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Identification of Novel Polyphenolic Secondary Metabolites from *Pistacia Atlantica* Desf. and Demonstration of their Cytotoxicity and CCl_4 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 CCl_4 induced hepatotoxicity in rodents was explored. Novel components 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'- dimethoxyellagicacid (2) and gallotannines, namely: 1,2,3,4,6-penta-*O*-galloyl-ß-$^4C_1$-glupyranose, (3); 1,6-digalloylglucopyranose (4) ;1,3-digalloylglucopyranose (5); 2,3-digalloyl-glucopyranose,nilocitin (6) ;2,3,6-trigalloylglucopyranose (7) and 2,3-di-*O*-galloyl-4,6-*O*-hexahydroxydiphenoyl-(α/β)- $^4C_1$-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 IC_{50} = 3.38 µg/ ml and PC3 cell line with IC_{50}=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) , IC_{50} of HEPG2 value = 4.5 µg/ml. the IC_{50} 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% (IC_{50}) 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 ameliorating of hepatotoxicity induced by CCL_4 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, CCL_4, 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]** . (CCl_4), 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, CCl_4 prompted fibrosis, cirrhosis and hepatocarcinoma **[5]** .The lethal impact of CCl_4 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, CCl_4 . 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 glycolsides), and anthocyanins. **[12]** . The wealth of the flavonoids glycosides was likewise noted in the aeronautical pieces *of P. lentiscus, P. atlantica, Pistacia vera, Pistacia chinensi 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-pentadecenyl-phenol ;3,4,5-tri-*O*-galloyl quinic corrosive;1,2,3,4,5 -penta-*O*-galloyl-ß-D-$^4C_1$-glucopyranose and gallic corrosive from *P.vear*, *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-ß-D-$^4C_1$-gluco pyranose (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 CCl_4 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-d_6 on a JEOL ECA 500 MHz, NMR spectrometer,^{1}H at 500 MHz and ^{13}C 125MHz. Standard pulse sequence and parameters were used to obtain one-dimensional. ^{1}H chemical shifts (δ) were measured in ppm, relative to TMS , ^{13}C - 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) H_2O; (2) 6% HOAc; (3) BAW (n-BuOH : HOAc : H_2O , 4:1:5, upper layer).

Plant Material

Fresh leaves of *Pistacia atlantica* Desf (Anacardiaceae) were collected on May 2016 from wadi sawfajjin, 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 H_2O/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 H_2O/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-BuOH 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: H_2O) (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 ^{1}H and ^{13}CNMR 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 cells .

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% CO_2 , 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 CCl_4 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 CCl_4 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 CCl_4 .

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 CCl_4 alone.
Group-3: Rats were intraperitoneally injected with CCl_4 followed by oral administration of *Pistacia* ethyl acetate extract, ET (200 mg/kg body weight).
Group-4: Rats were intraperitoneally injected with CCl_4 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.

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 assay: 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 60min 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 at 4°C 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_{max}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., Sumerduck, 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_4 alone; rats injected with CCl_4 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% para formaldehyde, and embedded in paraffin wax blocks. Tissue sections of 5 µm thick 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)

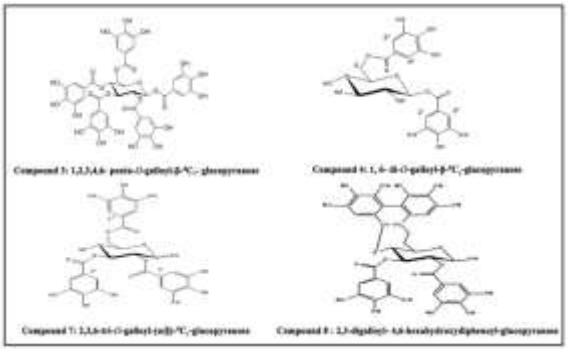


Figure 1 : The structures of bioactive isolated pure compounds : 1,2,3,4 penta-*O*-galloyl-β-D-$^4C_1$-glucopyranose 1,6-digalloylglucopyranose (4) ; 2,3,6-tri-*O*-galloyl-(α/β)-glucopyranose (7); 2,3-galloyl-4,6-*O*-hexahydroxyl diphenoyl-(α/β)-glucopyranose (8).

Compound (3): **1,2,3,4,6-penta-*O*-galloyl-ß-$_4C_1$-glupyranose**, Pure compound was isolatedas an off-white amorphous powder (86 mg), negative ESI-MS analysis, *m/z* 939.1146 $[M-H]^-$,(calc. for $C_{41}H_{31}O_{26}$, 939.1109). ^{1}H-NMR (CD_3OD) , glucose moiety δ_{ppm} 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-6α),4.37 (dd, 12.2, 4.2 Hz, H-6β). Galloyl moieties: δ_{ppm} 7.10, 7.04, 6.97, 6.94, 6.89 (s, each 2H) .^{13}C-NMR (CD_3OD) glucose moiety: δ_{ppm} 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, 110.44,110.38,110.36,110.3 (C-2, C-6).

