HEPATOPROTECTIVE AND HEPATOCURATIVE EFFECTS OF NABK HONEY IN PENICILLIN-INDUCED HEPATIC TOXICITY
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ABSTRACT
In our present research, we investigated the hepatoprotective and hepatocurative effects of nabk honey in penicillin-induced hepatotoxicity. Biochemical analysis of serum was done for all groups. Hepatotoxicity was confirmed by comparing the serum levels of AST, ALT, ALP, total protein and albumin in penicillin-treated group with that of normal control. Nabk honey considerably ameliorated the toxic effects of penicillin on livers. Nabk honey showed the ability to avert the elevated serum AST, ALT and ALP levels, and augmented the total protein and albumin, along with improved histopathological changes in livers. On comparing between hepatoprotective and hepatocurative effects, hepatoprotective effect of nabk honey showed a considerably significant [P < 0.01] improvement in biochemical parameters and morphological changes of livers in penicillin-induced hepatotoxicity. Accordingly, hepatoprotective effect of nabk honey was more effective than hepatocurative effect of nabk honey.

Keywords: Hepatoprotective and hepatocurative, nabk honey, penicillin.

INTRODUCTION
Liver plays a major role in the detoxification and excretion of many endogenous and exogenous compounds; any type of injury (due to systemic drugs, food preservatives, agrochemicals and addiction to alcohol) or impairments of its functions may lead to many complications in one's health1. Antibiotics are widely used nowadays to cure many types of diseases2. Antibiotics have different courses of administration depending upon the seriousness of the infection and the potency of the antibiotic taken. The recovery time of an individual may range from a week to a whole month3. One of the foremost concerns in modern medicines is antibiotic resistance. Over the last decade, almost every kind of bacteria has become stronger and less responsive to antibiotic treatment when it is really required4. Although health benefits of antibiotics are often emphasized, the side effects of antibiotics are not commonly known5. Antibiotics are considered as a common cause of drug-induced liver injury (DILI)6-8. Penicillin antibiotics are bacteriostatic agents with a broad spectrum of antimicrobial activity. Besides their antimicrobial activity, it has been shown that penicillin may be useful in the treatment of pathological conditions in which acute or chronic inflammations are involved, such as dermatological, periodontal, rheumatic and neurodegenerative diseases9. Although the penicillin retains important roles in both human and veterinary medicines, the emergence of microbial resistance has limited their effectiveness10. Large doses of penicillin have been shown to induce hepatic dysfunction in animal11 and human12. This dysfunction of the liver resulted in the disturbance of nitrogen metabolism, jaundice and other signs of hepatocellular damage, e.g., increase of serum transaminases. Al-Awar et al.13 and Al-Shaibani et al.14 observed a significant increase in the serum AST, ALT and ALP and a significant decrease in the serum total protein levels of rabbits treated with penicillin (10 mg/kg) for 10 days were also noted. Austin et al.15 recorded a significant decrease in the albumin in the serum of mice after treatment with penicillin (10000 IU and 250000 IU/kg) for 21 days. The side effects which associate with the therapy by penicillin is mainly due the generation of an excessive amount of reactive oxygen species (ROS), resulting in the detrimental effects of the cellular antioxidant defense system, as well as, enhancement of the lipid peroxidation (LPO) process16,17. Reactive oxygen species (ROS) are an inevitable byproduct of cellular respiration causing oxidation of lipids, nucleic acids, and proteins. The (ROS) damage is an underlying cause of disease, including cancer, inflammatory, and neurodegenerative diseases18,19. Liver disease20. Antioxidants protect key cell components from damage by neutralizing the free radicals21. Antioxidants that occur naturally in the body or are consumed through the diet may block damage to cells22. Therefore, supplementation of antioxidants can be considered as the alternative method to reduce such alterations. In fact, several studies demonstrated that the cellular antioxidant an activity is reinforced by the presence of dietary antioxidants23. Accordingly, interest has recently grown in the role of natural antioxidants used as a strategy to prevent oxidative damage as a factor in the pathophysiology of various health disorders24. Numerous of biological and pharmacological properties of honey have been noted, including antibacterial, anti-fungal, anti-inflammatory, antioxidant, immunomodulatory, antiviral and anti carcinogenic properties25-30. The mentioned properties of the honey are mainly due to the presence of many chemical compositions such as polyphenolic composites including flavonoids, tannins, terpenoids and phenolic compounds, that are known to have a free-radical scavenging activity and reduce the levels of ROS31,32. This study was designed to investigate the protective and curative effect of nabk honey to reduce the side effects induced by penicillin on some biochemical parameters as well as liver tissue structure.
MATERIALS AND METHODS

