On the other hand, a significant (p < 0.05) increase in concentrations of serum albumin was observed in rats received Pistacia extracts in addition to CCl₄ in contrast with rodents got CCl₄ alone. The increase of albumin concentration after treatment with Pistacia concentrates might be credited to the diminishing in lipid peroxidation processes and increase in the activities of plasma protein thiol's as a result of the treatment [30].
Table 4: Effect of oral administration of CCl₄ alone or with different Pistacia extracts, on the activities of serum aspartate aminotransferase (ASAT), alanine aminotransferase (ALAT) and alkaline phosphatase (ALP) and the levels of total bilirubin (TBil) and direct bilirubin (DBil) of male albino rats

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>CCl₄</th>
<th>Pist. Et + CCl₄</th>
<th>Pist. Me + CCl₄</th>
</tr>
</thead>
<tbody>
<tr>
<td>ASAT (UL⁻¹)</td>
<td>33.02 ± 1.30</td>
<td>118.7 ± 24.49</td>
<td>48.20 ± 8.01</td>
<td>52.7 ± 11.2</td>
</tr>
<tr>
<td>ALAT (UL⁻¹)</td>
<td>25.60 ± 1.50</td>
<td>75.60 ± 2.77</td>
<td>39.02 ± 5.52</td>
<td>38.9 ± 7.63</td>
</tr>
<tr>
<td>ALP (UL⁻¹)</td>
<td>55.30 ± 3.84</td>
<td>70.02 ± 8.08</td>
<td>53.22 ± 5.72</td>
<td>56.14 ± 7.61</td>
</tr>
<tr>
<td>TBil (mg dL⁻¹)</td>
<td>0.66 ± 0.02</td>
<td>0.89 ± 0.03</td>
<td>0.73 ± 0.05</td>
<td>0.77 ± 0.03</td>
</tr>
<tr>
<td>DBil (mg dL⁻¹)</td>
<td>0.11 ± 0.005</td>
<td>0.14 ± 0.006</td>
<td>0.10 ± 0.008</td>
<td>0.10 ± 0.004</td>
</tr>
</tbody>
</table>

Data are represented as mean ± standard error.

The activities of ASAT, ALAT and ALP and TBil, in serum of rodents were altogether influenced by the type of treatment, where the serum levels of DBil were not influenced by any of the contemplated variables. In contrast with the controls, the CCl₄ treated rodents demonstrated huge rises in the exercises of ASAT and ALAT and ALP just as the degrees of TBil. Despite what might be expected, the activities of ALP, ASAT and ALAT just as the degrees of TBil and DBil of Pistacia extracts in addition to CCl₄ treated rodents were not fundamentally unique in relation to those of the control gathering. In the present examination serum hepatic biomarkers, AST and ALT exercises were significantly expanded (p<0.05) in rats treated with the CCl₄ contrast with the control.

As in the present examination, the previous investigations have demonstrated that CCl₄ expanded essentially serum ALP levels and complete protein and egg whites’ levels [31, 32]. The increased serum levels of hepatic markers have been attributed to the liver damage, on the grounds that these enzymes are found in cytoplasmic region of the cell and they are discharged into circulation in the event of cell harm [33].

However, treatment with Pistacia extracts plus CCl₄ was found to suppress (p<0.05) the increase of serum AST and ALT activities. In accordance with the present results, [34], reported that oral administration of hydroalcoholic extract of Pistacia vera on experimentally induced hepatotoxicity in rats improves liver functional factors, including serum ALT, AST and LDL levels.

Moreover, many other plant extracts were reported to have considerable therapeutic effects on liver injury induced by chemical agents, for example, administration of poly phenolic extracts from chicory (Cichorium intybus) resulted in wholly normalization of the serum AST and ALT levels in mice exposed to thioacetamide, a hepatotoxic organosulfur compound [35], also reported similar effects from barberry extract upon administration to CCl₄ induced hepatotoxic animals [36]. These finding infers that challenge to shield liver tissue from CCl₄ damage.

