IJPSR (2020), Volume 11, Issue 2 (Research Article)

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 01 May 2019; received in revised form, 02 September 2019; accepted, 11 September 2019; published 01 February 2020

THE POSSIBLE PROTECTIVE EFFECT OF FRESH BEETROOT JUICE AGAINST PARACETAMOL INDUCED HEPATOXICITY IN ADULT ALBINO RATS

Aiman Al-Qtaitat [*1], Aiman Al-Maathidy [1], Sinan S. Farhan [2], Ghadeer Almuhaisen [3] and Jihad A. M. Alzyoud [4]

Department of Anatomy and Histology [1], Department of Physiology and Pathology [3], Faculty of Medicine, Mutah University, Karak, Jordan.
Department of Basic Sciences [2], Faculty of Pharmacy, Al-Rafidain University College, Baghdad, Iraq.
Faculty of Applied Medical Sciences [4], The Hashemite University, Zarqa, Jordan.

Keywords:

Hepatotoxicity, Acetaminophen, Paracetamol, Hepatocytes, Beetroot, *Beta vulgaris*

Correspondence to Author:
Aiman Al-Qtaitat

Department of Anatomy and Histology, Faculty of Medicine, Mutah University, Karak, Jordan.

E-mail: aimanaq@mutah.edu.jo

ABSTRACT: Hepatotoxicity in the form of acute liver failure is a serious side effect of several drugs most importantly paracetamol. Beetroot main ingredients are nitrate, betalains, phenolics and ascorbic acid, which are responsible for its protective against oxidative stress and inflammation. **Aim:** This study assessed the possible protective effect of beetroot against liver toxicity induced by paracetamol. **Method:** Forty adult male rats where divided into four groups (ten/each): control groups, group I and II (with and without beetroot juice), group III, paracetamol treated group (400 mg/kg/day), and group IV protected group (paracetamol concomitant with beetroot juice by gavage 2 ml/day) for one month. Blood samples and liver tissues were collected and subjected to biochemical and microscopic analysis, respectively. **Results:** Results demonstrated that paracetamol consumption, group III, induced toxicity to the liver evident by high ALT and AST. The intake of beetroot juice concomitantly with paracetamol (group IV) ameliorates its side effects supported by histological findings and liver function chemistry results. Group IV showed a decreased ALT and AST levels to normal levels. Liver tissue and cells appeared close to normal under light and transmission electron microscope. In conclusion, results suggest a complementary effect of beetroot to mediate anti-hepato-toxicity.

INTRODUCTION: Liver injury due to certain prescribed drugs is a recognized problem seen in health centers all over the world. This toxicity could be idiosyncratic related to normal treatment doses affect certain individuals after a period of intake, or a predictive type in which the medication was taken at high doses either deliberately or wrongly [1-5].

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.11(2).844-52

This article can be accessed online on www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.11(2).844-52

Acetaminophen (paracetamol) is a common drug that normally prescribe safely as anti-calor or anti-dolor symptom management with little anti-inflammatory effects. It is metabolized in liver and excreted by kidney. It is a common cause of idiosyncratic or predictive hepatotoxicity with the most important serious side effect in the form of acute liver failure as seen in western world [6-10].

Overdose of Acetaminophen and its metabolites N-acetyl-p-benzoquinone-imine (NAPQI) cause liver damage, which is usually cleared from liver by glutathione (GSH) [11]. Paracetamol hepatotoxicity is manifested either at cellular or tissue levels. Cellular changes include; necrosis or vacuolation of hepatocyte with karyolitic nuclei and increased

Kupffer cells, while tissue changes include; congestion of portal triad and central vein as a result of narrowing, inflammatory cell infiltrate [11, 12]. Biochemical changes include several alterations in enzymes and inflammatory mediators.

For example, increased levels of liver enzymes, alanine and aspartate aminotransferases (ALT and AST, respectively), widely used serum biomarkers for liver toxicity [13]. Studies have shown that ALT is more specific for liver than AST as the later was also seen in muscle damage [14, 15]. Remedies of herbal extracts have been used to reduce the adverse effects and protect against drug liver toxicity, and they have been used at preclinical and clinical levels [16]. Beetroot or *Beta vulgaris* or Shamandar, is a common vegetable used as a food, and prepared as herbal remedies in several medical conditions. Therapeutic effects were attributed to its active ingredients, such asnitrate, betalains, phenolics and ascorbic acid. Nitrate, for exampleis useful for hypertension through its conversion into nitric oxide, whereas betalains are important in inflammation and oxidative stress, *e.g.* phenolics and ascorbic acid [17]. Beetroot extract effect has been demonstrated to protect renal tissue in a rat model of drug induced toxicity using gentamicin by decreasing necrosis of cells and cell infiltrates [18].

Other pharmacological effects include; antitumor, carminative, hemostatic, cardiovascular and hypoglycemic, also it is used for improving sexual ability and as power drink for athletes (see [19-27] for review). Animal models were used to study liver toxicity and investigated new treatment options; consequently, findings were used to design clinical studies prior to its use [3, 7, 19, 28-30]. Most reported effects of beetroot supplements were focused on its effect over muscle injury. Those results obtained were supporting antioxidant and anti-inflammatory mechanisms that includes several biomarkers such as, CPK, ALT, AST, CRP, interleukin-6 (IL-6), interleukin-8 (IL-8), tumor necrosis factor-α (TNF-α), and others [31]. Further studies reported a potential beneficial positive effect of using beetroot juice in a carbon tetrachloride induced oxidative stress in an animal study. They showed that liver enzymes such as xanthine oxidase, catalase-CAT, peroxidase, glutathione peroxidase-GSHPx and glutathione reductase were moderated by the beetroot juice [32-34].

