



## Efficacy of White Honey in Attenuating Histological Changes Resulting from Multivitamin-induced Hepatotoxicity in Albino Rats

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### ABSTRACT

The body requires vitamins to keep it healthy and strengthen the immune system, but a serious health risk may result from its excessive intake. White honey is an important natural product used in traditional medicine as an alternative treatment for clinical conditions ranging from wound healing to removal of cytotoxicity. The present research was an attempt to evaluate the anti-toxicity effect of white honey against hepatotoxicity induced by high dose of multivitamins in albino rats. Sixty male albino rats were used in this study and were divided into four experimental groups: 10 rats served as normal control (Group 1); 10 rats were administered with white honey for one month (Group 2); 20 rats were treated with high dose of multivitamins for one month (Group 3); and 20 rats were treated with high dose of multivitamins for one month, followed by white honey for additional one month (Group 4). The results showed a significant increase in body weight of Group 3 rats (23.3 kg) compared to the control (18.7 kg), while a reduction in body weight was recorded in Group 4 (20.5 kg) in relation to Group 3. A similar observation was made for liver function test parameters. The histological analysis revealed that white honey was able to restore many pathological and harmful changes resulting from the high dose of multivitamins and emergence of a clear improvement of liver tissues. Our finding therefore indicated that white honey supplementation ameliorated the harmful effect induced by high dose of multivitamins.

**Keywords:** Hepatotoxicity, Liver, Multivitamins, Treatment, White honey.

### Introduction

The use of dietary supplements (multivitamins) among adults in the United States has increased over the past 30 years.<sup>1,2</sup> Multivitamins are a combination of many different vitamins that are normally found in foods and other natural sources. They are used to provide vitamins that are not taken through diet. These dietary supplements are also used to treat vitamin deficiencies (lack of vitamins) caused by illness, pregnancy, poor nutrition, digestive disorders, and many other conditions.<sup>3</sup> Many vitamins can cause serious or life-threatening side effects if taken in large doses. Overdose symptoms may include stomach pain, blood in the urine, pale skin, easy bruising or bleeding, vomiting, diarrhea, constipation, loss of appetite, hair loss and peeling skin.<sup>4</sup> Vitamin and mineral bioavailability dietary supplements lack a standard scientific and regulatory definition as well as validation through *in vitro* and animal models that accurately reflect human bioavailability.<sup>5</sup> Due to the several health benefits associated with multivitamins, has resulted to their indiscriminate use without consulting a doctor or adhering to the necessary dosage. This can cause severe damage to the liver by negatively affecting its functions and tissue composition of its cells.<sup>6,7</sup> In an attempt to finding a treatment for the harmful effects resulting from the indiscriminate use of multivitamins, white honey which is often used as a natural product has caught researchers' attention as an alternate therapy due to its antioxidant properties.<sup>8-10</sup>

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Moreover, white honey has been used as substances for treating wounds, burns, cancer and cardiovascular diseases.<sup>11-13</sup> White honey has been reported to have an ameliorative activity against all toxicity of the liver cells by increasing the immune capacity through a significant increase of the immunoglobulins.<sup>14</sup> Honey at a dose of 1.2 g/kg has been found to increase the activity and amount of antioxidants like vitamin C,  $\beta$ -carotene, uric acid, and glutathione reductase in healthy people.<sup>15</sup> However, bee products are known as possible sources of natural antioxidants, which can combat oxidative stress because of their phenolic compound content (phenolic acids and flavonoids) that expresses a scavenging activity of free radicals.<sup>16</sup> Additionally, these phenolics, sugars, proteins, carotene, amino acids, maillard reaction products, organic acids, and other minor compounds participate in the antioxidant effects of honey and other bee products.<sup>17,18</sup> The current research was therefore aimed at evaluating the effects of a high dose of multivitamins on liver tissues of male albino rats and also investigating the ameliorative potential of white honey in decreasing the multivitamin-induced harmful changes.

### Materials and Methods

#### Source of experimental animals

Sixty healthy male albino rats ( $120 \pm 5$  g) were obtained from the Animal House of the College of Pharmacy, King Saud University, Riyadh, Saudi Arabia and kept in an animal room at  $25 \pm 2^\circ\text{C}$  in an alternating 12 hours light and dark cycle. Two weeks before the experiment was conducted, the animals were allowed to adapt to the testing facility conditions. All animals were treated in compliance with the quality pointers for the laboratory animal care and protocol of the National Analysis Council 22 was used.

#### Ethical approval

Before the start of the research, an ethical approval for use of experimental animals was obtained from the Research Ethics Board of Princess Nourah Bint Abdulrahman University Animal Care and Use. The Ethical clearance number was IRB Log Number: 20-0329.

### Animal grouping and treatment

In this study, the animals were distributed in special cages equipped with bottles to drink water in a room that was ventilated and exposed to natural factors and fresh grass, hay, alfalfais, legume (HCDGP, 2007, GPCS, 2007 and ILAR 1995). The animals were selected with a mean age of 12-15 weeks and an average body weight of 60 g. Sixty acclimatized male albino rats were divided into 4 experimental groups: Group 1 (Control group of 10 albino rats that were given regular drinking water); Group 2 (Honey group of 10 albino rats that were administered with white honey at a dose of 300 mg/70 g body weight in drinking water for one month and fresh water as needed); Group 3 (multivitamin group of 20 albino rats that were treated with high dose of multivitamins at a dose of 600 g/kg body weight in drinking water for one month); and Group 4 (multivitamin with honey group of 20 albino rats that were treated with high dose of multivitamins at a dose of 600 g/kg body weight/ day in drinking water for one month, followed by white honey at a dose of 300 mg/70 kg of body weight for additional one month).