Impact on the Hepatic Lipid Peroxidation and Endogenous Antioxidants:

The impacts of CCl₄ alone or with Pistacia extracts organizations on the degrees of hepatic MDA and GSH and the activities of endogenous cancer prevention agent catalysts were appeared in (Table 5). The hepatic levels of MDA and GSH just as the activities of CAT, SOD and GR were fundamentally affected by the sort of treatment. In the liver of rodents directed CCl₄ alone, there was a significant rise in the degrees of MDA joined by a checked a marked reduction in the GSH substance, SOD and GR were fundamentally affected by the sort of treatment. In the rodsents of Pistacia extracts with CCl₄ treated gatherings, the mean estimations of hepatic MDA fixation were altogether lower than those of CCl₄ treated rodents and were not essentially unique, in relation to those of the controls. The mean estimations of hepatic GSH substance of Pistacia extracts plus CCl₄ treated rodents were fundamentally higher than those of CCl₄ treated gathering. When contrasted with the CCl₄ treated gathering, the rodents directed Pistacia extracts in addition to CCl₄ demonstrated a stamped rise in the exercises of CAT and SOD and

Table 5: Effect of oral administration of CCL₄ alone or with different Pistacia extracts, on the levels of hepatic malondialdehyde (MDA) and glutathione (GSH) and the activities of catalase (CAT),superoxide dismutase (SOD) and glutathione reductase (GR) of male albino rats

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>CCL₄</th>
<th>Pist. Et + CCL₄</th>
<th>Pist. Me + CCL₄</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDA (nmol g⁻¹ liver)</td>
<td>4.48±0.11</td>
<td>9.18±0.26</td>
<td>4.15±0.22</td>
<td>4.78±0.34</td>
</tr>
<tr>
<td>GSH (mg g⁻¹ liver)</td>
<td>40.04±e.10</td>
<td>19.72±0.98</td>
<td>37.34±2.84</td>
<td>38.91±2.31</td>
</tr>
<tr>
<td>CAT (U g⁻¹ liver)</td>
<td>104.3±17.1</td>
<td>39.40±8.27</td>
<td>99.03±13.38</td>
<td>101.56±14.74</td>
</tr>
<tr>
<td>SOD (U g⁻¹ liver)</td>
<td>9.56±0.17</td>
<td>4.36±0.19</td>
<td>9.41±0.16</td>
<td>10.23±.35</td>
</tr>
<tr>
<td>GR (U g⁻¹ liver)</td>
<td>73.20±2.71</td>
<td>27.80±1.28</td>
<td>68.40±3.48</td>
<td>69.76±3.93</td>
</tr>
</tbody>
</table>

Data are represented as mean±standard error.

GR, that did not essentially vary from those of the controls. Information of the present investigation is as per the discoveries of different laborors, for example, [37] who detailed that hepatotoxic impacts by CCL₄ are lipid peroxidation inception, and are to a great extent due to its dynamic metabolite of CCL₃ (This metabolite can extract hydrogen from unsaturated fats, starting the lipid peroxidation), lead to cell damage, lastly liver harm. Additionally, [38], expressed that the viability of any hepatoprotective medication is reliant on, its ability of either decreasing the unsafe impact or reestablishing the ordinary hepatic physiology that has been circulated by a hepatotoxin. In this association, the present examination uncovered that Pistacia extracts decreased (p<0.05) CCL₄ instigated raised compound levels in tried gatherings, demonstrating the security of basic respectability of hepatocytic cell film or recovery of harmed liver cells.

Furthermore, the present results are in agreement with the work done by [39,40], who found that Pistacia vera leaves, seeds and resins have notable amounts of antioxidant substances with hepatoprotective effects and their antioxidant properties may be attributable to its flavonoid and polyphenolic contents.

Moreover, previous studies have reported that pistachio elicits significant antioxidant activity like the synthetic antioxidant [41-43].As previously noted and like the results achieved for other plants in the literature [44-46]

Our observations and findings can be attributed to the antioxidant ingredients of Pistacia extracts that probably inhibit lipid peroxidation and consequently inhibition of oxidative stress. Therefore, the cell membranes remain intact and as a result cells are prevented to enter the necrosis step.