Using carbon tetrachloride- and N-nitrosodiethylamine in doses that produce liver injury in rats have showed a ameliorate effect of beetroot juice upon liver enzymes metabolic activity. Including, for instance, glutathione peroxidase, glutathione reductase, and superoxide dismutase, all are markers of oxidative stress. Similarly, glutathione and thiobarbituric acid reactive substances (TBARS) assays as markers for lipid peroxidation, and a comet assay as a marker for DNA damage in white blood cells [35]. Beetroot protective effects were investigated for liver toxicity induced by many factors, such as, carbon tetrachloride [36, 37], ethanol mediated [38, 39], N-nitro-sodiethylamine [40], 7,12-dimethylbenzanthracene (DMBA) [41], high-fat diet [42], and Organophosphates (OP) [43] which all provide an evidence-based protective effect against liver toxicity.

The aim of this study to evaluate the potential ameliorating effects of beetroot against paracetamol-induced injury in the liver of male rats using blood biochemistry and tissue histology methodology. The experiments were conducted according to the ethical forms approved by the Ethics and Scientific Research Committees in Mu'tah University (2010-2).

MATERIAL AND METHODS:

Study Design: This study was approved by the Scientific and Ethics Committee at Faculty of Medicine, Mutah University (2018/7). All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. Forty adult male Sprague-Dawley rats (150-200 gm) were housed in cages under standard laboratory conditions with dark and light cycle. All animals were permitted free access to standard diet and water *ad libitum* and they were randomly divided into 4 groups 10 animals each; Group I: served as a control group received standard diet, free access to water with no treatment throughout the study period. Group II: rats received fresh beetroot juice 2 ml/rat/day *via* gastric gavage for a one month[18, 32]; the fresh beetroots were obtained from a local market. Group III: experimental group were rats received paracetamol 400 mg/kg/day of BW (Body Weight) for one month based on the previously identified toxic dose *in-vivo* [1, 2, 12]; Paracetamol were purchased from (Sigma-Aldrich, USA). Group IV:

served as a protected group and were received paracetamol 400 mg/kg/day of BW, concomitant with beetroot juice (2 ml/rat/day by gavage) for one month. At the end of the experiment, animals were anesthetized and blood sample were collected by cardiac puncture in sterilized centrifuged tubes. Serum samples were harvested following centrifuging at 3000 rpm for 10 min for measuring liver biochemical parameters. The animals were then sacrificed by cervical dislocation and the liver was removed for histopathological studies.

Liver Enzymes Assessment: Serum samples were used to measure the levels of alanine amino-transferase (ALT), and aspartate aminotransferase (AST) using the Hitachi 902 Automatic Chemical Analyzer.

Histopathology: Two specimen were excised from the left lobe of the dissected liver. The first specimen processed for light microscope, briefly, samples were fixed in 10% neural buffered formalin solution, dehydrated in gradual ethanol solutions (50 to 100%), then cleared in xylene and embedded in paraffin wax. Thin sections of 5-6 mm thick, were prepared for staining. Hematoxylin and eosin were used to stain sections and examined under a light microscopic to detect changes within liver tissue. The second sample was prepared for transmission electron microscope after being immediately fixed in 3% glutaraldehyde solution.

Statistical Analysis: Analyses were performed using IBM SPSS Statistics v.19. Biochemical parameters were compared according to the distribution of our data and homogeneity of variances. A one way-ANOVA test with Post hoc multiple comparisons if significant were done for parametric data and Kruskal Wallis and man Whitney U for non-parametric data. $P<0.05$ was accepted as statistically significant.

RESULTS:

Liver Function: Liver function enzymes ALT (serum glutamate-pyruvate transaminase, SGPT) and AST (serum glutamic oxaloacetic transaminase, SGOT) serum levels were increased in rats treated with paracetamol (group III) compared to both the control (group I) and beetroot administered group (group II). Interestingly, rats received paracetamol concomitant with beetroot (group IV) showed normal values of both enzymes similar to control group. Statistically, adminis-tration of beetroot juice (group II) alone does not produce any significant differences in both enzymes, while administration of paracetamol alone (group III) significantly increases both enzymes levels when compared with the control group. Therefore, taking beetroot juice concomitantly with paracetamol significantly decreases the bad effect of paracetamol on both enzymes' levels (**Fig. 1** and **2**, **Table 1** and **2**).

TABLE 1: SHOWING ALT (SGPT) SERUM LEVELS (AS RANGE AND AS MEAN ± SD) FOR ALL GROUPS AFTER 30 DAYS AND STATISTICAL COMPARISON BETWEEN STUDIED GROUPS

SGPT (ALT) IU/l	Groups				P^0 value
	Group I	Group II	Group III	Group IV	
Min. – Max.	85.0 – 91.0	80.0 – 96.0	140.0 – 180.0	82.0 – 95.0	<0.001*
Mean ± SD	88.0 ± 2.05	89.10 ± 5.55	158.50 ± 12.81	88.40 ± 3.92	
P^1 value		0.998	<0.001*	1.000	
P^2 value			<0.001*	1.000	
P^3 value			<0.001*		

: p value for F test (ANOVA) for comparing between the different studied groups. P^1: p value for Post Hoc test (Scheffe) for comparing between group I and each other group. P^2: p value for Post Hoc test (Scheffe) for comparing between group II and groups III and IV. P^3: p value for Post Hoc test (Scheffe) for comparing between group III and group IV. *: Statistically significant at $p \leq 0.05$, N =10 for each group.

TABLE 2: SHOWING SGOT (AST) SERUM LEVELS (AS RANGE AND AS MEAN ± SD) FOR ALL GROUPS AFTER 30 DAYS AND STATISTICAL COMPARISON BETWEEN STUDIED GROUPS

SGOT (AST) IU/l	Groups				P^0 value
	Group I	Group II	Group III	Group IV	
Min. – Max.	120.0 - 150.0	120.0 -158.0	215.0 – 266.0	120.0 – 166.0	<0.001*
Mean ± SD	133.90 ±11.97	136.20 ± 10.76	235.70 ± 19.21	142.80 ± 15.03	
P^1 value		0.998	<0.001*	0.773	
P^2 value			<0.001*	0.910	
P^3 value				<0.001*	

P^0: p value for F test (ANOVA) for comparing between the different studied groups. P^1: p value for Post Hoc test (Scheffe) for comparing between group I and each other group. P^2: p value for Post Hoc test (Scheffe) for comparing between group II and groups III and IV. P^3: p value for Post Hoc test (Scheffe) for comparing between group III and group IV. *: Statistically significant at $p \leq 0.05$, N =10 for each group.