Compound (4):**1, 6-di-*O*-galloyl-β-$^4C_1$-glucopyranose** Pure material as a non-crystalline creamy white amorphous Powder (75mg) . It exhibited an negative ESI-MS $[M-H]^-$ at *m/ z*=483 in its. ^{1}H- NMR Spectral Data (DMSO-d_6) δ_{ppm} 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-5),4.35 (1H, *d*, *J*=12 Hz, H-6α),4.2 (1H, *dd*, *J* =12 Hz and *J*=4.5, H-6β). Galloyl moieties in β-anomer 6.89(2H,*s*) and 6.95(2H,*s*).^{13}C-NMR Spectral Data (DMSO-d_6) δ ppm, β-glucose moiety 94.9 (C-1), 75.2 (C-2), 76.7(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',6',C-2'', 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)-$_4C_1$-glucopyranose ,** was isolated as an off-white amorphous powder (75 mg).UV spectral data at 275 nm which is gallotannins . It exhibited a molecular ion at $[M-H]^-$: *m/z*= 635; HRESI-MS, negative mode: *m/z* = 635.4616, corresponding to a molecular formula of C_{27} H_{24} O_{18} .^{1}H- NMR Spectral Data (DMSO-d_6)δ_{ppm} α-glucose moiety : 5.37 *(*d, *J*=3.5 Hz, H-l), 4.95 (dd, *J*=8 and 3.5 Hz, H-2), 5.70 (t, *J*=8 Hz, H-3), 3.70 (m, H-4), 3.95 (m, H-5), 4.38 (d, *J*=12.5 Hz, H- 6), 4.25 (dd, *J*=12.5 Hz and 4.5 Hz, H-6').β-glucose moiety: 5.05 (d, *J*=8 Hz. H-l),5.10 (t, *J*=8 Hz, H-2), 5.33 (t, *J*=8 Hz, H-3), 3.80 (m, H-4), 3.92 *(m*, H-5), 4.42 (d, *J*=12.5 Hz, H-6), 4.29 (dd. *J*=12.5 and 4.5 Hz, H-6').Galloyl moieties in α- and β-anomers: 7.0 (s), 6.99 (s), 6.98 (s), 6.95 (s), 6.93 (s), 6.88(s). ^{13}C-NMR Spectral Data (DMSO-d_6) δ_{ppm}, α-glucose moiety 91.2 (C-l), 72.2 (C-2), 72.8 (C-3), 69.3 (C-4), 72.3 (C-5). 64.3 (C-6). β-glucose moiety 95.3 (C-l), 73.5 (C-2), 75.5 (C-3), 70.8 (C-4), 74.9 (C-5), 64.4 (C-6). Galloyl moieties in α- and β-anomers:121.6,121.7,121.8(C-1',C-1'',C-1'''),109.8, 109.9,110.2(C-2',6',C-2'',6'',C-2''',6'''),146.1, 146. 0, 145.9,145.4(C-3',5',C-3'', 5'', C3''', 5'''),139.3 , 138.6, 138.8 (C-4',C-4'', C-4'''),167.0,166.8, 166.6, 165.0 (C-7',C-7'',C-7''').

Compound(8)**:2,3-di-*O*-galloyl-4,6-*O*-hexahydroxyldiphenoyl-(α/β)-$^4C_1$–glucopyranose,** isolated pure as off-white amorphous powder (91 mg), was found to possess chromatographic characters , dark blue response towards $FeCl_3$ spray reagent, UV at λ_{max} 275 nm and comparable for galloylatedhexahydroxydiphenoyl glucoses . The $[M-H]^-$ at m/z proved a molecular weight is 786. $_1$H- NMR Spectral Data (DMSO-d_6)δ_{ppm} α-glucose moiety, 5.38 (1H,d, *J*=2.5, H-1),5.01 (1H,dd, *J*=8 and 2.5, H-2), 5.69 (1H,t, *J*=8, H-3),4.89 (1H, m, H-4),4.57 (1H, m, H-5),5.12 (m, H-6 α) and 3.81 (d, *J*=12.5, H-6 β). β - glucose moiety , 4.94 (1H, d, *J*=8, H-1),5.03 (1H, t, *J*=8, H-2), 5.53 (1H, t, *J*=8, H-3),4.34 (1H, t, *J*=8, H-4),4.34 (1H, m, H-5), 5.12 (m, H-6α) & 3.74 (d, *J*=12.5, H-6β). Galloyl moieties in α-and β-anomers: 6.89 ,6.88, 6.82, 6.77 (s, H-2 and H-6) Hexahydroxydiphenoyl moiety in α- and β-anomers : 6.40, 6.36, 6.26, 6.22 (s, H-3 and H-3') . ^{13}C-NMR Spectral Data (DMSO-d_6) δ_{ppm} α - glucose moiety : 90.22 (C-1), 72.07 (C-2), 70.56 (C-3), 70.43 (C-4), 69.96 (C-5), 62.92 (C-6) , b-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). Hexahydroxydiphenoyl moiety in α- and β-anomers: 116.07 (C-1&C-1'),124.79, 124.21(C-2&C-2'), 105.6,105.8 (C-3&C-3'), 144.37 (C-4& C-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'-dimethoxyellagicacid (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 cytotoxic:

The ethyl acetate extract showed a higher cytotoxicity to Caco-2 cell line with IC_{50} =3.38 µg/ml and PC_3 cell line with IC_{50}=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), IC_{50} of HEPG2 value = 4.5 µg/ml. the IC_{50} for Caco-2 was 11 µg/ml. and MCF7, IC_{50}=13.5 µg/ml. as well , in comparison with pure compounds (4,7,8). (Figure 4)

