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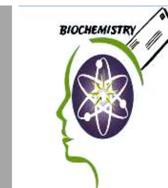

Effect of N-Acetyl cysteine and Pomegranate Peel Water Extract on Hepatotoxicity Induced by Paracetamol.

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ARTICLE INFO

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ABSTRACT

Background: One of the safest antipyretics, analgesics is paracetamol when used at therapeutic doses but overdoses caused hepatic necrosis. Recent researches were proven the ability of pomegranate to treat many diseases. For new ways to reduce paracetamol toxicity pomegranate peel extract was used in comparison to N- Acetyl cysteine a standard antidote for paracetamol toxicity. **Methods:** Seventy two adult male albino rats were divided equally into nine groups; Negative control group; Solvent (distilled water) group; N-Acetyl cysteine treated group (a single dose 150 mg/kg. B.w/day orally); Pomegranate peel extract treated group (a single dose 430mg/kg. b. W/ day orally); N-Acetyl cysteine and Pomegranate peel extract treated group (each rat was given same previous doses); Paracetamol group (a single dose of 400 mg/ kg. B.w / day orally); N-Acetyl cysteine; Pomegranate peel extract and combination of N-Acetyl cysteine and Pomegranate peel extract with paracetamol were three treatment groups received the same previous doses. Treatment Continued for 28 days. These samples were collected for matrix metalloproteinase assessment in blood serum; Hepatic antioxidants and Oxidative stress marker assessment in plasma and liver tissue homogenates and Routine liver injury marker assessment in blood serum. **Res49ults:** Treatment with paracetamol caused hepatic toxicity, the Pomegranate peel extract showed a mild hepatoprotective effect, N-acetyl cysteine showed the moderate hepatoprotective effect and combination treatment of N-Acetyl cysteine and Pomegranate peel extract showed the best hepatoprotective effect against paracetamol toxicity. **Conclusion:** Paracetamol is a hepatotoxic and combination of N-Acetyl cysteine and the Pomegranate peel extract is the best treatment against Paracetamol hepatic toxicity.

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INTRODUCTION

Many pharmaceuticals induce liver injury due to capability of liver to concentrate and biotransform xenobiotics [1, 2]. One of safe analgesics is paracetamol described for all ages with no side effects at curative

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doses [3]. Paracetamol metabolized in liver forming sulfate and glucuronoides esters which excreted in bile and urine [4] and Small amount was oxidized by cytochrome P450 forming N-acetyl-p-benzoquninoamine (NAPQI) which detoxify with glutathione (GSH) [5, 6]. Hepatic glutathione inhibition occurred in the paracetamol overdose lead in association of NAPQI to cellular proteins [7]. Damaging of hepatocytes elevate matrix metalloproteinase (MMPs) levels, zinc dependent proteases present in the extracellular matrix [8, 9]. N-Acetylcysteine is able to detoxify directly and indirectly reactive metabolites in addition to it is a glutathione precursor so used as an effective antidote against paracetamol hepatotoxicity [4]. . Nowadays attention has been attracted by natural products [10, 11], such as pomegranate fruit, it is rich with active compounds which give it the ability to treat many diseases [12]. This work aims to investigate the effect of N-Acetyl cysteine and pomegranate peel extract against paracetamol hepatotoxicity.

MATERIALS:

Plant materials

Pomegranate (Punica Granatum L.) Obtained from local markets. The peels were manually removed, sun dried and ground.

Chemicals and kits:

Paracetamol in the form of Cetal 500 mg tablets manufactured by EIPICO Pharmaceutical Industries Company (10th of Ramadan, Egypt). N-Acetyl cysteine in the form of Acetyl cysteine 200mg effervescent instant manufactured by SEDICO drug industries company (6 October city, Egypt). Mouse Matrix Metalloproteinase-1 (MMP-1) and Rat Glutathione Peroxidase (GSH-PX) sandwich enzyme -linked immunosorbent assay (ELISA) kit was purchased from (CUSABIO Biotech Co. Ltd, china). Total Glutathione (GSSG/ GSH) assay kit was purchased from (CELL BIOLABS, Inc. USA). Biochemical kits were purchased from the Biodiagonistic Company (Dokki, Giza, Egypt).

Animals:

Animals were purchased from veterinary medicine faculty, Zagazig University, Egypt. Seventy two adult male swiss albino rats their weights ranging from 150-200 g were housed in plastic cages free from any source of crucial contamination under controlled conditions with an ambient temperature degree 22±2 0C, relative humidity of 50 ±5% and a 12 h light – cycle at the animal house faculty of medicine, Zagazig university. They were maintained for one week before starting the experiment as an acclimatization period on a normal diet containing small amounts of fruit, vegetables and grains and adlibtum.

Experimental design:

This study carried out on 72 adult male rats, divided equally into nine groups (8 rats) in each group (according to an ethical committee of Institutional Review Board (IRB) instructions).

Group I (Negative control group): Each rat received nothing.

Group II (Solvent group) (Distilled water group): Each rat received only 1mL distilled water once daily orally by gavage.