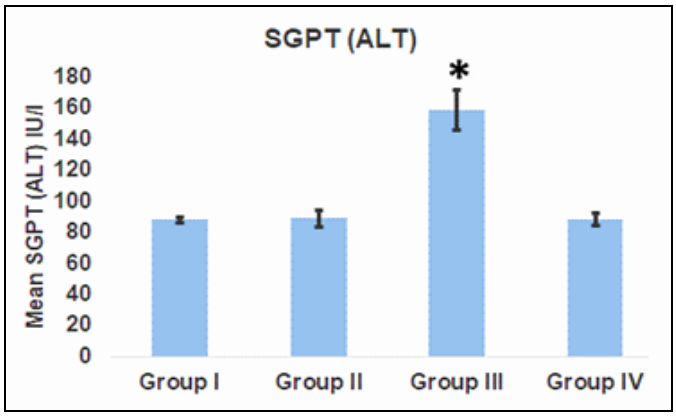

FIG. 1: SGPT (ALT) SERUM LEVELS (MEAN ± SD) FOR ALL GROUPS AFTER 30 DAYS. *: Statistically significant at $p \leq 0.05$, N =10 for each group.

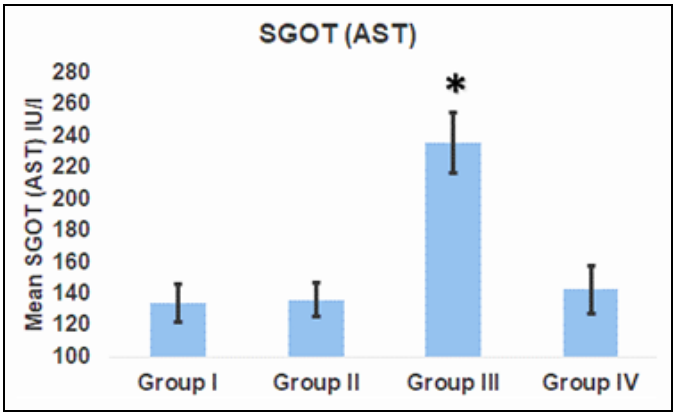

FIG. 2: SGOT (AST) SERUM LEVELS (MEAN ± SD) FOR ALL GROUPS AFTER 30 DAYS. *: Statistically significant at $p \leq 0.05$, N =10 for each group.

Histological Findings:

Light Microscope: Liver tissues form groups I and II showed under light microscope a normal histological structure of lobules and hepatocytes **Fig. 3**. Rats who received only paracetamol (group III) showed alterations in the structure of liver lobules such as congested central vein and prominent cellular changes in hepatocytes and increased number of Kupffer cells **Fig. 4**. Group IV, which received beetroot with paracetamol, the detrimental effect of paracetamol was ameliorated as a smaller number of hepatocytes showed vacuolation and liver lobules preserved their organization **Fig. 5**.

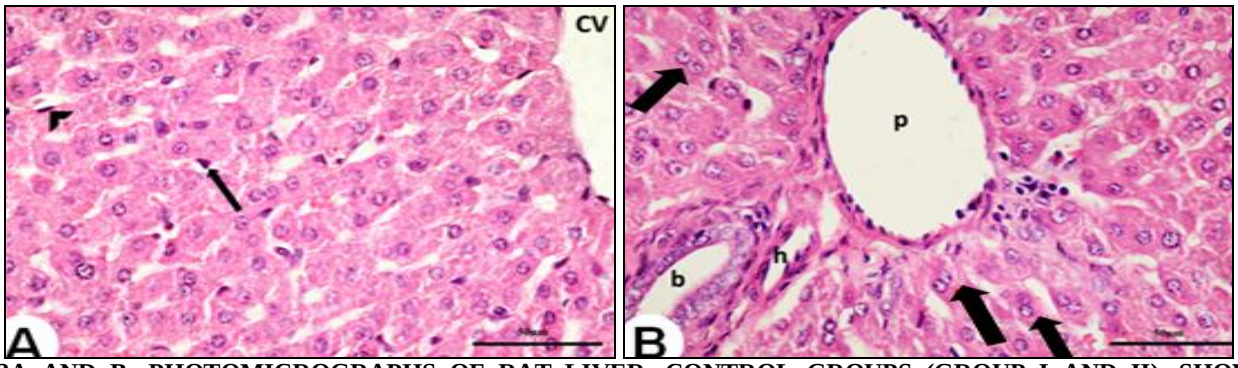

FIG. 3A AND B: PHOTOMICROGRAPHS OF RAT LIVER, CONTROL GROUPS (GROUP I AND II). SHOWING NORMAL HISTOLOGICAL FEATURES. HEPATOCYTES (WIDE ARROW) ORGANIZED IN RADIAL CORDS STARTING FROM CENTRAL VEIN (CV), BLOOD SINUSOIDS (ARROW HEAD) AND KUPFFER CELLS (ARROW), PORTAL TRIAD FORMED OF A PORTAL VEIN BRANCH (P), HEPATIC ARTERY (H) AND BILE CANALICULI (B). H AND E STAIN, X400