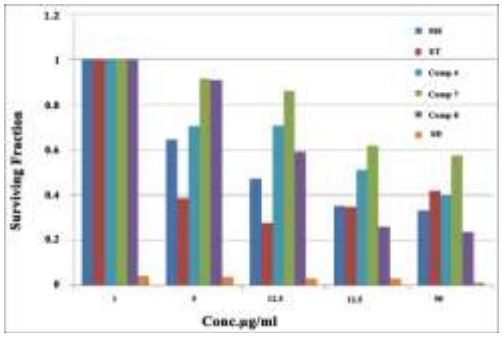


Figure 2: In *vitro* cytotoxic activity of methanol, ethyl acetate of *Pistacia atlantica* and compounds (4,7,8) on colorectal adenocarcinoma cell line (Caco-2 cell line)

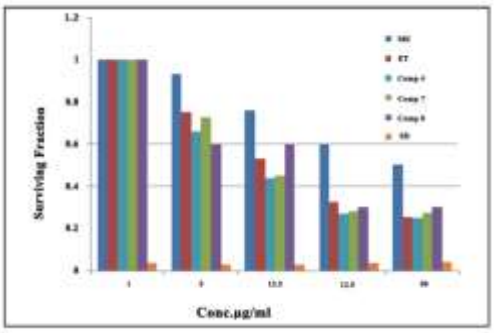


Figure 3: In *vitro* cytotoxic activity of methanol, ethyl acetate of *Pistacia atlantica* and compounds (4,7,8) to prostate carcinoma cell line (PC_3) cell line

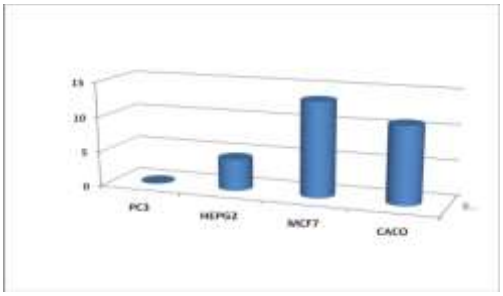


Figure 4: The cytotoxic activity of pure compound 1,2,3,4,6- penta-*O*-galloyl-β-$^4C_1$-glucopyranose (3) to all three cancer cell lines (HEPG2, Caco-2, MCF-7)

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 1: Effect of oral administration of CCL_4 alone or with different *Pistacia* extracts, on certain hematological parameters of male albino rats

Parameters	Experimental groups			
	Control	**CCL_4**	***Pist* Et + CCL_4**	***Pist* Me + CCL_4**
Red blood cell count (×1012 L^{-1})	5.99± 0.31	6.71±0.62	6.91±0.51	6.06±0.4
White blood cell count (×109 L^{-1})	5.9± 0.82	15.5±1.91	14.7±0.87	14.4±0.74
Platelet count (×109 L^{-1})	471±43.3	783.5±41.6	483.3±33.7	435.6±34.84
Hemoglobin content (g d L^{-1})	12.6 ±0.38	9.3±0.32	12.36±0.85	11.6±0.92
Packed cell volume (%)	34.2±1.23	39.1±1.91	36.7±1.78	36.1±2.46

Data are represented as mean ± standard error.

Table 2: Effect of oral administration of CCL_4alone 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

Parameters	Experimental groups			
	Control	CCL_4	*Pist.* Et + CCL_4	*Pist.* Me + CCL_4
TL (mgdL^{-1})	512.04 ±43.06	658.8 ±50.38	488.40±38.07	440.80±31.76
TC (mgdL^{-1})	118.20±2.97	228.8 ± 20.31	122.40± 13.68	103.80±4.54
TG (mgdL^{-1})	104.40±7.34	164.80±14.59	106.00± 9.39	101.40± 8.33
LDL-C (mgdL^{-1})	61.20±9.87	159.02 ± 16.76	55.60± 8.03	43.80± 4.49
HDL-C (mgdL^{-1})	36.60±6.40	27.06 ± 3.95	40.00± 5.52	39.80± 4.73

Data are represented as mean±standard error.

Glutathione reductase (GR) of male albino rats

The present study has established that CCl_4 treatment could have affected the lipid metabolism of liver (triglyceride and cholesterol levels). This is evidenced from the present observations in which CCl_4 caused a significant ($p < 0.05$) increase in the levels of lipid parameters. In this connection, **[26]** stated that CCl_4 intoxication is similar to hepatitis in case of the triglycerides catabolism. This situation could be also attributed to the reduction of lipase activity, which could lead to decrease in triglyceride hydrolysis **[27]**

On the other hand, it very well may be expected that hypercholesterolemia in CCl_4 inebriated rodents was come about because of harm of hepatic parenchymal cells that lead to unsettling influence of lipid digestion in liver . In any case, rodents treated with *Pistacia* ethyl acetate derivation and methanolic concentrates demonstrated a huge ($p<0.05$) decline in triacylglycerol and cholesterol esteems in contrast with CCl_4 inebriated rodents. The mechanism of lipid bringing down impacts of *Pistacia* concentrates may be ascribed to an inhibitory movement on microsomalacyl coenzyme A, cholesterol acyl transferease in *vitro*. This enzyme is responsible for acylation of cholesterol to cholesterol esters in liver **[28]** . Serum protein profile of various gatherings of rodents (Table 3) was observably influenced by the kind of treatment as rodents managed CCL_4 alone displayed checked decreases in the degrees of egg whites synchronous with a huge increment in the degrees of globulin, when contrasted with the controls. 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 CCl_4 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 CCl_4 treated gathering.