Group III (N- acetyl cysteine treated group): Each rat was gavaged orally with N-Acetyl cysteine at dose 150 mg/kg b.w once daily [13]

Group IV (Pomegranate peel extract treated group): Each rat was gavaged

orally with pomegranate peel extract at dose 430 mg/ kg b.w once daily [14]

Group V (N-acetyl cysteine and pomegranate combined treated group): Each rat was gavaged orally with N-acetyl cysteine and pomegranate peel extract at the same previously mentioned doses once daily

Group VI (Paracetamol group): Each rat was gavaged orally with paracetamol at dose 400 mg / kg b.w once daily [4]

Group VII (paracetamol + N-acetyl cysteine treated group): Each rat was gavaged orally with paracetamol and N-acetyl cysteine at the same previously mentioned doses once daily

Group VIII (paracetamol +Pomegranate peel extract treated group): Each rat was gavaged orally with paracetamol and pomegranate peel extract at the same previously mentioned doses once daily

Group IX (paracetamol + N-Acetylcysteine and pomegranate peel extract treated group): each rat received paracetamol, N-Acetyl cysteine and pomegranate peel extract at the same previously mentioned doses once daily.

Rats were treated for 28 days. On the 29th day, venous blood samples were collected from animals by means of micro capillary glass tubes from the retro-orbital plexus to prepare serum samples [15] and rat liver tissues were collected to prepare liver samples [16]

METHODS:

Preparation of Aqueous Extract of Pomegranate peel.

5g of pomegranate peel powder was separately blended for 2 min with 300 ml of 80% distilled water. This mixture was then left in the dark; at room temperature for 1 h then filtrated using (Whatman No. 1) and centrifuged at 3500 rpm for 10 min. Then kept on- 200 C till analysis [17].

High performance liquid chromatography (HPLC) analysis of pomegranate peel extract

Extraction was performed by taking 0.5 g of dried pomegranate peel in a 100 ml Erlenmeyer flask, then dispersed in 40 ml of water solution containing 2 g /L of 2, (3) - tert butyl-4-hydroxyanisole (BHA). The mixture was then ultra-sonicated for 5 minutes. 10 ml of 6M HCL was added to this extract.Then the sample was bubbled with nitrogen for 40 -60s after which the flask was sealed tight and the sample was filtered through a 0.2 µm membrane filter before quantification by Agilent High-performance liquid chromatography (HPLC) [18].

Liver tissue homogenates preparation

0.2 g of each liver tissue was homogenized in 4 ml of cold buffer (50mM phosphate buffer (pH 7.0), containing 5mM Ethylene diamine tetra acetic acid (EDTA), and 1mM 2 mercaptoethanol.) Then centrifuged at 3000 r.p.m. for 10 minutes at 20C.The supernatant fluids were removed to use for the measurement of liver antioxidants [19].

Assessment of antioxidant parameters:

Hepatic reduced glutathione (GSH) and Hepatic glutathione peroxidase (GPX) were measured in the liver homogenate [20-21]. Glutathione-S-transferase (GST), Catalase (CAT) and Superoxide dismutase (SOD) were measured in liver tissue homogenate [22-24] calorimetrically using ECO308 biochemistry analyzer. Lipid peroxidation as malonaldhyde (MDA) and Nitric oxide (NO) were measured in liver tissue homogenate [25,26] .

Assessment of Biochemical Parameters

Matrix metalloproteinase (MMP-1) activity was measured in serum [27]. Routine analysis for liver, Serum alanine amino transferase (ALT) and aspartate amino transferase (AST) levels were assayed kinetically [28], Albumin, Total protein, Total bilirubin were assayed calorimetrically [29-31] using a UV-200-Rs spectrophotometer (L w scientific, Umedic, Germany).

Histopathological Study

A part of liver tissue was immediately fixed at 10% buffered formalin solution in normal saline, dehydrated in graded concentrations of ethanol (50–100%), cleared in xylene and embedded in paraffin. Liver sections (4–5 lm) were prepared and stained with hematoxylin-eosin (H&E) dye using standard techniques for photo microscopic observations [32].

Statistical Analysis

Results were expressed as means of 6–8 rats± standard error of the mean (SEM). All statistical analyses were performed using one way analysis of variance (ANOVA) test using the statistical package for social sciences (SPSS; version 19.0) computer software program (SPSS Inc., Chicago, IL, USA) with the value of $p < 0.05$ considered statistically significant [33].

RESULTS:

Plant Material Extraction:

High performance liquid chromatography (HPLC) analysis of plant material showed more active compounds represented in (Table 1), Fig (1, 2). The major active ingredient identified where Phenolic acids category (Gallic acid, Pyrogallol, 4-amino – benzoic, Protocatchuic; Vanillic acid; Ellagic acid; Benzoic acid; Salycilic acid; Rosmarinic acid. Phenylpropanoids Category (Caffeic acid, p-coumaric acid; Ferulic acid; Iso- ferulic acid; e-vanillic; Alpha-coumeric acid; 3, 4, 5 methoxy – cinnamic; Coumarin; Cinnamic acid.