Determination of Percent of DNA Damage by Comet Assay in Liver Tissues:

The data in (Table 6) and (Figures 5, 6) revealed that CCL₄ liver intoxication produced a significant elevation in tail moment compared to control group of rats. On the other hand, administration of either Pistacia ethyl acetate extract or methanolic extracts plus CCL₄ significantly reduced tail moment and consequently significant reduction in the percent of DNA damage as compared to CCL₄ intoxicated group in comparison to the control group this attributed to phenolics and gallotannine compounds in the extracts.

These results are in connection with a recent study reporting that the presence of phenolic compound decreased the severity of acrylamide induced DNA damage in the rat liver [47].

Histopathological Results

Brain:

Microscopic investigation of control brain sections of rats shows highly active neurons which having huge pale-stained nuclei, nuclear chromatin

Table 6: Effect of oral administration of CCL₄ alone or with different Pistacia extracts, on the rate of DNA damage in brain tissues of rats using comet assay.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>*No. of cells</th>
<th>²Class of comet</th>
<th>DNA damaged cells (mean ± SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>500</td>
<td>34</td>
<td>466 23 11 0</td>
</tr>
<tr>
<td>CCL₄</td>
<td>500</td>
<td>124</td>
<td>376 32 44 48</td>
</tr>
<tr>
<td>Pist Et + CCL₄</td>
<td>500</td>
<td>83</td>
<td>417 35 26 22</td>
</tr>
<tr>
<td>Pist Me + CCL₄</td>
<td>500</td>
<td>79</td>
<td>421 33 25 21</td>
</tr>
</tbody>
</table>

*No. of cells analyzed were 100 per an animal.
and prominent nucleoli disappeared. The glial cells surrounded the neurons and support it. These cells have small densely stained nuclei with condensed chromatin and no visible nucleoli. Neuropl or background substances are shown in the cortex (Figure 7).

Examination of sections of brain cortex of rats administered with CCl₄ alone showed dark neurons with irregular shape and glial cells that appeared inside white vacuoles. Neurofibrillar tangles stained with magenta color and looking like flames were founded. The tangle appears as long pink filaments in the cytoplasm.

The neuropil is appeared vacuolated (Figure 8). Photomicrograph of section in brain cortex of rat administered with CCl₄ and Pistacia ethyl acetate extract showing the structure of neurons appeared like normal and regular shape (Figure 9). Photomicrograph of section in brain cortex of rat administered with CCl₄ and Pistacia methanol extract showing dark neurons with irregular shape and surrounded by pericellular halos (blue arrows). No extracellular vacuoles are found in the neuropil (Figure 10).

Liver:

Microscopic examinations of sections of liver from normal control rats show the normal architecture of hepatic lobules. The focal veins lie at the focal point of the lobules encompassed by ropes of hepatocytes, between the strands of hepatocytes which the hepatic sinusoids are seen (Figure 11). Histopathological examination of liver from rodents directed with CCl₄ alone indicating interruption of the liver tissue with loss of lobular game plan, spanning fibrosis with collagenous septa arrangement extended entryway tract to focal vein with mononuclear cells, vascular degeneration and corruption of hepatocytes (Figure 12).

Liver segments of rodents managed with CCl₄ and Pistacia ethyl acetate extract indicating mellow fiery cells invasions around focal vein, vacuolar degeneration, and putrefaction of hepatocytes. Binucleated and enacted Kupffer cells were seen (Figure 13).

If there should arise an occurrence of rodents managed with CCl₄ and Pistacia methanol extract was seen that liver segment kept up hepatic design, with just couple of incendiary cells penetrations around focal vein, and centrilobular hepatic necrosis with mellow vacuolar degeneration of hepatocytes. (Figure 14).

In the present investigation, the biochemical discoveries were additionally affirmed by histopathological perceptions. The progressions generally incorporate hepatocellular corruption or apoptosis, greasy collection, provocative cells

invasion and other histological signs which were additionally predictable with the discoveries of different creators [47,32]

Conclusions

The *P. atlantica* leaves extract is a promising source for bioactive compounds which exhibit potent cytotoxic activity and capable of synthesizing and accumulating different types of phenolics. The cytotoxic activities of isolated pure four gallotannin compounds were investigated for the first time from *P. atlantica* leaves.