Table 3: Effect of oral administration of CCL_4 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

Parameters	Experimental groups			
	Control	CCL_4	*Pist.* Et + CCL_4	*Pist.* Me + CCL_4
TP (g dL^{-1})	6.68±0.22	6.52±0.30	6.24±0.05	6.19 ± 0.08
A (g dL^{-1})	4.42±0.13	3.42±0.15	4.12±0.09	4.36 ± 0.07
G (g dL^{-1})	2.46±0.24	3.70±0.18	2.62±0.19	2.59 ± 0.11
A/G ratio	1.72±0.16	0.85± 0.09	1.38±0.16	1.36 ± 0.13

Data are represented as mean±standard error.

In this examination the huge ($p<0.05$) decline in serum albumin of rats treated with CCl_4 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 concentration of serum albumin was observed in rats received *Pistacia* extracts in addition to CCl_4 in contrast with rodents got CCl_4 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 thiols as a result of the treatment **[30]** . Liver capacity markers, as impacted by the organization of CCl_4 . *Pistacia* extracts alone and blended, were exhibited in (Table 4).

Table 4: Effect of oral administration of CCL_4 alone or with different *Pistacia* extracts, on the activities of serum aspartate aminotransferase (ASAT), alanine aminotransferase (ALAT) and alkalinephosphatase (ALP) and the levels of total bilirubin (TBil) and direct bilirubin (DBil) of male albino rats

Parameters	Experimental groups			
	Control	CCL_4	*Pist.* Et + CCL_4	*Pist.* Me + CCL_4
ASAT (UL^{-1})	33.02 ±1.30	118.7 ± 24.49	48.20± 8.01	52.7 ±11.2
ALAT (UL^{-1})	25.60±1.50	75.60±2.77	39.02 ± 5.52	38.9 ± 7.63
ALP (UL^{-1})	55.30±3.84	70.02± 8.08	53.22± 5.72	56.14 ± 7.61
TBil (mg dL^{-1})	0.66±0.02	0.89±0.03	0.73±0.05	0.77 ± 0.03
DBil (mg dL^{-1})	0.11±0.005	0.14 ±0.006	0.10±0.008	0.10± 0.004

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 CCl_4 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 CCl_4 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 CCl_4 contrast with the control.

As in the present examination, the previous investigations have demonstrated that $CC1_4$ 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 CCl_4 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 (Cichoriuminty bus) 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 CCl_4 induced hepatotoxic animals **[36]**.These finding infers that challenge to shield liver tissue from CCl_4 damage.

Impact on the Hepatic Lipid Peroxidation and Endogenous Antioxidants:

The impacts of CCl_4 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 CCl_4 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 activities when contrasted with the controls. In the rodents of *Pistacia* extracts with CCl_4 treated gatherings, the mean estimations of hepatic MDA fixation were altogether lower than those of CCl_4 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 CCl_4 treated rodents were fundamentally higher than those of CCl_4 treated gathering. When contrasted with the CCl_4 treated gathering, the rodents directed *Pistacia* extracts in addition to CCL_4 demonstrated a stamped rise in the exercises of CAT and SOD and

Table 5: Effect of oral administration of CCL_4 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

Parameters	Experimental groups			
	Control	CCL_4	*Pist.* Et + CCL_4	*Pist.* Me + CCL_4
MDA (nmol g^{-1} liver)	4.48±0.11	9.18± 0.26	4.15±0.22	4.78 ± 0.34
GSH (mg g^{-1} liver)	40.04±5.10	19.72±0.98	37.34±2.84	38.91 ± 2.31
CAT (U g^{-1} liver)	104.3±17.1	39.40±8.27	99.03 ±13.38	101.56 ± 14.74
SOD (U g^{-1} liver)	9.56±0.17	4.36±0.19	9.41±0.16	10.23 ± .35
GR (U g^{-1} liver)	73.20±2.71	27.80±1.28	68.40±3.48	69.76 ± 3.93

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 laborers, for example, [37] who detailed that hepatotoxic impacts by CCl_4 are lipid peroxidation inception, and are to a great extent due to its dynamic metabolite of CCl_3 (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 ($p<0.05$) CCl_4 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 CCl_4 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 CCl_4 significantly reduced tail moment and consequently significant reduction in the percent of DNA damage as compared to CCl_4 in toxicated 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 CCL_4alone or with different *Pistacia* extracts, on the rate of DNA damage in brain tissues of rats using comet assay.