Catechols Category (Chlorogenic acid; Catechol; Rhamnetin.Resveratrol, Caffeine. Flavanones category (Catechein ,Epicatechein; Quercetin; Naringenin; Hespirtin; Kampferol; Rhamnetin; Apegnin; Acacetin) Flavone glycosides category (Luteo.6-arbinose 8- glucose, Luteo.6 glucose 8-arbinose , Apig. 6-arbinose 8- glactose, Apig. 6-rhamnose 8-glucose, Apig. 6-glucose 8-rhamnose, Luteo. 7- glucose , Narengin , Rutin, Hespirdin, Quercetin -3- o- glucoside, Apig. 7-o- neohespiroside, Kamp. 3,7-dirhamoside, Apig. 7- glucose, Quercetrin.

Assessment of antioxidant parameters:

Solvent group, N-Acetyl cysteine treated group, pomegranate peel extract treated group and N-Acetyl cysteine and pomegranate peel extract treated group showed no significant difference with negative control group $P>0.05$

GSH content, GPX, CAT, SOD and GST activities in negative control group were (8.87±0.99 (µmol/g tissue), 132.23±3.99 (U/g tissue), 23.77±2.38 (U/g tissue), 102.48±4.49 (U/g tissue) and 263.16±8.56 (µmol/g tissue).

A significant decrease in GSH content, GPX, CAT, SOD and GST activities were shown in the paracetamol group by (78.8%, 22.1%, 25.0%, 46.6% and 14.1%) to be (1.88±0.32 (µmol/g tissue), 103.01±3.07 (U/g tissue), 17.83±0.77 (U/g tissue), 54.68±2.07 (U/g tissue) and 226.01±4.29 (µmol/g tissue)) as compared to negative control group $P<0.0001$. N-Acetyl cysteine treated group, pomegranate peel, water extract treated group and combination treated group with

paracetamol were showing a significant increase in GSH content, GPX, CAT, SOD and GST activities by (229.8%, 16.9%, 26.8%, 64.8%and8.6%); (183.0%, 14.6%, 22.2%, 47.6% and 4.8%) and (329.3%, 24.1%, 32.4%, 79.6% and 14.3%). respectively as compared to paracetamol group P<0.0001. Meanwhile, MDA and NO levels were significantly increased from 11.79±1.01 (nmol/g tissue) and 47.78±2.56 (µmol/ g tissue) in the negative control group to 18.17±0.43 (nmol/g tissue) and 77.80± 5.87 (µmol/g tissue) by 54.1% and 62.8% in paracetamol group P<0.0001. While, their concentration was decreased by (26.1%, 22.2% and 33.7%) and (27%,19.15 and 34.4%) in N-Acetyl cysteine treated group, pomegranate peel, water extract treated group and combination treated group with paracetamol respectively as compared to paracetamol group P<0.0001.Table (2).

Assessment of Biochemical Parameters

Solvent group, N-Acetyl cysteine treated group, pomegranate peel extract treated group and N-Acetyl cysteine and pomegranate peel extract treated group showed no significant difference with negative control group P>0.05 .MMP-1, ALT, AST, Albumin, Total protein and Total bilirubin levels were (1490.53±237.65 (pg/ml), 42.94 ± 1.12 (U/L), 137.60 ±2. 32 (U/L), 3.19±0.12 (g/dl), 6.33 ±0. 24 (g/dl) and 0.78 ±0. 03 (mg/dl)) in the negative control group. After administration of paracetamol levels MMP-1, ALT, AST and Total bilirubin were increased to be (3044.80±27.60 (pg/ml) 108.13 ±2. 41(U/L), 215.18 ±7. 97 (U/L) and 5.14 ±0. 40 (mg/dl)) by (104.3%, 151.8%, 56.4 % and 559.0 %) respectively as compared to the negative control group. P < 0.0001.Treatment with N-Acetyl cysteine or/ and pomegranate peel, water extract with paracetamol were showed significantly decreased in their levels by (29.2%, 43.5%, 25.2% and 82.3%), (18.5%, 38%, 16.6% and 75.3%) and (50.2%, 53.1%, 34.5% and 84.4%) respectively as compared to paracetamol group P<0.0001.

On the other hand Albumin and Total protein levels were significantly decreased after administration of paracetamol (1.91± 0.29(g/dl), 3.64 ±0. 17 (g/dl)) by (40.12%, 42.5%) respectively, compared to the negative control group. P < 0.0001. Albumin and Total protein were showed significantly increased in treatment groups Albumin and Total protein concentrations in N-Acetyl cysteine treated group, pomegranate peel, water extract treated group and combination treated group with paracetamol were (26.3% and 45.9%); (21.2% and 33.8%) and (28.3% and 64.6%) Table (3).

Histopathological results:

Light microscopic examinations of liver sections of all control groups revealed that the liver parenchyma was formed of multiple classic lobules with indistinct boundaries. Each lobule was formed of cords of hepatocytes radiating from central vein. These cords were lined by flat endothelial cells. At portal areas, bile ducts were lined by simple cuboid endothelium (Fig3). In paracetamol treated group most of hepatocyte had darkly stained shrunken nuclei and vacuolated cytoplasm. Cellular infiltrations among hepatocytes were observed. Markedly congested hepatic sinusoids as well as central and portal veins were detected. In portal areas, inflammatory cells with deeply small nuclei were deposited (Fig 4).Treatment with N-acetyl cysteine revealed a preservation of normal hepatic lobular architecture in some lobules. Some hepatocytes had central rounded nuclei with prominent nucleoli and their cytoplasm still had small vacuoles. In portal areas, congested blood vessels, bile ducts lined by cuboidal epithelium and a few cellular infiltrations were observed