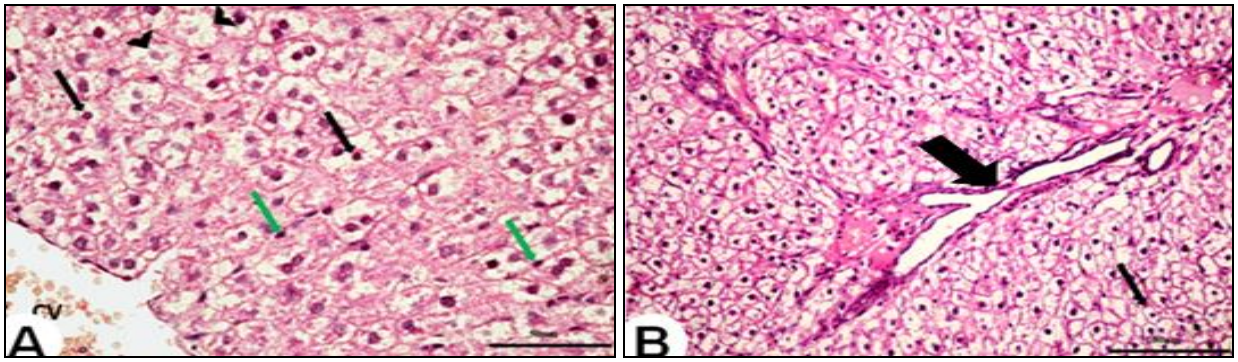

FIG. 4A AND B: PHOTOMICROGRAPHS OF RAT LIVERS STAINED WITH H AND E, GROUP III (PARACETAMOL GROUP). SHOWING CHANGES OF LIVER ARCHITECTURE, DISTURBANCE OF THE HEPATOCYTES: BALLOONED AND VACUOLATED CYTOPLASM, DENSE ECCENTRIC NUCLEI (BLACK ARROW), AND KARYOLYTIC NUCLEI (ARROWHEAD). BLOOD SINUSOIDS: NARROWING AND OBLITERATION LINED BY KUPFFER CELLS (GREEN ARROW). PORTAL TRACT: PROLIFERATION OF BILE DUCTS (WIDE ARROW), AND CONGESTED CENTRAL VEIN (CV)

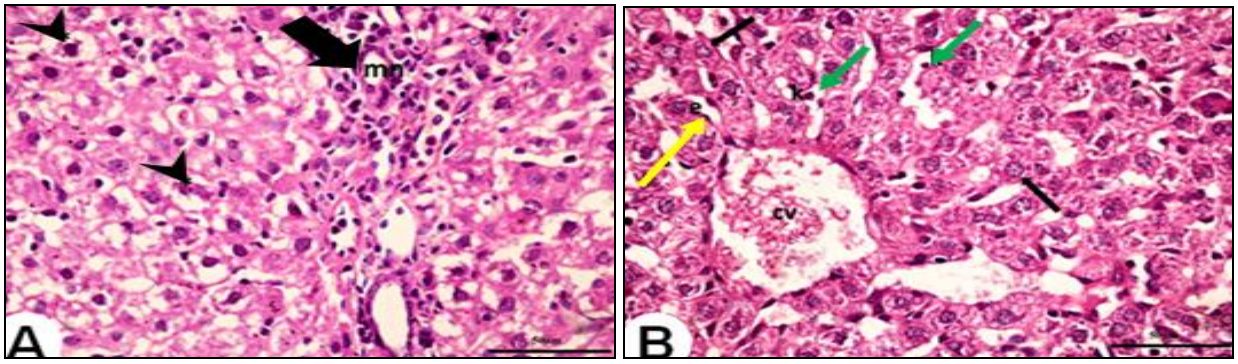

FIG. 5A AND B: PHOTOMICROGRAPHS OF RAT LIVERS STAINED WITH H AND E, GROUP IV (PARACETAMOL AND BEETROOT GROUP) SHOWING MILD DISTURBANCES OF THE HEPATIC LOBULES WITH PRESERVED HEPATIC ARCHITECTURE. HEPATOCYTES: ACIDOPHILIC GRANULAR CYTOPLASM WITH CENTRAL VESICULAR BASOPHILIC NUCLEI (ARROWED) AND FEW SWOLLEN HEPATOCYTES WITH VACUOLATED CYTOPLASM (ARROWHEADS). PORTAL TRACT: MONONUCLEAR CELLULAR INFILTRATION (WIDE ARROW). BLOOD SINUSOIDS: LINED BY ENDOTHELIAL CELLS (YELLOW ARROW) AND PROMINENT KUPFFER CELLS (GREEN ARROW)

Electron Microscope: Ultrastructurally the result obtained were supporting to the results found optically. Liver tissues form groups I and II showed normal feature of hepatocytes **Fig. 6** while rats of group III who received only paracetamol showed marked alterations in hepatocytes organelles. For example, cells have dense nuclei, marginated nucleoli, vacuolated cytoplasm, pleomorphic mitochondria, and dilated smooth and rough endoplasmic reticulum **Fig. 7**. These alterations in cells were improved in group IV (beetroot and paracetamol) reversing the detrimental effect of paracetamol alone **Fig. 8**.