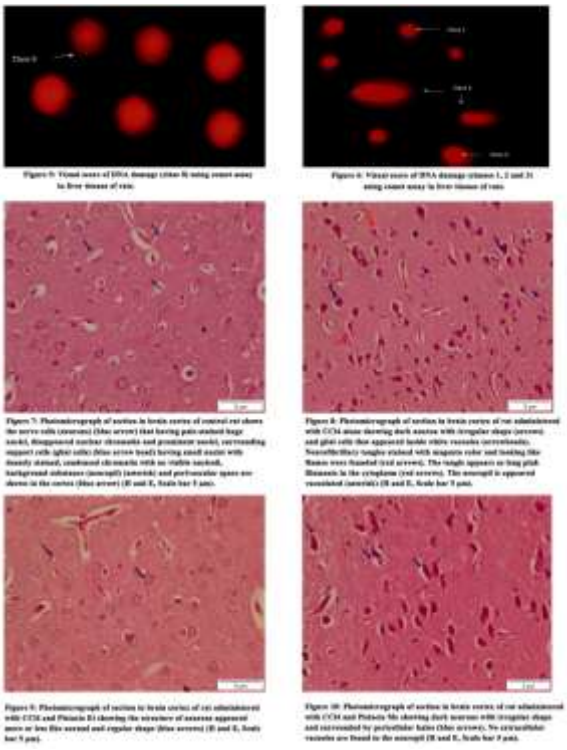
Treatment	*No. of cells		¥Class of comet				DNA damaged cells (mean ± SEM)
	Analyzed	Total comets	0	1	2	3	
Control	500	34	466	23	11	0	6.83±0.11
CCL_4	500	124	376	32	44	48	24.81±0.82
Pist Et + CCL_4	500	83	417	35	26	22	16.62±0.46
Pist Me + CCL_4	500	79	421	33	25	21	15.82±0.61

¥: 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 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. Neuropil or background substances are shown in the cortex (Figure 7).

Examination of sections of brain cortex of rats administered with CCl_4 alone showed dark neurons with irregular shape and glial cells that appeared inside white vacuoles. Neurofibrillary 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 CCl_4 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 CCl_4 and *Pistacia* methanol extract showing dark neuronswith irregular shape and surrounded by pericellular halos (blue arrows). No extracellular vacuoles are found in the neuropil (Figure 10).

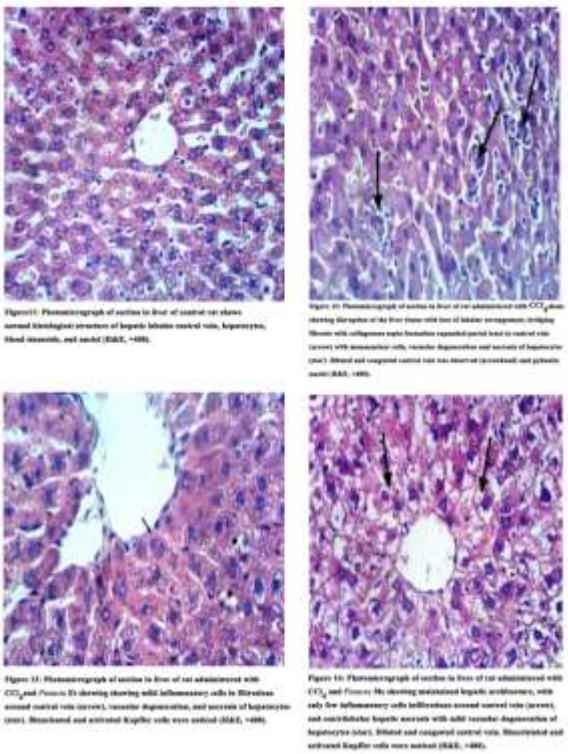


Liver:

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 CCl_4 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, vacuolar degeneration and corruption of hepatocytes (Figure 12).

Liver segments of rodents managed with CCl_4 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 CCl_4 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 hepaticnecrosis with mellow vacuolar degeneration of hepatocytes. (Figure 14).



In the present investigation, the biochemical discoveries were additionally affirmed by histpathological 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 CCl_4 induced hepatic damage in male Wistar albino rats were observed in the present study. Probably, due to the antioxidative properties of *P. atlantica* extracts and accumulating of different types of phenolic compounds which helped hepatic cells to obviate CCl_4 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.

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References

[1] Iauk, L., Ragusa, S., Rapisarda, A., Franco, S. and Nicolosi, V.M.. In vitro antimicrobial activity of *P. lentiscus* L. extra*cts: Preliminary report.* Journal of Chemotherapy, 1996, 8, 207-209, doi: 10.1179/ joc.1996.8.3.207

[2] Yousfi M, Nadjemi B, Belal R, Bombarda I, Gaydou E. Triacylglycerol composition of oil from *P.atlantica* fruit growing in Algeria. *Journal of the American Oil Chemists Society*. 2005 doi: 10.1007/s11746-005-1048-7. [CrossRef] [Google Scholar]

[3] Delazar A, Reid RG, Sarker SD. GC-MS analysis of the essential oil from the oleoresin of *Pistacia atlantica* var. *Mutica*. *Chem. Nat. Compd*. 2004 ; 40 (1): 24-27

[4] Nunez M. Hepatotoxicity of antiretrovirals: Incidence, mechanisms and management. *J Hepatol.* 2006;44:132–139.doi:10.1016/j. jhep. 2005. 11.027.[PubMed][CrossRef]

[5] Karakus E, Karadeniz A, Simsek N. Protective effect of Panax ginseng against serum biochemical changes and apoptosis in liver of rats treated with carbon tetrachloride J.Hazard, Mater. 2011;195:208213.doi:10.1016/j.jhazmat.2011.08. 027.[PubMed][CrossRef] [Google Scholar]