(Fig.5).On another hand, treatment with pomegranate peel extract revealed preservation of normal hepatic lobules architecture. Others had disrupted hepatic lobules. Most of hepatocytes had rounded pale nuclei with prominent nucleoli. Few of them contained small deeply stained nuclei. Cytoplasm of hepatocytes was vacuolated. Congested blood sinusoids and also central vein were observed. The portal areas contained bile ducts lined by cuboidal epithelium, portal venule and infiltrated cells (Fig6). Combination treatment with N-acetyl cystreine and pomegranate peel revealed improvement with minimal affection of few hepatic lobules. This improvement was in the form of preserved lobular architecture that most of liver cells began to arrange into hepatic cords that usually radiate from central vein and separated by blood sinusoids, they had rounded, vesicular and pale nuclei with prominent nucleoli. The cytoplasm was acidophilic and slightly vacuolated especially in the portal areas (Fig7).

DISCUSSION:

Paracetamol is widely consumed as an antipyretic drug that is safe in therapeutic doses but can cause fatal hepatic damage at higher accumulated doses [3]. This was clear by highly oxidative stress observed in groups treated with Paracetamol in this investigation as there was a very highly significant increase in NO, MDA, MMP-1, ALT, AST and Total bilirubin and a very highly significant decrease in SOD, CAT, GST, GSH, GPx, Albumin and Total protein mean values in paracetamol treated group when compared with their corresponding values in the control group. This refer to Paracetamol metabolised in liver forming sulphate and glucuronoides esters excreted in bile and urine [4]. Small amount oxidized by cytochrome P450 forming N-Acetyl-P-benzoquninoamine (NAPQI) which detoxify with glutathione (GSH) [5]. Accumulated doses of Paracetamol caused hepatic glutathione inhibition which give NAPQI the chance to association with cellular proteins [7]. These results were in agreement with **(Senthilkumar, 2014**[34] **;Mohammed et al., 2015**[35]) who demonstrated that documented that there was a significant acute liver injury in rats treated with paracetamol that was demonstrated by increase in liver enzymes (ALT, AST), and lipid per oxidation (MDA), nitric oxide level (NO). And significant decrease in level of glutathione (GSH), Catalase activity, [also showed significant increase in MMP-1 Administration of either N-Acetyl cysteine or pomegranate peel extract with paracetamol resulted in a significant decrease in NO, MDA, MMP-1, ALT, AST and Total bilirubin and a significant increase in SOD, CAT, GSH, GPX albumin and total protein mean values when compared with paracetamol administrated group, however, the improvement in the mean values of these parameters were incomplete when compared with those of the control group. Furthermore, combined administration of both N-Acetyl cysteine and pomegranate peel extract with paracetamol resulted in a very highly significant decrease in NO, MDA, MMP-1, ALT, AST and Total bilirubin and a very highly significant increase in SOD, CAT, GSH, GPX albumin and total protein mean values when compared with their values in paracetamol group and a non- significant difference when compared with control group values. Relative to glutathione availability one of the most important consideration has been to properly maintain the availability of cysteine in the blood as that is known to be the rate limiting substrate for glutathione re-synthesis [36], N-acetyl cysteine (NAC) is an acetylated cysteine residue able to maintain cysteine pool so maintained biosynthesis of reduced glutathione this the reason as N-Acetyl cysteine is the standard antidote of Paracetamol toxicity. Analysis of pomegranate peel with HPLC lighted more active compounds in

pomegranate peel as Gallic acid, ellagic acid and Resveratrol and more others , which known as stronger antioxidants. Pomegranate inhibited phosphorylation and activation of mitogen-activated protein kinases (signal transduction molecules involved in MMP expression. On other hand researches observed ability of Resveratrol and Gallic acid to inhibit cyclooxgenase-2 (COX-2) enzyme and 5-lipooxygenase enzyme activities the two mechanical action pathways for Paracetamol [3]. **(Parmar and Kar,2008)** [37] showed that P. granatum peel extract decreased lipid per oxidation in hepatic, cardiac, and renal tissues and had a facilitatory effect on the scavenging ability of superoxide anion and hydrogen peroxide. Another study in rats with carbon tetrachloride-induced liver damage demonstrated that pretreatment with pomegranate peel extract resulted in the reduction of lipid per oxidation, while the free-radical scavenging activity of catalase, superoxide dismutase, and peroxidase were significantly enhanced

Conclusion:

Accumulated doses of Paracetamol caused hepatic toxicity ,administration of N-acetyl cysteine and pomegranate peel extract reduced the toxicity of Paracetamol.