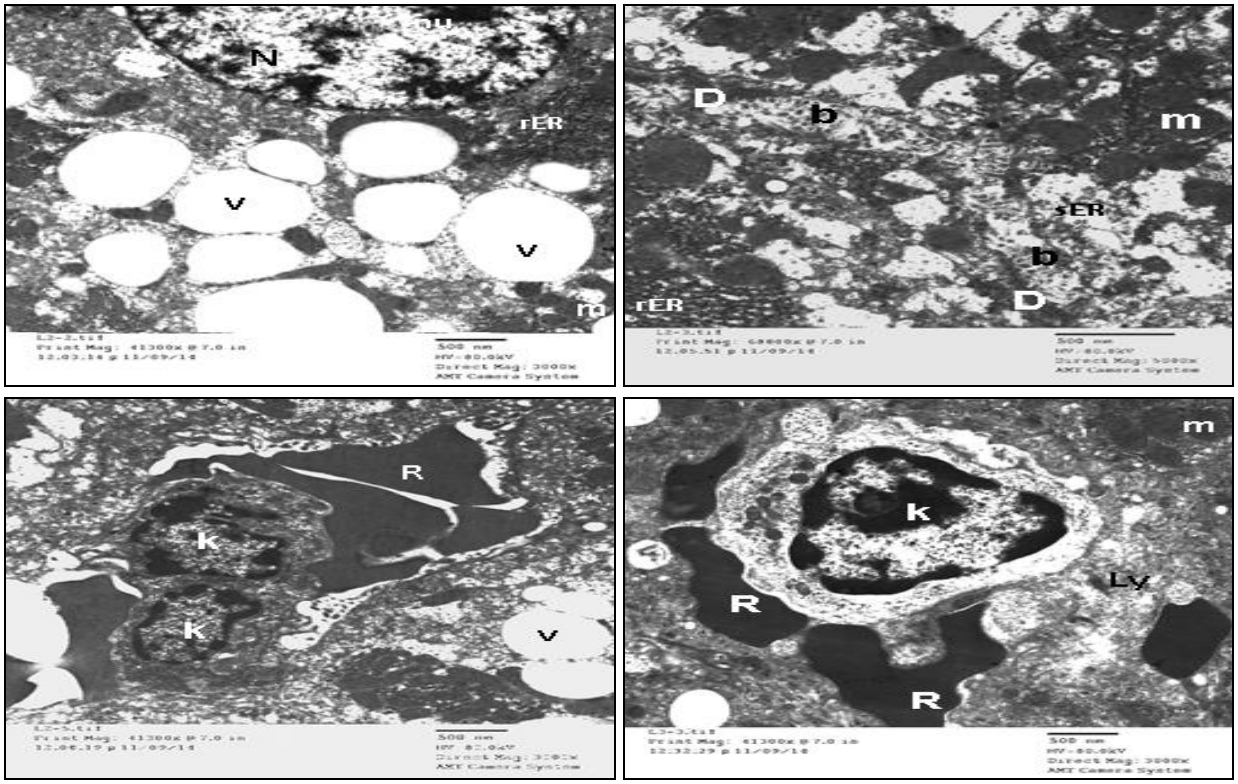

FIG. 7A, B, C AND D: ELECTROMICROGRAPHS OF RAT LIVER OF GROUP III REVEALED MARKED ULTRASTRUCTURAL CELLULAR CHANGES. MOST OF THE HEPATOCYTES' NUCLEI (N) WERE IRREGULAR AND ELECTRON DENSE. SOME OF THE NUCLEOLI WERE MARGINATED (NU). THE CYTOPLASM OF MOST CELLS SHOWED MARKED RAREFACTION COMPRESSING THE ORGANELLES AROUND THE NUCLEUS AND TOWARDS THE PERIPHERY OF THE CELL MEMBRANE. IT REVEALED MANY VACUOLES (V), PLEOMORPHIC MITOCHONDRIA (M) WITH DENSE MATRIX, DILATED ROUGH ENDOPLASMIC RETICULUM (RER) WITH PARTIAL DEGRANULATION AND DILATED SMOOTH ENDOPLASMIC RETICULUM (SER). DILATED BILE CANALICULUS (B) BOUNDED BY DESMOSOMES (D) WITH NEARBY LYSOSOMES (LY) ARE SEEN. NOTE: CONGESTED BLOOD SINUSOIDS (R) LINED BY PROMINENT KUPFFER CELLS (K)

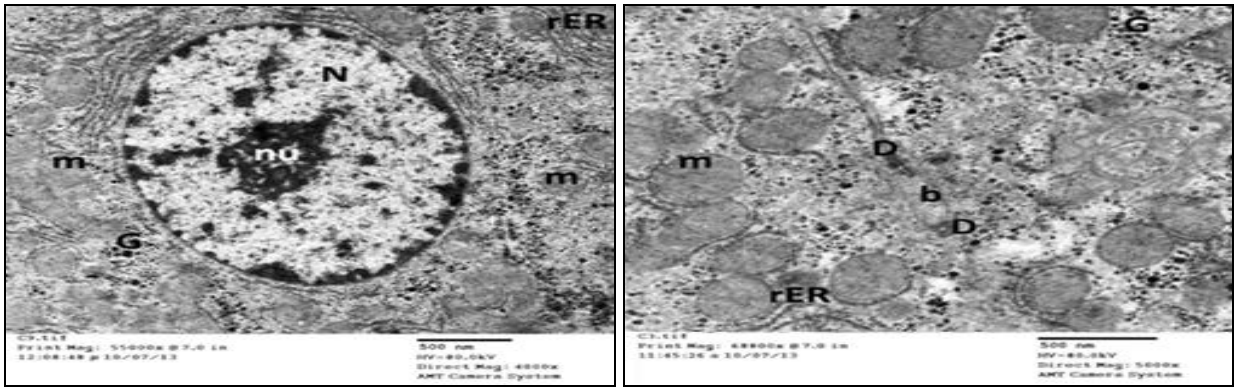

FIG. 6A AND B: ELECTROMICROGRAPHSOF RAT LIVER OF GROUP I AND II SHOWING, THE CLASSICAL HEPATIC ULTRASTRUCTURE. HEPATOCYTES' NUCLEI (N) HAD REGULAR SMOOTH CONTOUR WITH PROMINENT NUCLEOLI (NU). THE CYTOPLASM SHOWED ABUNDANT ORGANELLES; NUMEROUS MITOCHONDRIA (M) WITH LAMELLAR CRISTAE AND GLYCOGEN GRANULES (G). MULTIPLE ARRAYS OF ROUGH ENDOPLASMIC RETICULUM STUDDED WITH RIBOSOMES (rER) AND SMOOTH ENDOPLASMIC RETICULUM WERE OBSERVED. BILE CANALICULI (B) WERE SEEN AS NARROW SPACES AND FIRMLY BOUNDED BY DESMOSOMES (D)