Moreover, the hepatoprotective effects of *Pistacia* extracts on CCl₄ induced hepatic damage in male Wistar albino rats were observed in the present study. Possibly, due to the antioxidative properties of *P. atlantica* extracts and accumulating of different types of phenolic compounds which helped hepatic cells to obviate CCl₄ induced necrosis and inflammation which can be also observed in histopathological discoveries.

The outcomes acquired here and the reports from past examinations propose that *Pistacia* concentrates may work as a decent contender for the treatment or anticipation of liver disappointment.

However, further investigations are required to unveil the molecular, identification of the active ingredients, and elucidation of the mechanisms involved in the effect.

Acknowledgement

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References


المحتوى البوليفيىولي، كمضببد لألكسذة و الميكروبث الىشطه
لتمتيع أوراق نبات البطم الطليسي والسمية الخلوي و CCl4
النام عن الكبد في الفئران

"سحرعوض الله حسيه
أمبوي محمذ المسلمي،
سبلمت عثمبن عثمبن ،
عبذ المحسه محمذ سليمبن

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أقسم كيمياء، كلية العلوم، جامعة الزقازيق، الزقازيق، مصر.

هذا البحث هو البحث عن مستخلص جديد نشط بولوجي
لوراق نبات البطم الطليسي، وقد تم قياس الآثار البوليفيائية
لحالة
حول الميثانول وليلس أوراق هذا النبات و محاولة و بعض
المركبات المعروفة من، تم استكشاف التأثير الداعمي لمستخلصات
CCl4
النام والمصنعل من اوراق نبات الكبد الكبي و سطح
في الفئران. تم عزل المكونات الجيدة باستخدام
الفئران. تم توصيف هيئات المكونات الجيدة، وتم
ملاحظة النباتات البوليفيائية لأشعة فوق البنفسجية. بالنسبة
لأصوات الطيفية الانعكاسية، نحن نشير إلى تحلية الانتقادات
العالية في سمك المركبات المعروفة بالأطباق الطيفية، انحلال
نطاق البوليفيائية، تحتل البوليفيات، البحث عن مستخلص
جديد يقلل من انتشالات بولوجياء أكثر فعالية، بعد تحديد جمع
المركبات في هذا المرجع.

النشاط العام هو من مستخلصات الميثانول وحلول اوراق
نبات البطم الطليسي وسرد من مركبات جيدة علي أربع خطوط
خلايا سرطان بشري لتكن محددة. خط خالية سرطان الفة القولون
والمستفي خط خاليا 2، خط خاليا سرطان البروستاتا خط خاليا
SRB (HEPG2) (MCF7) (PC3) (HEPG2) (MCF7) (PC3)
تم استخدام الخصص
.

قياس السمية الخلوي المحتمة.

أظهر مستخلص خاليا خاليا سمية خاليا على خط خاليا
IC50 = 3.38
ميكروغرام / ملغ و خاليا
IC50 = 14.3
ميكروغرام / ملغ. و لكن ذلك، كان انتخراج الميثانول
أقل السمية للخلايا إلى خطوط الخالية الطبيعة. وقد لوحظت انتكاسات
فوقية لتحلية الخلوي في حروف انزامل (الفايرو) 3 (جميع
خطوط خالية السرطانية الخاليا للمحتمة.

HEPG2 = 4.5
MCF7، Caco-2
HEPG2 = 11
Caco-2
IC50 = 13.5
MCF7، Caco-2
IC50 = 3.38

المكروغرام / ملغ. كان
Caco-2
IC50 = 11
ميكروغرام / ملغ. مع
IC50 = 13.5
MCF7
و Caco-2
IC50 = 3.38

المركبات الأفقية (7.8% تم حساب تقييم في نظرة
50% ) من الكل
(IC50) مستخلص من الكبد الكبي وفقاً للخلايا المحتمة. و رفض المعايير
على ذلك، تتم تمكين مستخلصات الميثانول وحلول اوراق نبات
CCl4
البطم الطليسي شبيهًا بتختين المسماة الكبي و التي يسببها
في الفئران من خلال جذور النبات وتحت وظائف الكبد.

ملاحظة الكبد التشنجية.