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Table (1): High performance Liquid Chromatography (HPLC) analysis of (Pomegranate Peel Wtaer Extract)

Active compound	Retention time (RT)	Active compound	Retention time (RT)
Gallic acid	7.304	Quercetin -3- o- glucoside	12.424
Pyrogallol	7.334	Resveratrol	12.599
4-amino – benzoic	7.892	Rosmarinic acid	12.627
Protocatchuic	8.574	Apig. 7-o- neohespiroside	12.820
Catechein	8.680	Kamp. 3,7- dirhamoside	12.873
Chlorogenic acid	9.288	Ellagic acid	12.970
Luteo.6-arbinose 8- glucose	9.338	e-vanillic acid	13.066
catechol	9.538	Apig. 7- glucose	13.091
Epicatechein	9.640	Quercetrin	13.260
Caffeine	9.814	Alpha- coumeric acid	13.317
Caffeic acid	10.221	Benzoic acid	13.498
Vanillic acid	10.333	3,4,5 methoxy- cinnamic	13.947
Luteo.6 glucose 8-arbinose	10.563	coumarine	14.047
Apig. 6- arbinose 8- glactose	11.420	Salycilic acid	14.339
p-coumaric acid	11.687	Quercetin	14.680
Apig. 6-rhamnose 8-glucose	11.776	Naringenin	14.948
Ferulic acid	11.900	Cinnamic acid	15.176
Apig. 6-glucose 8-rhamnose	11.987	Hespirtin	15.222
Luteo. 7- glucose	12.073	Kampferol	15.963
Iso-ferulic acid	12.192	rhamnetin	16.160
Naregin	12.192	Rhamnetin	16.160
Rutin	12.280	Apegnin	16.262
Hespirdin	12.320	Acacetin	18.321

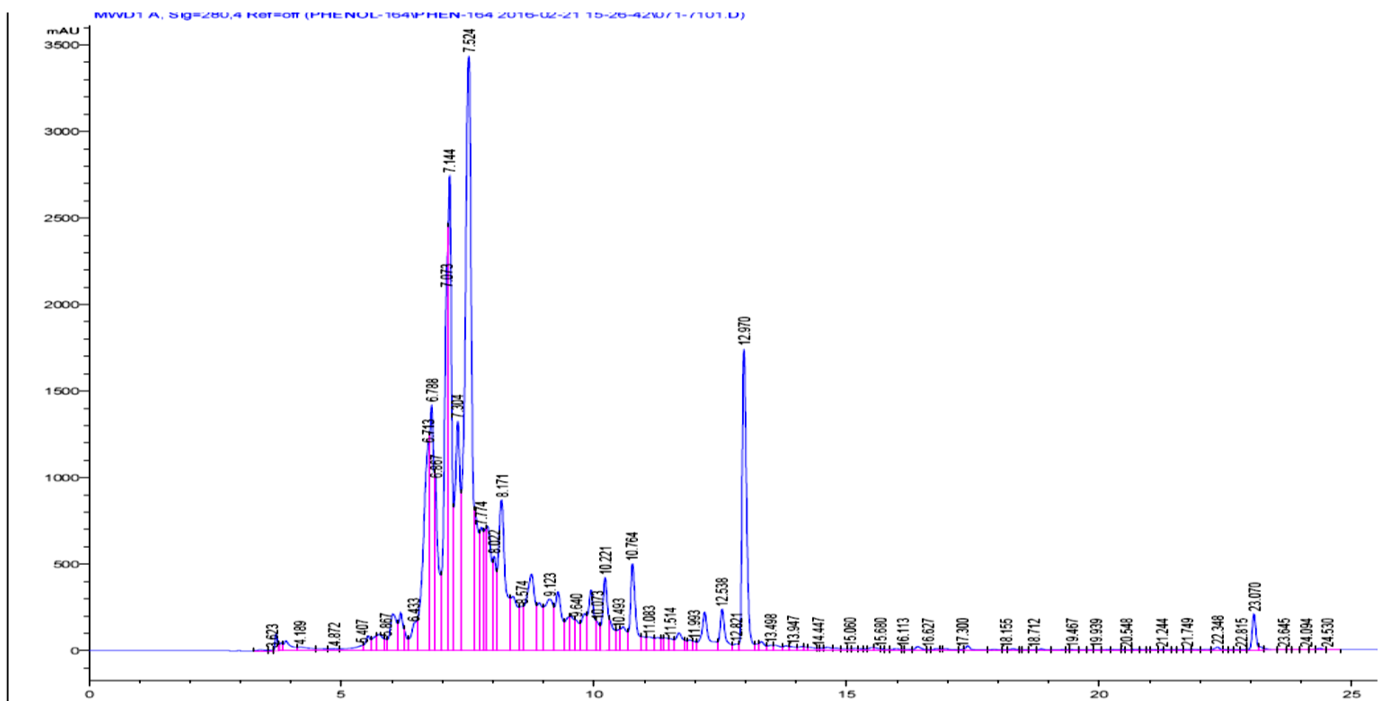

Figure (1): A chromatogram of pomegranate peel extract (phenolics ingredients) obtained by HPLC.