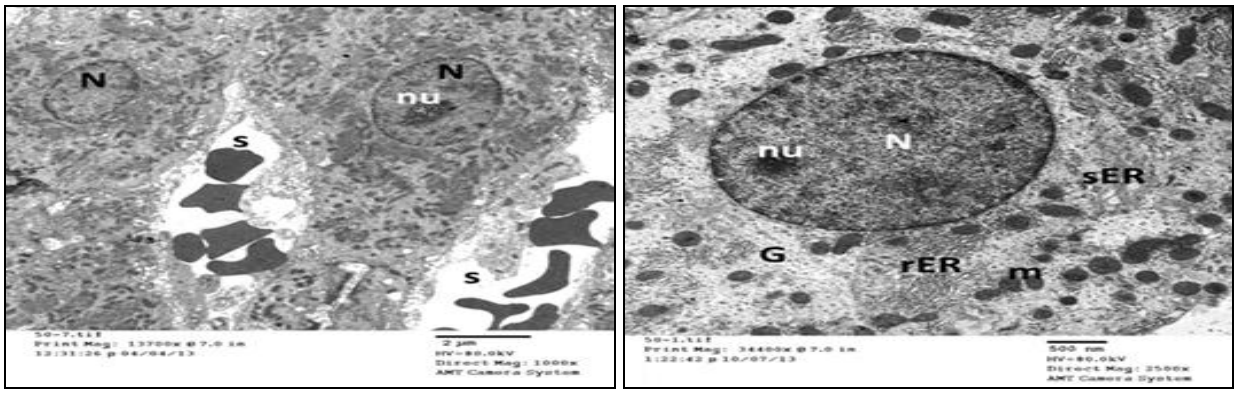

FIG. 8A AND B: ELECTROMICROGRAPHS OF RAT LIVER OF GROUP IV SHOWING ALMOST NORMAL HEPATOCYTES AROUND BLOOD SINUSOIDS (S), WITH MILD CONGESTION. THE NUCLEI ARE EUCHROMATIC WITH REGULAR CONTOUR (N), AND DEPICT PROMINENT NUCLEOLI (NU). THEIR CYTOPLASM SHOWS PROFILES OF ROUGH (RER), SMOOTH ENDOPLASMIC RETICULUM (SER), NUMEROUS MITOCHONDRIA (M) AND GLYCOGEN GRANULES (G)

DISCUSSION: Many agents were tested for its protective effect against paracetamol-induced liver toxicity such as isoquercitrin and results showed a decreased oxidative stress, less inflammation and nitrosative stress [44]. Paracetamol is considered the most common drug causing toxicity in liver [4, 5]. Many studies use ALT and AST as indicators to evaluate the protective effect of certain herbal extracts on drugs induced toxicity in liver [45, 46]. Our serum biochemical data showed increased liver enzymes levels as a result of paracetamol administration, that is ALT and AST, both are considered as widely serum biomarkers for liver toxicity [13]. This increased effect was in accordance with other studies. For instance, studies have shown that ALT is more specific for liver than AST as the later was also seen in muscle damage [14, 15]. Adding beetroot juice to the food of rats along with paracetamol results in decrease AST and ALT significantly to normal levels in rats. On the other hand, in human taking beetroot juice following a marathon decreased AST serum levels but not significantly compared to a placebo [31, 47]. Beetroot mechanisms of action are through its anti-oxidant and anti-inflammatory properties and by enhances energy production *via* nitrate [24]. Our results were in accordance with previous study that used juice of beetroot, which emphasized on its role as supportive antioxidant and protective effect on liver tissue *via* assayed enzymes within a carbon tetrachloride induced stress [32].

Histological changes presented in group III, may be attributed to the toxic effect of paracetamol, which occurs through different postulated cellular pathways, including an inhibition of the COX enzymes pathway selectively in brain tissue, which is mediates through peroxide [8].

A previous study demonstrates necrosis of hepatocytes as a result of paracetamol overdose inmouse [11]. A further study confirms changes of congestion of blood, cell death and inflammatory cell infiltrate [12].

Morphologically, the liver architectural alterations appeared in group III are almost identical to those produced due to lead toxicity in rat, with the exception of fatty cell infiltration [48, 49]. Beetroot extract effect has been demonstrated to protect both liver and renal tissues as a source of different components [17]. In rat model renal toxicity induced by gentamicin, histological profile showed necrosis of cells and cell infiltrates similar to those in liver profile [18]. Many remedies have been used to improve liver induced toxicity, for example, Genistein (a phytoestrogen present in soy products) have shown alleviation of liver symptoms of drug toxicity [25]. Most of these positive effect were mediated through antioxidant, anti-inflammation effect of the active components of beetroot [25, 26].

Liver toxicity is changes under electron microscope including, alterations in hepatocytes organelles, vacuolated cytoplasm, dilated bile canaliculus and congested blood sinusoids, presented in our results, mostly agreed with other studies [48-50]. Furthermore, those changes may be attributed to the effect of drug upon the mitochondria, producing pleomorphic mitochondria [50]. Concomitant use of beetroot juice with paracetamol ameliorated the hepatic toxicity induced by paracetamol and resulted in the restoration of histological changes, as shown above.

CONCLUSION: In conclusion, exposure to paracetamol induces injury in the liver tissue; results suggest a complementary effect of beetroot to mediate paracetamol-induced liver toxicity histologically and *via* modulation of liver enzymes. The examination of only two biomarkers may be limitations of our, an investigation of more other liver biomarkers would confirm liver changes.

ACKNOWLEDGEMENT: The author is thankful to the Faculty of Medicine, Mutah University, Faculty of Pharmacy, Al-Rafidain University and Faculty of Applied Medical Sciences, The Hashemite University for providing the necessary facilities to carry out this study.

CONFLICTS OF INTEREST: The author declares no conflict of interest.