Chemicals
Penicillin (Procaine penicillin) in form white powder was obtained from (Ave Group-USA-Colombia-Mexico). Diagnostic kits for the aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), total protein and albumin were obtained from Spinreact, S.A. Ctra. Santa Coloma, 7 E-17176 Sant Esteve DE Bas (GI) Spain. All other chemicals and reagents were of highest purity commercially available.

The honey used
The used honey (Nabk honey) was obtained from beekeeper, Mabian-Hajjah-Yemen.

Animals and experiment design
In this experiment 70 healthy Adult male guinea pigs, weighing 800 to 900 g, were obtained from the zoo, Sana’a-Yemen. Animals were housed in the animal house - Department of Biology- Faculty of Science- Sana’a University, under standard conditions in room temperature. Animals were allowed to acclimatize to the laboratory environment for 30 days. The animals were feeding fresh grass hay, alfalfais, legume, cabbage, carrot, celery and spinach as recommended by HCDGP33 and GPCS34 and tap water ad libitum. This experiment was divided into two parts:

Part 1
This part was mentioned to examine the protective effect of nabk honey on the Biochemical parameters (ALT, AST, ALP, total protein and albumin), in addition to their effect on the normal structure of liver tissues against the side effects of penicillin, for this goal, 40 guinea pigs were randomly divided into 4 groups as follows:-

<table>
<thead>
<tr>
<th>Groups n = 10 guinea pigs</th>
<th>Dose / kg body weight</th>
<th>No. of Days / Route</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group-1 Normal control</td>
<td>Normal saline Solution 5 ml/kg</td>
<td>For 30 days / Oral.</td>
</tr>
<tr>
<td>Group-2 Nabk honey control</td>
<td>Nabk honey 600 mg/kg</td>
<td>For 30 days / Oral.</td>
</tr>
<tr>
<td>Group-3 Penicillin Control</td>
<td>Penicillin 50000 IU/kg</td>
<td>For 30 days / Intraperitoneal injection (i.p).</td>
</tr>
<tr>
<td>Group-4 Nabk honey and Penicillin</td>
<td>Nabk honey 600 mg/kg and Penicillin 50000 IU/kg</td>
<td>For 30 days / Nabk honey / Oral and Penicillin / i.p.</td>
</tr>
</tbody>
</table>

The selected dose of Penicillin was based on previous studies14-16, the selected dose of Nabk honey was according to Al-Awar et al.33 and Gharzouli35. At the end of every part of experiment, guinea pig in all group were fasted overnight for 12 h and sacrificed under ether anesthesia, the blood was immediately collected and centrifuged and plasma was discarded and kept at - 21°C for the biochemical tests. The liver of each guinea pig were removed, small pieces of liver were taken, then fixed by using a 10 % neutral formalin for 24 hours and were kept in alcohol for the tissue preparation.

Biochemical assay
Estimation of alanine- aminotransferase (ALT) and aspartateaminotransferase (AST)
The estimation was carried out according to the method originally developed by Reitman and Frankel18.

Estimation of alkaline phosphatase
ALP was determined using a colorimetric method as described by Kind and King37.

Estimation of total protein
The total protein was determined by Biuret method explained by Tietz38.

Estimation of albumin
Serum albumin was determined according to the method of Doumas et al.39.

Histological procedure
The liver of each guinea pig were removed. After the organs were removed, they were fixed by using a 10 % neutral formalin fixation for 24 hours. The fixed tissues were dehydrated in series of alcohol concentrations 70 %, 80 %, 90 % and 100 %. The dehydrated tissues were then cleared by using xylain as clearing agents. Then the cleared tissues were embedded in paraffin wax at 60°C. Blocks were cut at 5 mm thick and stained with hematoxylin and eosin40.