[6] Stoyanovsky D, Cederbaum AI. Metabolism of carbon tetrachloride to trichloromethyl radical: an ESR and HPLC-EC study. *Chem Res Toxicol.* 1999;12:730–736. https://doi.org/10.1021/ tx9900371

[7] Ramadori G, Saile B. Portal tract fibrogenesis in the liver. Lab Invest. 2003;84:153–59. doi: 10.1038/ labinvest.3700030. [PubMed] [CrossRef] [Google Scholar]

[8] Canbay A, Feldstein A, Baskin-Bey E. The caspase inhibitor IDN-6556 attenuates hepatic injury and fibrosis in the bile duct ligated mouse. *J Pharm Exp Ther.* 2004;3:1191–1196. [PubMed] [Google Scholar]

[9] Lee CH, Park SW, Kim YS. Protective mechanism of glycyrrhizin on acute liver injury induced by carbon tetrachloride in mice. *Biol Pharm Bull.* 2007;30:1898–1904. doi:10.1248/ bpb.30.1898. [PubMed] [CrossRef] [Google Scholar]

[10] Aghili Khorasani Shirazi MH. Makhzan-al-Advia. Edited by Roja Rahimi, Mohammad Reza Shams Ardekani, Fatemeh Farjadmand. The Principal's of Traditional Iranian Medicine; 2009. http:// journals.sbmu.ac.ir/en-mh/article/view/14626

[11] Pell , S. K. , J. D. Mitchell , A. J. Miller , And T. A. Lobova Anacardiaceae. In K. Kubitzki [ed.] *The families and genera of vascular plants*, 2011; 7–45. Springer-Verlag, Berlin, Germany

[12] Romani A, Pinelli P, Galardi C, Mulinacci N, Tattini M . Identification and quantification of galloyl derivatives, flavonoid glycosides and anthocyanins in leaves of *P.lentiscus* L. *Phytochem. Anal.* 2002. 13(2):79-86 , DOI:10.1002/pca.627

[13] Kawashty S, Mosharrafa S, El-Gibali M, Saleh N. The flavonoids of four *Pistacia* species in Egypt. *Biochemical Systematics and Ecology*. 2000;28(9):9157. [PubMed]

[14] Abdelwahed A, Bouhlel I, Skandrani I, Valenti K, Kadri M, Guiraud P, Steiman R, Mariotte AM, Ghedira K, Laporte F, Dijoux-Franca MG, Chekir-Ghedira L. Study of antimutagenic and antioxidant activities of Gallic acid and 1,2,3,4, 6-pentagalloylglucose from *Pistacia lentiscus* Confirmation by microarray expression profiling.

Chemico-Biological Interactions 2007. 165: 1–13. https://doi.org/ 10.1016/ j. cbi.2006.10.003

[15] Bhouri, W. Derbel, S. Skandrani, I.. Study of genotoxic, antigenotoxic and antioxidant activities of the digallic acid isolated from *P.lentiscus* fruits. *Toxicology in vitro*,2010 ; 24 (2) :509-515. doi: 10.1016/j.tiv.2009.06.024

[16] Salma.O.K.Othman,Maher.A.El-Hashash,Sahar. A.M.Hussein,Amani.M.D.El-Mesallamy,Sameh. A.Rizk Fakhri A. Elabbar. Phenolic content as Antioxidant and Antimicrobial Activities of *Pistacia Atlantica* Desf. (Anacardiaceae) extract from Libya, *Egyptian Journal of Chemistry* ,019,62:1.21-28,doi:10.21608/ ejchem.2018. 4577.1403 http:// ejchem.journals. ekb.eg/article _11269.html

[17] Ait Said S, Fernandez C, Greff S, Derridj A, Gauquelin T, Mevy J-P. Inter-population variability of leaf morpho-anatomical and terpenoid patterns of Pistacia atlantica Desf. Ssp. atlantica growing along an aridity gradient in Algeria. *Flora*. 2011; 206(4):397–405. https://www.sciencedirect.com/science/article/pii/ S036 7253010001659

[18] Skehan, P., Storeng R.,Scudiero D.,Monks A., McMahon J.(1990): New colorimetric cytotoxicity assay for anticancer drug screening. *J. Nat. Cancer Inst.*, 82: 1107-1112, https://doi. org/10.1093/jnci/ 82.13.1107Al-Fartosi KG, Majid A, Auda MA. The role of Camel's milk against some oxidant-antioxidant markers of male rats treated with CCl_4. Int J Res Pharmaceut Biomed Sci. 2012;3(1):385–389. [Google Scholar]

[19] Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Anal Biochem.* 1979;95:351–8. doi: 10.1016/0003-2697(79)90738-3

[20] Beutler, E., O. Duron and B.M. Kelly. Improved method for the determination of blood glutathione. *J. Lab. Clin. Med.*, 1963, 61: 882-888.

[21] Nishikimi, M., N.A. Roa and K. Yogi,. The occurrence of superoxide anion in the reaction of reduced phenazine methosulfate and molecular oxygen. *Biochem. Biophys. Res. Commun.*, 1972;46: 849-854.

[22] Goldberg, D.M. and R.J. Spooner. Method for the determination of glutathione reductase. *Methods Enzymatic Anal.*, 1983, 3: 258-265.