REFERENCES:

1. Das J, Ghosh J, Manna P and Sil PC: Acetaminophen induced acute liver failure *via* oxidative stress and JNK activation: protective role of taurine by the suppression of cytochrome P450 2E1. Free Radic Res 2010; 44: 340-55.
2. Gu X and Manautou JE: Molecular mechanisms underlying chemical liver injury. Expert Rev Mol Med 2012; 14, e4.
3. Aubert J, Begriche K, Delannoy M, Morel I, Pajaud J, Ribault C, Lepage S, Mcgill MR, Lucas-Clerc C, Turlin B, Robin MA, Jaeschke H and Fromenty B: Differences in early acetaminophen hepatotoxicity between obese ob/ob and db/db mice. J Pharmacol Exp Ther 2012; 342: 676-87.
4. Jaeschke H, Xie Y and Mcgill MR: Acetaminophen-induced Liver Injury: from Animal Models to Humans. J Clin Transl Hepatol 2014; 2: 153-61.
5. Ikeda T: Idiosyncratic drug hepatotoxicity: strategy for prevention and proposed mechanism. Curr Med Chem 2015; 22: 528-37.
6. Mcgill MR and Jaeschke H: Metabolism and disposition of acetaminophen: recent advances in relation to hepato-toxicity and diagnosis. Pharm Res 2013; 30: 2174-87.
7. Ostapowicz G, Fontana RJ, Schiodt FV, Larson A, Davern TJ, Han SH, Mccashland TM, Shakil AO, Hay JE, Hynan L, Crippin JS, Blei AT, Samuel G, Reisch J and Lee WM: Results of a prospective study of acute liver failure at 17 tertiary care centers in the United States. Ann Intern Med 2002; 137: 947-54.
8. Ghanem CI, Perez MJ, Manautou JE and Mottino AD: Acetaminophen from liver to brain: New insights into drug pharmacological action and toxicity. Pharmacol Res 2016; 109: 119-31.
9. Fontana RJ: Pathogenesis of idiosyncratic drug-induced liver injury and clinical perspectives. Gastroenterology 2014; 146: 914-28.
10. Uetrecht J and Naisbitt DJ: Idiosyncratic adverse drug reactions: current concepts. Pharma Rev 2013; 65: 779-08.
11. Gardner CR, Mishin V, Laskin JD and Laskin DL: Exacerbation of acetaminophen hepatotoxicity by the anthelmentic drug fenbendazole. Toxicol Sci 2012; 125: 607-12.
12. Hasanein P and Sharifi M: Effects of rosmarinic acid on acetaminophen-induced hepatotoxicity in male Wistar rats. Pharm Biol 2017; 55: 1809-16.
13. Mcgill MR: The past and present of serum amino-transferases and the future of liver injury biomarkers. Excli J 2016; 15: 817-28.
14. Kullak-Ublick GA, Andrade RJ, Merz M, End P, Benesic A, Gerbes AL and Aithal GP: Drug-induced liver injury: recent advances in diagnosis and risk assessment. Gut2017; 66: 1154-64.
15. Tonomura Y, Kato Y, Hanafusa H, Morikawa Y, Matsuyama K, Uehara T, Ueno M and Torii M: Diagnostic and predictive performance and standardized threshold of traditional biomarkers for drug-induced liver injury in rats. J Appl Toxicol 2015; 35: 165-72.
16. Budnitz DS, Lovegrove MC and Crosby AE: Emergency department visits for overdoses of acetaminophen-containing products. Am J Prev Med 2011; 40: 585-92.
17. Clifford T, Howatson G, West DJ and Stevenson EJ: The potential benefits of red beetroot supplementation in health and disease. Nutrients 2015b; 7: 2801-22.