Statistical analysis
The statistical analysis was performed by SPSS; continuous data are expressed as mean ± S.D. Data were compared using one – way ANOVA. P value <0.01 was considered to be statistically significant. post hoc analysis of grope differences was performed by LSD test. The treated groups were compared both with each other and with treated and untreated control groups.

RESULTS

Biochemical results
We measured biochemical parameters AST, ALT, ALP, total protein and albumin in order to determine the effect of nabk honey to reduce the toxic induced by penicillin. Group 2 animals which received penicillin (i.p) in a single dose 50000 IU/kg for 30 days showed significant rise in the levels of serum ALT, AST and ALP and significant fall in serum total protein and albumin as compared to control group (Table 1). Treatment with nabk honey alone in a single dose 600 ml/kg/day period of 30 day (group 3) showed comparable
Histological results

Results in Figure 1, 6: The control livers show normal lobular architecture with central vein and radiating cords of hepatocytes, separated by blood sinusoids. Hepatocytes are large and polyhedral in shape with slightly acidophilic granular cytoplasm. They have large, rounded, vesicular nuclei with prominent nucleoli. Results in Figure 2: The liver cells of group 2 animals showed normal liver picture as in control group. Results in Figure 3, 4, 7: The liver cells of group 3, 6 animals showed obvious histological changes, in the form of distortion in the hepatic organization, dilatation and congestion of the blood sinusoids and central vein, infiltration, hemorrhage, congestion, inflammation, metaplasia, hyperplasia, hypertrophy, necrosis, vasodilatation. Some hepatocytes showed signs of degeneration in the form of hypertrophy with highly vacuolated cytoplasm and deeply stained nuclei. Other hepatocytes exhibited hyalinized cytoplasm with pale nuclei, and some hepatocytes showed signs of degeneration in the form of hypertrophy with highly vacuolated cytoplasm and deeply stained nuclei.

Table 1: Hepatoprotective effect of nabk honey on penicillin-induced changes in biochemical parameters

<table>
<thead>
<tr>
<th>Groups N = 10</th>
<th>Treatment given</th>
<th>AST U/L</th>
<th>ALT U/L</th>
<th>ALP U/L</th>
<th>Total protein g/dl</th>
<th>Albumin g/dl</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
<td>Control N.S for 30 days</td>
<td>21.95 ± 1.7</td>
<td>27.41 ± 1.8</td>
<td>52.91 ± 2.2</td>
<td>7.53 ± 0.28</td>
<td>3.78 ± 0.15</td>
</tr>
<tr>
<td>Group 2</td>
<td>Honey 600 mg/kg for 30 days</td>
<td>20.71 ± 1.9</td>
<td>27.33 ± 1.8</td>
<td>54.28 ± 1.8</td>
<td>7.69 ± 0.21</td>
<td>3.88 ± 0.22</td>
</tr>
<tr>
<td>Group 3</td>
<td>Penicillin 50000 IU/kg for 30 days</td>
<td>46.88 ± 4.9</td>
<td>63.06 ± 5.6</td>
<td>78.06 ± 4.3</td>
<td>6.03 ± 0.45</td>
<td>2.96 ± 0.18</td>
</tr>
<tr>
<td>Group 4</td>
<td>Honey Penicillin 600 mg/kg + 50000 IU/kg for 30 days</td>
<td>24.58 ± 2.5</td>
<td>30.71 ± 2.3</td>
<td>55.33 ± 2.5</td>
<td>7.38 ± 0.42</td>
<td>3.68 ± 0.12</td>
</tr>
</tbody>
</table>

The values are given as Mean ± Standard Deviation (M ± SD) (in each group); [*] = Low significance; [**] = high significance. *P < 0.01, when compared with values of Control [Group 1] N.S (Normal saline) #P < 0.01, when compared with values of penicillin Control [Group 3].

Group 6 animals which received penicillin (1p) in a single dose 50000 IU/kg for 20 days showed significant rise in the levels of serum ALT, AST and ALP and significant fall in serum total protein and albumin as compared to control group (Table 2). Nabk honey has curative role against the side effects of penicillin in liver as demonstrated by the improvement in the tested biochemical parameters. Treatment with Honey for 20 days after withdrawal of penicillin therapy (group IV, 40th day) significantly reversed levels of AST, ALT, ALP, albumin and total protein as compared to penicillin treated group 6 (Table 2).