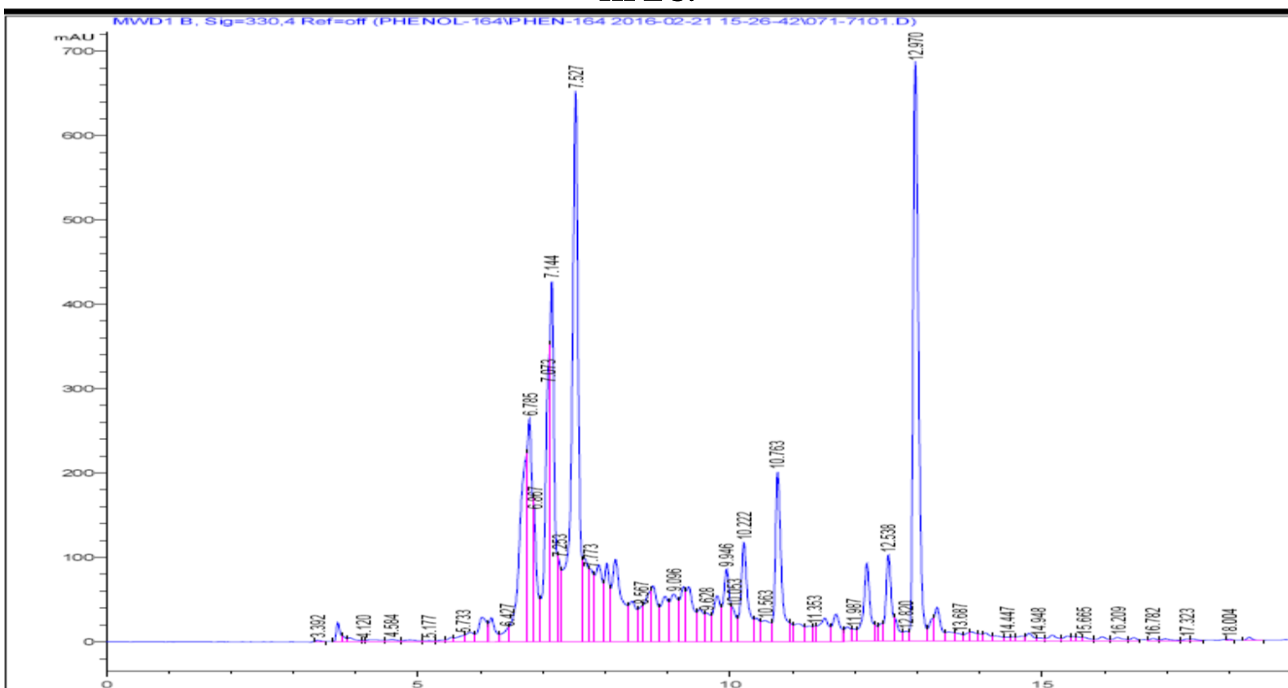

Figure (2): A chromatogram of pomegranate peel water extract (flavonoids ingredients) obtained by HPLC.

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Table (2):Statistical comparison of oxidative stress parameters (Plasma NO, Hepatic MDA, Hepatic SOD, Hepatic CAT, Hepatic GST, Hepatic GSH, and Hepatic GPx) in different studied groups by (ANOVA Test).

		NO (µmol/g tissue)	MDA (n mol/g tissue)	SOD (U/g tissue)	CAT (U/g tissue)	GST (µmol/g tissue)	GSH (µmol/g tissue)	GPX (U/g tissue)
Negative control group (I) M±SD	Mean ±SD	47.78± 2.56	11.79± 1.01	102.48± 4.49	23.77± 2.38	263.16± 8.56	8.87± 0.99	132.23± 3.99
	% Change	-	-	-	-	-	-	-
N-acetyl cysteine treated group (III) M±SD	Mean ±SD	47.73± 2.84	11.69± 0.62	102.74± 2.31	23.65± 2.53	263.54± 3.23	8.86± 1.54	132.17± 2.74
	% Change	-	-	-	-	-	-	-
Pomegranate peel extract treated group (IV) M±SD	Mean ±SD	47.46± 2.69	11.65± 0.89	102.38± 2.28	23.28± 2.81	263.35± 3.93	8.90± 1.83	132.23± 2.39
	% Change	-	-	-	-	-	-	-
N-acetyl cysteine + Pomegranate peel extract treated group (V) M±SD	Mean ±SD	47.39± 2.54	11.67± 0.69	102.56± 2.06	23.47± 2.40	263.95± 3.49	8.88± 1.52	132.20± 2.30
	% Change	-	-	-	-	-	-	-
Paracetamol group (VI) M±SD	Mean ±SD	77.80± 5.87	18.17± 0.43	54.68± 2.07	17.83± 0.77	226.01± 4.29	1.88± 0.32	103.01± 3.07
	% Change	62.8%	54.1%	46.6%	25.0%	14.1%	78.8%	22.1%
Paracetamol + N-acetyl cysteine treated group (VII) M±SD	Mean ±SD	56.76± 56.76	13.43± 0.39	90.13± 3.06	22.61± 0.30	245.50± 1.31	6.20± 0.85	120.43± 1.03
	% Change	27%	26.1%	64.8%	26.8%	8.6%	229.8%	16.9%
Paracetamol + Pomegranate peel extract treated group (VIII) M±SD	Mean ±SD	62.92± 2.21	14.14± 0.72	80.69± 3.87	21.78± 0.42	236.95± 1.20	5.32± 0.80	118.03± 0.60
	% Change	19.15%	22.2%	47.6%	22.2%	4.8%	183.0%	14.6%
Paracetamol + N-acetyl cysteine + Pomegranate peel extract treated group (IX) M±SD	Mean ±SD	51.04± 2.69	12.05± 0.78	98.19± 1.90	23.60± 1.23	258.22± 4.89	8.07± 0.49	127.86± 3.71
	% Change	34.4%	33.7%	79.6%	32.4%	14.3%	329.3%	24.1%
P Value	-	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001

M= Mean

SD= Standard deviation

P < 0.0001 = extremely significant.