[23] Blasiak, J., Arabski, M., Krupa, R., Wozniak, K., Zadrozny, M., Kasznicki, J., et al. (2004). DNA damage and repair in type 2 diabetes mellitus. *Mutat. Res.*554,297–304.doi:10.1016/j.mrfmmm .2004.05.011

[24] Sahar A. M. Hussein , (2004): Flavonoid and methoxyellagic acid sodium sulphates from *Frankenia* leaves *L.Die Pharmazie* 59(6),484-487https://www.ingentaconnect.com/ content/ govi/pharmaz/2004/00000059/00000006/ art00013?

[25] Meral I., Kanter M. Effects of Nigella sativa L. and Urticadioica L. on selected mineral status and hematological values in CCl_4treated rats. Biol Trace Elem Res. 2003; 96(1-3): 263-70.Al-Fartosi KG, Majid A, Auda MA. The role of Camel's milk against some oxidant-antioxidant markers of male rats treated with CCl_4. *Int J Res Pharmaceut Biomed Sci.* 2012;3(1):385–389. [Google Scholar]

[26] Meral I., Kanter M. Effects of Nigella sativa L. and Urticadioica L. on selected mineral status and hematological values in CCl_4treated rats. Biol Trace Elem Res. 2003; 96(1-3): 263-70.Al-Fartosi KG, Majid A, Auda MA. The role of Camel's milk against some oxidant-antioxidant markers of male rats treated with CCl_4. *Int J Res Pharmaceut Biomed Sci.* 2012;3(1):385–389. [Google Scholar]

[27] Jahn CE, Schaefer EJ, Taam LA, Hoofnagle JH,Lindgren FT, Albers JJ, Jones EA,Brewer HB Jr.Lipoprotein abnormalities in primary biliary cirrhosis. Association with hepatic lipase inhibition as well as altered cholesterol esterification . *Gastroenterol* 1985,89:1266-12 78

[28] Matsuda K. ACAT inhibitors as antiather osclerotic agents: compounds and mechanisms. Med Res Rev.1994;14:271–305. doi:10.1002/ med.2610140302.[PubMed][CrossRef]

[29] Al-Fartosi KG, Khuon OS, Al-Tae HI. Protective role of camel's milk against paracetamol induced hepatotoxicity in male rats. *Int J Res Pharmaceut Biomed Sci.* 2011;2:1795–1799.[Google Scholar]

[30] Sreepriya M, Devaki T, Nayeem M.Protective effects of *Indigo feratinctoria* L. against D-Galactosamine and carbon tetrachloride challenge on'in situ'perfused rat liver *IndianJ. Physiol.Pharmacol* 2001,45:428-434https://www.ijpp.com/IJPP % 20archives/200145/4/428-434.pdf

[31] Khan AA, Alzohairy M. Hepatoprotective effects of camel milk against CCl4 induced hepatotoxicity in rats. *Asian J. Biochem.* 2011;6 (2):171–180.doi:10. 3923 /ajb.2011. 171.180 [CrossRef] [Google Scholar]

[32] Arıcı O, Cetin N. Protective role of ghrelin against carbon tetrachloride (CCl_4 induced coagulation disturbances in rats. *Regul Pept.* 2011;166:139–142.doi:10.1016/j.regpep.2010.10.009. [PubMed] [CrossRef] [Google Scholar]

[33] Iranmanesh F, Mousaei Amin A, Shamsizadeh A, Fatemi I, Malaki Rad A, Rahnama A. Effects of P.vera Hydro-Alcoholic Extract on Carbon Tetrachloride-Induced Hepatotoxicity in Male Rats. *Iranian Journal of Pharmacology and Therapeutics* 2016;14 (2) : 35-40.

[34] Madani H, Asgary S, Naderi G, Talebolhoseini M. Hepatoprotective effect of *Chichoriuminty bus* L. on liver toxicity in rat. *J Medicinal Plants* 2006; 1:32-8

[35] Rafiee F, Heidari R, Ashraf H, Rafiee P. Protective effect of *Berberis integerrima* fruit extract on carbon-tetrachloride induced hepatotoxicity in rats. *J Fasa Univ Med Sci* 2013;3:179-87.

[36] Park WH, Lee SK, Kim CH. A Korean herbal medicine, panax notoginseng, prevents liver fibrosis and hepatic microvascular dysfunction in rats. *Life Sci.* 2005;76:1675–1690. doi: 10.1016/j.lfs.2004.07.030

[37] Palanive MG, Rajkapoor B, Kumar RS. Hepatoprotective and antioxidant effect of pisonia aculeata L. against CCl_4 induced hepatic damage in rats. *Sci Pharm* 2008, 76:203–215, doi:10.3797/scipharm.0803-16 https://pdfs. semanticscholar.org/ d6a6/655 aa 9b5e81739a756e 0c4aa03be4c27f877.pdf

[38] Silva EM, Souza JNS, Rogez H, Rees JF. Larondelle Y. Antioxidant activities and polyphenolic contents of fifteen selected plant species from the Amazonian Region. *Food Chem.*2007;101:1012–8.doi:10.1016/j.foodchem.2006.02.055 . [CrossRef]

[39] Ballistreri G, Arena E, Fallico B. Influence of ripeness and drying process on the polyphenols and tocopherols of *P.vera* L. *Molecules*,2009;14(11):4358–4369.doi:10.3390 / molecules 14114358

[40] Gentile C, Tesoriere L, Butera D, Fazzari M, Monastero M,Allegra M. Antioxidant activity of *P.vera* hull extracts. *Food Chem* 2005; 92:521-5

[41] Hossein zadeh H, Sajadi Tabassi SA, Milani Moghadam N,Rashedinia M, Mehri S. Antioxidant activity of P. vera fruits, leaves and gum extracts. Iran J Pharmaceut Res. 2012;11:879-87.