Table 2: Hepatocurative effect of Nabk honey on Penicillin-induced changes in Biochemical Parameters

<table>
<thead>
<tr>
<th>Groups N=10</th>
<th>Treatment given</th>
<th>AST U/L</th>
<th>ALT U/L</th>
<th>ALP U/L</th>
<th>Total protein g/dl</th>
<th>Albumin g/dl</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 5</td>
<td>Control N.S for 40 days</td>
<td>22.76 ± 2.1</td>
<td>26.87 ± 2.5</td>
<td>53.45 ± 1.9</td>
<td>7.54 ± 0.43</td>
<td>3.90 ± 0.21</td>
</tr>
<tr>
<td>Group 6</td>
<td>Penicillin for 20 day + N.S for next 20 days</td>
<td>38.48 ± 5.8</td>
<td>51.47 ± 4.1</td>
<td>69.86 ± 4.9</td>
<td>6.53 ± 0.67</td>
<td>3.27 ± 0.31</td>
</tr>
<tr>
<td>Group 7</td>
<td>Penicillin for 20 day + Honey for next 20 days</td>
<td>28.31 ± 3.7</td>
<td>35.43 ± 3.7</td>
<td>58.18 ± 2.5</td>
<td>6.96 ± 0.40</td>
<td>3.49 ± 0.28</td>
</tr>
</tbody>
</table>

The values are given as Mean ± Standard Deviation (M ± SD) (in each group); [*] = Low significance; [**] = high significance. *P < 0.01, when compared with values of Control [Group 5] N.S (Normal saline) #P < 0.01, when compared with values of penicillin Control [Group 6].
Figure 3. Light micrograph of a vertical section in the liver of guinea pigs after treatment with penicillin for 30 days, showing obvious histopathological changes. Hemorrhage (H), Congestion (J), Infiltration (E). (HE) stain (X400).

Figure 4. Light micrograph of a vertical section in the liver of guinea pigs after treatment with penicillin for 30 days, showing obvious histopathological changes. Edema (M), Necrosis (N). (HE) stain (X400).

Figure 5. Light micrograph of a vertical section in the liver of guinea pigs after treatment with Nabk honey and penicillin for 30 days, showing a normal liver picture as in control group. Spherical nucleus (B), Sinusoids (D), Blood vessel (A), and Kupffer cells (C). (HE) stain (X400).

Figure 6. Light micrograph of a vertical section in the liver of guinea pigs after treatment with normal saline for 40 days, showing a normal architecture without pathological alterations. Spherical nucleus (B), Sinusoids (D), Blood vessel (A), and Kupffer cells (C). (HE) stain (X400).

Figure 7. Light micrograph of a vertical section in the liver of guinea pigs after treatment with penicillin for 20 days + normal saline for next 20 days, showing obvious histopathological changes. Congestion (J), Infiltration (E). (HE) stain (X400).
DISCUSSION