Table (3):Statistical comparison of Matrix metalloproteinase (MMP-1); Liver enzymes (ALT, AST); Albumin; Total protein and Bilirubin in different studied groups by (ANOVA Test).

		MMP-1 (pg/ml)	**ALT (U/L)**	**AST (U/L)**	**ALBUMIN (g/dl)**	**Total Protein (g/dl)**	**Total Bilirubin (mg/dl)**
Negative control group (I) M±SD	Mean ± SD	1490.53 ± 237.65	42.94 ± 1.12	137.60 ± 2.32	3.19 ± 0.12	6.33 ± 0.24	0.78 ± 0.03
	% Change	-	-	-	-	-	-
N-acetyl cysteine treated group (III) M±SD	Mean ± SD	1495.27 ± 316.16	42.73 ± 0.88	137.93 ± 3.68	3.19 ± 0.18	6.37 ± 0.19	0.80 ± 0.01
	% Change	-	-	-	-	-	-
Pomegranate peel extract treated group (IV) M±SD	Mean ± SD	1490.95 ± 116.56	42.13 ± 0.85	137.88 ± 3.53	3.17 ± 0.20	6.38 ± 0.10	0.79 ± 0.04
	% Change	-	-	-	-	-	-
N-acetyl cysteine + Pomegranate peel extract treated group (V) M±SD	Mean ± SD	1503.18 ± 214.43	41.93 ± 0.56	137.59 ± 3.31	3.14 ± 0.15	6.47 ± 0.14	0.80 ± 0.03
	% Change	-	-	-	-	-	-
Paracetamol group (VI) M±SD	Mean ± SD	3044.80 ± 27.60	108.13 ± 2.41	215.18 ± 7.9	1.91 ± 0.29	3.64 ± 0.17	5.14 ± 0.40
	% Change	104.3%	151.8%	56.4%	40.12%	42.5%	559.0%
Paracetamol + N-acetyl cysteine treated group (VII) M±SD	Mean ± SD	2156.43 ± 113.57	61.08 ± 1.18	160.87 ± 1.74	3.62 ± 0.06	5.31 ± 0.2	0.91 ± 0.05
	% Change	29.2%	43.5%	25.2%	26.3%	45.9%	82.3%
Paracetamol + Pomegranate peel extract treated group (VIII) M±SD	Mean ± SD	2482.72 ± 96.02	67.04 ± 2.33	179.37 ± 1.37	3.87 ± 0.06	4.87 ± 0.17	1.27 ± 0.1
	% Change	18.5%	38%	16.6%	21.2%	33.8%	75.3%
Paracetamol + N-acetyl cysteine + Pomegranate peel extract treated group (IX) M±SD	Mean ± SD	1516.08 ± 16.74	50.73 ± 4.65	141.02 ± 18.84	3.52 ± 0.07	5.99 ± 0.24	0.80 ± 0.1
	% Change	50.2%	53.1%	34.5%	28.3%	64.6%	84.4%
P Value		<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.001

M= Mean SD= Standard deviation
P<0.001 = very highly significant difference
P < 0.0001 = extremely significant.

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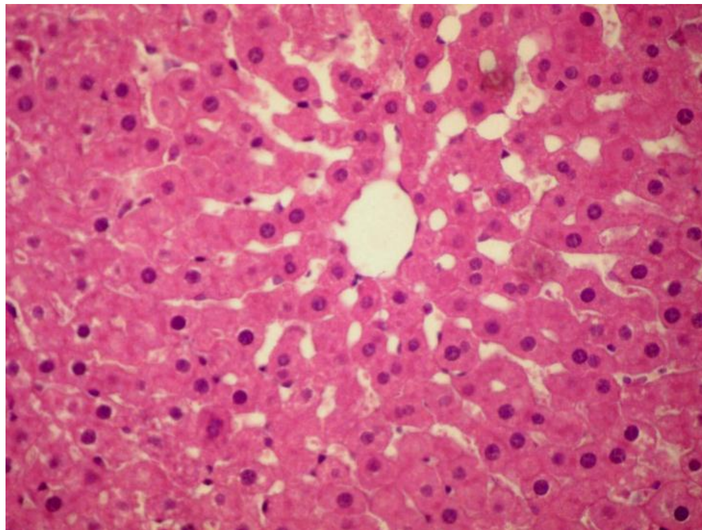

Figure (3): A photomicrograph of a section in the liver obtained from an adult male albino rat of the control group showing hepatocytes arranged in plates radiating from the central vein with the central rounded vesicular nuclei and acidophilic cytoplasm, separated by blood sinusoids. (H&E X 400)

