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

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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-hepatoxity.

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.

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 as nitrate, betalains, phenolics and ascorbic acid. Nitrate, for example, is 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-nitrosodiethylamine 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 aminotransferase (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, administration 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

<table>
<thead>
<tr>
<th>SGPT (ALT) IU/l</th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
<th>Group IV</th>
<th>( P^2 ) value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Min. – Max.</td>
<td>85.0 – 91.0</td>
<td>80.0 – 96.0</td>
<td>140.0 – 180.0</td>
<td>82.0 – 95.0</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>88.0 ± 2.05</td>
<td>89.10 ± 5.55</td>
<td>158.50 ± 12.81</td>
<td>88.40 ± 3.92</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>( P^1 ) value</td>
<td>0.998</td>
<td></td>
<td>1.000</td>
<td></td>
<td>1.000</td>
</tr>
<tr>
<td>( P^2 ) value</td>
<td>&lt;0.001*</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>( P^3 ) value</td>
<td>&lt;0.001*</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*: : 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

<table>
<thead>
<tr>
<th>SGOT (AST) IU/l</th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
<th>Group IV</th>
<th>( P^2 ) value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Min. – Max.</td>
<td>120.0 – 150.0</td>
<td>120.0 – 158.0</td>
<td>215.0 – 266.0</td>
<td>120.0 – 166.0</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>133.90 ±11.97</td>
<td>136.20 ±10.76</td>
<td>235.70 ± 19.21</td>
<td>142.80 ± 15.03</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>( P^1 ) value</td>
<td>0.998</td>
<td></td>
<td>&lt;0.001*</td>
<td>0.773</td>
<td></td>
</tr>
<tr>
<td>( P^2 ) value</td>
<td>&lt;0.001*</td>
<td></td>
<td>0.910</td>
<td></td>
<td></td>
</tr>
<tr>
<td>( P^3 ) value</td>
<td>&lt;0.001*</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\( P^2 \): 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.
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.

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**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)**
Electron Microscope: Ultrastructurally the results 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. 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)

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: ELECTROMICROGRAPHS OF 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 in mouse. A further study confirms changes of congestion of blood, cell death and inflammatory cell infiltrate.

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. Beetroot extract effect has been demonstrated to protect both liver and renal tissues as a source of different components. In rat model renal toxicity induced by gentamicin, histological profile showed necrosis of cells and cell infiltrates similar to those in liver profile. 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. Most of these positive effect were mediated through antioxidant, anti-inflammation effect of the active components of beetroot.

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. Furthermore, those changes may be attributed to the effect of drug upon the mitochondria, producing pleomorphic mitochondria. 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.

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

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