18. El Gamal AA, Alsaid MS, Raish M, Al-Sohaibani M, Al-Massarani SM, Ahmad A, Hefnawy M, Al-Yahya M, Basoudan OA and Rafatullah S: Beetroot (*Beta vulgaris* L.) extract ameliorates gentamicin-induced nephrotoxicity associated oxidative stress, inflammation, and apoptosis in rodent model. Mediators Inflamm 2014; 983952.
19. Clifford T, Howatson G, West D and Stevenson E: The potential benefits of red beetroot supplementation in health and disease. Nutrients 2015a; 7: 2801-22.
20. Chakole RD, Zade S and Charde CM: Antioxidant and anti-inflammatory activity of ethanolic extract of *Beta vulgaris* Linn. roots. International J 2011; 2(4): 124-30.
21. Sharma N, Tanwer BS and Vijayvergia R: Study of medicinal plants in Aravali regions of Rajasthan for treatment of kidney stone and urinary tract troubles. International Journal of Pharm Tech Research 2011; 3; 110-13.
22. Wylie LJ, Bailey SJ, Kelly J, Blackwell JR, Vanhatalo A and Jones AM: Influence of beetroot juice supplementation on intermittent exercise performance. Eur J Appl Physiol 2016; 116: 415-25.
23. Ormsbee MJ, Bach CW and Baur DA: Pre-exercise nutrition: the role of macronutrients, modified starches and supplements on metabolism and endurance performance. Nutrients 2014; 6: 1782-08.
24. Gallardo EJ and Coggan AR: What's in Your Beet Juice? Nitrate and nitrite content of beet juice products marketed to athletes. Int J Sport Nutr Exerc Metab 2018; 1-17.
25. Mansour DF, Saleh DO and Mostafa RE: Genistein ameliorates cyclophosphamide-induced hepatotoxicity by modulation of oxidative stress and inflammatory mediators. Open Access Macedonian Journal of Medical Sciences 2017; 5: 836.
26. Teschke R and Danan G: Molecular research on drug induced liver injury. Multidisciplinary Digital Publishing Institute 2018; 19(1): 216.
27. Ninfali P, Antonini E, Frati A and Scarpa ES: C-glycosyl flavonoids from *Beta vulgaris* cicla and betalains from *Beta vulgaris* rubra: Antioxidant, anticancer and anti-inflammatory activities - a review. Phytotherapy Research 2017; 31: 871-84.
28. Wojdyla K, Wrzesinski K, Williamson J, Fey SJ and Rogowska-Wrzesinska A: Acetaminophen-induced S-nitrosylation and S-sulfenylation signalling in 3D cultured hepatocarcinoma cell spheroids. Toxicol Res (Camb) 2016; 5: 905-20.
29. James LP, Mccullough SS, Lamps LW and Hinson JA: Effect of N-acetylcysteine on acetaminophen toxicity in mice: relationship to reactive nitrogen and cytokine formation. Toxicol Sci 2003; 75: 458-67.
30. Larson AM, Polson J, Fontana RJ, Davern TJ, Lalanie, Hynan LS, Reisch JS, Schiodt FV, Ostapowicz G, Shakil AO and Lee WM: Acetaminophen-induced acute liver failure: results of a United States multicenter, prospective study. Hepatology 2005; 42: 1364-72.
31. Clifford T, Allerton DM, Brown MA, Harper L, Horsburgh S, Keane KM, Stevenson EJ and Howatson G: Minimal muscle damage after a marathon and no influence of beetroot juice on inflammation and recovery. Appl Physiol Nutr Metab 2017a; 42: 263-70.
32. Vulić JJ, Ćebović TN, Čanadanović-Brunet JM, Ćetković GS, Čanadanović VM, Djilas SM and Šaponjac VTT: *In-vivo* and *in-vitro* antioxidant effects of beetroot pomace extracts. Journal of Functional Foods 2014; 6: 168-175.
33. Ninfali P and Angelino D. Nutritional and functional potential of *Beta vulgaris* cicla and rubra. Fitoterapia2013; 89: 188-99.
34. Jain S, Garg VK and Sharma PK: Anti-inflammatory activity of aqueous extract of *Beta vulgaris* L. J Basic Clin Pharm 2011; 2: 83-6.
35. Kujawska M, Ignatowicz E, Murias M, Ewertowska M, Mikolajczyk K and Jodynis-Liebert J: Protective effect of red beetroot against carbon tetrachloride- and N-nitrosodiethylamine-induced oxidative stress in rats. J Agric Food Chem 2009; 57: 2570-5.
36. Cho BO, Ryu HW, So Y, Jin CH, Baek JY, Park KH, Byun EH and Jeong IY: Hepatoprotective effect of 2, 3-dehydrosilybin on carbon tetrachloride-induced liver injury in rats. Food Chemistry 2013; 138: 107-15.
37. Lu YH, Tian CR, Gao CY, Wang WJ, Yang WY, Kong X, Chen YX and Liu ZZ: Protective effect of free phenolics from *Lycopus lucidus* Turcz. root on carbon tetrachloride-induced liver injury *in-vivo* and *in-vitro*. Food and Nutrition Research 2018; 62.
38. Singh M, Hussain T, Firdous H, Shaikh S, Danish Rizvi SM, Moin A, Khan M and Kamal MA: Preclinical hepatoprotective effect of herbalism against ethanol induced hepatotoxicity: a review. Current Drug Metabolism 2018; 19: 1002-11.
39. Jain NK and Singhai AK: Protective role of *Beta vulgaris* L. leaves extract and fractions on ethanol-mediated hepatic toxicity. Acta Pol Pharm 2012; 69: 945-50.
40. Krajka-Kuźniak V, Szaefer H, Ignatowicz E, Adamska T and Baer-Dubowska W: Beetroot juice protects against N-nitrosodiethylamine-induced liver injury in rats. Food and Chemical Toxicology 2012; 50: 2027-33.
41. Szaefer H, Krajka-Kuźniak V, Ignatowicz E, Adamska T and Baer-Dubowska W. Evaluation of the effect of beetroot juice on DMBA-induced damage in liver and mammary gland of female sprague–dawley rats. Phytotherapy Research2014; 28: 55-61.
42. Lorizola I, Furlan C, Portovedo M, Milanski M, Botelho P, Bezerra R, Sumere B, Rostagno M and Capitani C: Beet stalks and leaves (*Beta vulgaris* L.) Protect Against high-fat diet-induced oxidative damage in the liver in mice. Nutrients 2018; 10: 872.
43. Ahmadian E, Khosroushahi AY, Eghbal MA and Eftekhari A: Betanin reduces organophosphate induced cytotoxicity in primary hepatocyte *via* an anti-oxidative and mitochondrial dependent pathway. Pesticide Biochemistry and Physiology 2018; 144: 71-78.
44. Xie W, Wang M, Chen C, Zhang X and Melzig MF: Hepatoprotective effect of isoquercitrin against acetaminophen-induced liver injury. Life Sci 2016; 152: 180-9.
45. Habibi E, Shokrzadeh M, Chabra A, Naghshvar F, Keshavarz-Maleki R and Ahmadi A: Protective effects of *Origanum vulgare* ethanol extract against cyclophosphamide-induced liver toxicity in mice. Pharmaceutical Biology 2015; 53: 10-15.
46. Bahashwan S, Hassan MH, Aly H, Ghobara MM, El-Beshbishy HA and Busati I: Crocin mitigates carbon tetrachloride-induced liver toxicity in rats. Journal of Taibah University Medical Sciences 2015; 10: 140-49.
47. Clifford T, Bell O, West DJ, Howatson G and Stevenson EJ. Antioxidant-rich beetroot juice does not adversely affect acute neuromuscular adaptation following eccentric exercise. J Sports Sci 2017b; 35: 812-19.
48. Jarrar BM and Taib NT: Histological and histochemical alterations in the liver induced by lead chronic toxicity. Saudi Journal of Biological Sciences 2012; 19: 203-10.
49. Metwally E, Negm F, Shams El-Din R and Nabil E: Anatomical and histological study of the effect of lead on hepatocytes of albino rats. International Journal of Biomedical Materials Research 2015; 3: 34-45.

50. Chwiki S, Campos MM, Mclaughlin ME, Kleiner DE, Kovacs JA, Morse CG and Abu-Asab MS: Adverse effects of antiretroviral therapy on liver hepatocytes and endothelium in HIV patients: an ultrastructural perspective. Ultrastructural Pathology 2017; 41: 186-95.

How to cite this article:
Al-Qtaitat A, Al-Maathidy A, Farhan SS, Almuhaisen G and Alzyoud JAM: The possible protective effect of fresh beetroot juice against paracetamol induced hepatoxicity in adult albino rats. Int J Pharm Sci & Res 2020; 11(2): 844-52. doi: 10.13040/IJPSR.0975-8232.11(2).844-52.