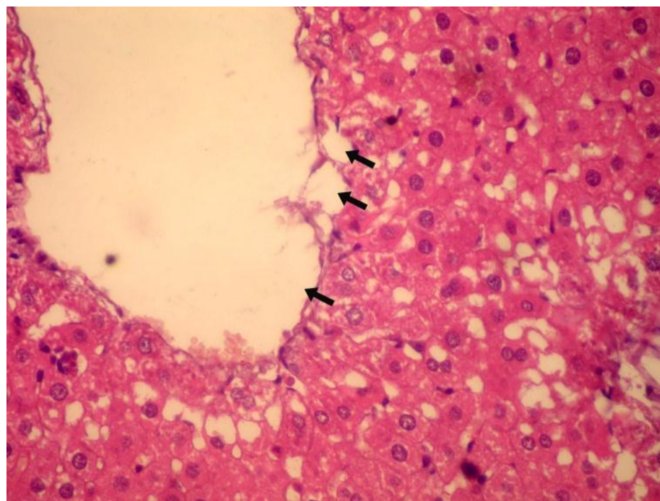

Figure (4): a photomicrograph of a section in the liver obtained from an adult male albino rat of the paracetamol group showing dilated central vein ,hepatocytes have pale vacuolated cytoplasm and deeply stained shrunken nuclei and inflammatory cellular infiltration . (H&E X 400)

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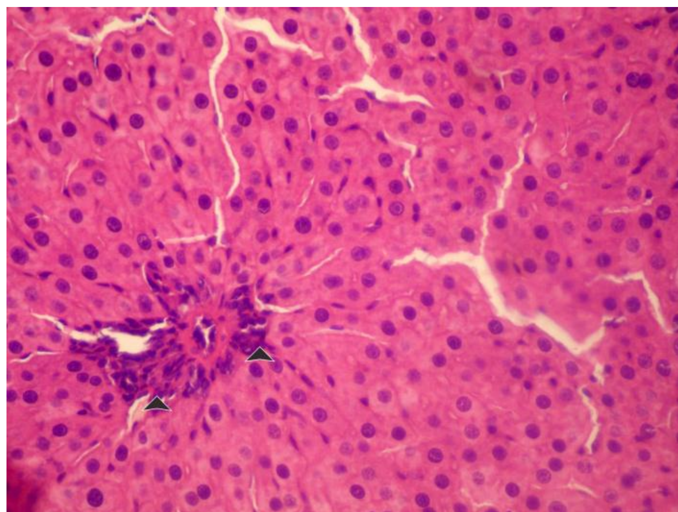

Figure (5): a photomicrograph of a section in liver obtained from an adult male albino rat of the paracetamol +N-Acetyl cysteine group showing hepatocytes have central rounded nuclei with prominent nucleoli, their cytoplasm has small vacuoles , portal area with portal venule ,bile duct and some apoptotic cells containing darkely stained nuclei .

(H&E X 400)

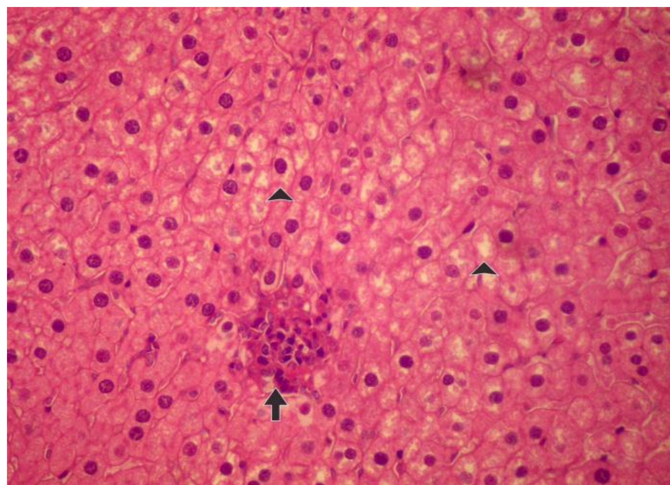

Figure (6): a photomicrography of a section in the liver obtained from an adult male albino rat of the paracetamol +pomegranate peel extract group showing the portal area with portal venule and few cellular infiltrations. Also slightly shrunken hepatocytes with deeply stained nuclei are seen. (H&E X 400)

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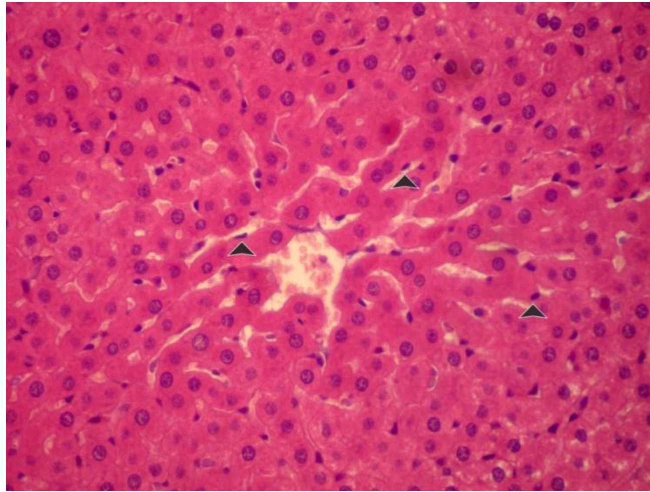

Figure (7): a photomicrograph of a section in the liver obtained from an adult male albino rat of the paracetamol + N-Acetyl cysteine + pomegranate peel extract group showing hepatocytes with rounded pale nuclei that are arranged as cords radiating from central vein and separated by blood sinusoidal spaces. (H &E X 400)