The noticed increase in the levels of aminotransferase (ALT and AST) and the level of ALP in the serum are the major diagnostic symptoms of liver diseases. In our study, administering penicillin to guinea pigs resulted in a statistically highly significant increase in the enzymes AST, ALT and ALP in the serum of the guinea pigs injected of penicillin only compared with the control group. These results may indicate to degenerative changes and hypofunction of liver as well as hepatic cell necrosis which increase the releasing of these enzymes in the blood stream. Elevated levels of these enzymes in the serum are presumptive markers of drug-induced necrotic lesions in the hepatocytes. The enhanced susceptibility of hepatocyte cell membrane to drug-induced peroxidative damage might have resulted in an increase releasing of these diagnostic marker enzymes into the systemic circulation. An increase in the AST and ALT levels indicates a reversible change of the cell membrane permeability. Our observations are highly supported by the other studies which suggest effect penicillin on liver function tests. In this study also, administering penicillin to guinea pigs resulted in a statistically highly significant decrease the level total protein and albumin in the serum of the guinea pigs injected of penicillin only compared with the control group. The reduction of total protein and albumin levels indicates that the administration of drugs has caused an impairment of liver function, e.g. its capacity to synthesize albumin from the hepatic parenchyma. Khan et al. reported that there was a differential binding of penicillin with serum albumin, while Shen et al. observed that albumin secretion of gel entrapped hepatocytes was reduced by penicillin. The decrement of alpha 1-globulin in the serum of penicillin-administrated animals could be due to liver dysfunction which affects the synthesis of alpha protein fractions in the liver. The increment of gamma-globulin level in the serum of tetracycline-treated animals may be due to hyperplasia of the reticulo-plasmic tissue of the bone marrow induced by penicillin administration. Our results are in agreement with. Histopathological changes in liver cells following injection of penicillin was the marked changes occurring in the liver in this study. This feature could be explained according the suggestion both of Tayala et al. ; Al-Awar et al.; Al-Saibani they reported that histopathological changes in liver cells due to free radical generating and free radical scavenging enzymes may be disturbed and leading to disrupt signal transduction pathway and increases the cellular permeability by acting on membrane phospholipids, resulting into a significant hepatic tissue injury. Dilatation and marker congestion of the hepatic vasculature of liver tissue which was noticed in the present investigation may be due the
failure of the heart which produces changes in different organs via two ways. Firstly, excessive blood in venous system increases blood pressure in the veins and capillaries which may exert undue pressure on the neighboring structures. Secondly, this is usually accompanied be a diminished blood supply, thus become subject to malnutrition, deficient oxygenation and the accumulation of excreatory and metabolic products\(^6\). Interpretation of vacuolar formation following chemical treatments has been subjected to wide speculation by many investigators. Robbins and Angel\(^6\) regarded such vacuolation to represent primary morphologic response to many forms of cell injury. They also attributed it to the noxious effects of treatment on the cell membranes, both structurally and functionally, causing market disturbances in its permeability system. This presumably leads to enhanced imbibition of water into the cells. When it sufficiently accumulates in the cells, such intracellular water produced clear cytoplasmic vacuoles indication the occurrence of the pathologic symptoms commonly referred to as hydropic degeneration or fatty degeneration caused by lipid abundance in such instance. Other authors are of the opinion that cytoplasmic vacuolation is most probably brought about by the increase of lysosome elements\(^5\). The lysosomes contain hydrolytic enzymes, when these organelles are disrupted under certain pathological conditions: they liberate their powerful enzymes, which bring about considerable autolysis of various cellular parts\(^4\). Necrosis and degeneration of the hepatic cells following injection of penicillin was the marked changes occurring in the liver in this study. This feature could be explained according the suggestion of Curran\(^5\) who reported that cells liver necrosis may be either due to progressive degenerative action of intracellular enzymes of the injured cells or to a metabolic disturbance and inhibition of synthesis needed of DNA and hence protein synthesis for the growth and maturation of the liver. On the other hand, the present histological study showed that of Nabhk honey reduced the cellular changes induced by penicillin treated groups in both hepatoprotective and hepatocurative studies, indicating that Nabhk honey contributed to the protection against penicillin induced liver toxicity. Our observations are highly supported by the other studies which suggest that Nabhk honey exert their protective and curative effects against some drugs, \(^{59,66}\) (doxorubicin, penicillin, streptomycin, ibuprofen, acetaminophen and paracetamol)- induced hepatotoxicity\(^3,32,56,59,66\). On hypothesis to explain the beneficial effects of honey in ameliorating biochemical parameters and histological changes is that honey may contains flavonoids, ascorbic acid, tocopherols, catalase and phenolic compounds. All of which work together to provide a synergistic antioxidant effect, scavenging and eliminating free radicals\(^13\).

CONCLUSION

In conclusion, we suggest that nabk honey may give beneficial results in the prevention of hepatic damage induced by the use of penicillin. On comparing between hepatoprotective and hepatocurative effects, hepatoprotective effect of Nabk honey showed a considerably significant \(P < 0.01\) improvement in biochemical parameters and morphological changes of livers in penicillin-induced hepatotoxicity. Accordingly, hepatoprotective effect of Nabk honey was more effective than hepatocurative effect of Nabk honey.


Guinea Pigs Care Sheet. Canyon Lake Veterinary Hospital; 2007.


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