[42] Yousef MI, Saad AA, El-Shennawy LK. Protective effect of grape seed proanthocyanidin extract against oxidative stress induced by cisplatin in rats. Food Chem Toxicol.2009,47(6):1176- 1183. doi: 10.1016/j.fct.2009.02.007.

[43] Thabrew M, Joice PD, Rajatissa W.A Comparative Study of the Efficacy of Pavetta indica and Osbeckia octandra in the Treatment of Liver Dysfunction. Planta Med 1987; 53(3): 239-241 https://doi.10.1055/s-2006-962691

[44] Abdullev FI. Biological effects of saffron. Biofactors 1993; 4(2):83–86.,Havel RJ: Functional activities of hepatic lipoproteins receptors. Annual Review of Physiology, 1986,48:19-134.10.1146,https://doi.org/10.1146/annurev.ph.4 8 .030186.001003.

[45] Ansar, S., Siddiqi, N. J., Zargar, S., Ganaie, M. A. and Abudawood, M.. Hepatoprotective effect of Quercetin supplementation against Acrylamide-induced DNA damage in wistar 30 rats Bmc Complement Altern Med, 2016,16.327-332.https://doi.org/10.1186/s12906-016 1322-7

[46] Sun F, Hamagawa E, Tsutsui C.Evaluation of oxidative stress during apoptosis and necrosis caused by carbon tetrachloride in rat liver. Biochim Biophys, Acta.2001; 1535:186–191.https://doi:10.1016/S0925-4439(00)00098-3. [PubMed] [CrossRef]

المحتوى البوليفينولى، كمضادات لألكسدة و الميكروبات النشطه لمستخلص اوراق نبات البطم االطلسي والسمية الخلوية و CCl_4 الناجم عن الكبد في الفئران

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الهدف من هذا البحث هو البحث عن مستخلص جديد نشط بيولوجيًا الوراق نبات البطم االطلسي، وقد تم قياس االثار البيولوجية لخالصة كحول الميثانول وايثيل اسيتات الوراق هذا النبات و مجزئاتة و بعض المركبات المفصولة منة . تم استكشاف التأثير الدفاعي لمستخلصات الميثانول المائي وخلأت ايثيل تجاه السمية الكبدية التي يسببه CCl_4 في الفئران. تم عزل المكونات الجديدة باستخدام أنظمة الفصل الكروماتوجرافي . تم توضيح هياكل المكونات الجديدة اعتمادًا على معلومات البيانات الطيفية لألشعة فوق البنفسجية ، بالرنين المغناطيسي النووي إلى جانب التحقيقات الطيفية الجماعية.تحديد المركبات المعزولة بالطرق التقليدية ، التحليل الطيفي ، البحث عن مستخلص جديد يحتمل أن يكون نشطًا بيولوجيًا أكثر فعالية بعد تحديد جميع المركبات في هذا المزيج.

النشاط السام للخاليا من مستخلصات الميثانول وخلأت ايثيل من اوراق نبات البطم االطلسي وأسفر عن مركبات جديدة على أربعة خطوط خاليا سرطان بشرية لتكون محددة: خط خلية سرطان الغدة القولون والمستقيم خط خاليا Caco-2 ، خط خاليا سرطان البروستاتا خط خلية سرطان PC_3 (HEPG2) (MCF-7) تم استخدام الفحص SRB لقياس السمية الخلوية المحتملة.

أظهر مستخلص خالت االيثيل سمية خلوية أعلى لخط خلية Caco-2 IC_{50}= 3.38 ميكروغرام / مل وخط خلية PC3 مع IC_{50}= 14.3ميكروغرام / مل. وعالوة على ذلك ، كان استخراج الميثانول أقل السمية للخاليا إلى خطوط الخاليا الطبيعية. وقد لوحظت إمكانات قوية للتسمم الخلوي في جلوكوبرانوز بنتاجالويل النقي) 3 (لجميع خطوط الخاليا السرطانية الثالثة
HEPG2 = 4.5 بقيمة IC_{50} ، MCF-7 ، HEPG2 ,Caco-2 ميكروغرام / مل. كان IC_{50} ل 11 Caco-2 ميكروغرام / مل.
و MCF7 ، 13.5 = IC_{50} ميكروغرام / مل. كذلك ، بالمقارنة مع المركبات النقية) 4،7،8 (تم حساب تثبيط نمو 50 ٪ (IC_{50}) لكل مستخلص من الكثافة البصرية للخاليا المعالجة وغير المعالجة. عالوة على ذلك ، نتج عن مستخلصات الميثانول وخلأت ايثيل اوراق نبات البطم االطلسي ترشيحًا جذابًا لتحسين السمية الكبدية التي يسببها CCl_4 في الفئران من خلأل مسح الجذور الحرة وتحسين وظائف الكبد وتطبيع بنية الكبد التشنجية.