### Collection of blood samples

Blood samples were obtained from the orbit pocket 27 of the experimental animals every 20 days, over a period of 60 days of the approved research duration. The collected blood was divided into two; one part was placed in a heparin tube for biochemical blood analysis, while the second part was centrifuged at 1000 rpm for a period 10 minutes at room temperature to recover the serum. The sera were all stored at -25°C until required for analysis. Blood liver enzymes, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) and nitric oxide were determined using a special reagent (Roche Diagnostics, KSA) following the manufacturer's instructions.

### Histological procedure

At the end of the experiment, the experimental albino rats were fasted overnight for 12 h. They were sacrificed under diethyl ether anaesthesia and dissected. Liver tissues were removed as small pieces, weighed and kept in a 10 % neutral buffered solution for two days to make tissue slices. The tissues were washed in running water and were dehydrated with increasing concentrations of ethyl alcohol (50, 75, 96, and 100%). During the process of dehydration, the specimens were placed into xylene, then infiltrated and embedded in paraffin wax. The embedded tissues were cut into sections of 5 µm thickness by Leica RM 2125 RT and stained with hematoxylin and eosin. Histopathological examinations were performed and photographed with Nikon Eclipse E 60023,24.

### Measurement of parameters

Food intake and body weight for each of the experimental animals in each cage were registered once a week. Average body weight for each group was calculated by dividing the total weight of all surviving animals by the total number of surviving animals. Liver index was calculated as follow:

$$\text{Liver index: Liver weight/body weight} \times 100$$

### Statistical analysis

Statistical analysis was performed using SPSS. Data were presented as mean  $\pm$  S.E. Means were compared using one-way ANOVA.  $P < 0.01$  was considered to be statistically significant. Post hoc analysis of group differences was performed by LSD test. Treatment groups were both compared with each other as well as with untreated control groups.

## Results and Discussion

### Changes in body weight and liver index ratio of experimental male albino rats

The results obtained for body weight and liver index ratio of experimental albino rats are presented in Table 1. There was a significant increase ( $P < 0.001$ ) in body weight of albino rats treated with excessive vitamin intake (Group 3; 23.3 kg), compared to the control (Group 1; 18.7 kg). A reduction in body weight was observed in Group 4 albino rats (20.5 kg), in relation to Group 3. In contrast to

body weight measurement, there was a significant decrease ( $P < 0.001$ ) in liver index ratio of Group 3 albino rats ( $0.86 \pm 0.22$ ) compared with the control, Group 1 ( $1.78 \pm 0.22$ ). The liver index ratio was observed to increase to  $1.09 \pm 0.17$  in the experimental Group 4 animals, where white honey was administered after high dose of multivitamins.

### Changes in liver function test parameters in experimental male albino rats

There were relative changes in the parameters of liver function test in Group 3 albino rats with hepatic changes induced by multivitamin-toxicity. There was a significant increase in the levels of liver function test parameters such as total protein ( $7.09 \pm 1.26$ ), AST ( $158 \pm 3.46$ ), ALT ( $42.81 \pm 2.33$ ) and nitric oxide ( $0.39 \pm 0.14$ ) in Group 3 albino rats, compared to the control which had  $6.18 \pm 0.31$ ,  $120.88 \pm 5.16$ ,  $29 \pm 1.22$  and  $0.28 \pm 0.08$ , respectively. Administration of white honey had an ameliorative effect on the multivitamin-induced toxicity. It was observed in Group 4, where the albino rats were administered with white honey after a high dose of multivitamins, a reduction in liver function test parameters with values of  $6.65 \pm 0.22$ ,  $133.49 \pm 1.92$ ,  $35.74 \pm 0.59$  and  $0.22 \pm 0.04$ , respectively for total protein, AST, ALT and nitric oxide, compared to Group 3 animals.

### Effects of multivitamins and white honey on histological structure of liver tissues obtained from experimental male albino rats

As shown in Figure 1a, in the control albino rats, the liver parenchyma consists of little lobules of a roughly polygonal shape with portal tracts at the apices. Within the lobules, the hepatocytes area unit was organized as cords of cells connecting the portal tracts within the outer boundary to the central veins (terminal branches of viscus veins). In the white honey group (Group 2), the liver tissues seemed within the white honey just like the natural composition as depicted in Figure 1b. The impact of multivitamins at high dose was evident in Group C within the liver tissue where a rise within the inflammatory cells, infiltration round the central vein, hemorrhage in central blood vessel with death of most liver cells resulting in death space (Figure 1c). It was observed in Group 4 (Figure 1d) that treatment with white honey led to the disappearance of many pathological and harmful changes that occurred in the ovaries as a result of multivitamin-induced toxicity and the emergence of a clear improvement in liver tissue.

In an attempt to increasing the body's immunity against diseases towards living a healthy life style has necessitated the over reliance on the use of multivitamins. These multivitamins are often taken at a high dose or without a doctor's prescription. This possess a serious health hazard on various organs of human body when not controlled. Several studies have revealed harmful effects on several organs such as the liver and kidneys.<sup>3,25,26</sup> The current research was undertaken to investigate the ameliorative effect of white honey on harmful changes caused by high dose of multivitamins in the liver tissue of albino rats. The impact of multivitamins was evident on the hepatic tissue, where tissue degradation and amendment in structure of liver cells showed signs of cellular death (necrosis). Additionally, there was a marked deterioration of the inner lining of the liver veins, epithelial tissue lining rupture of congestion, dilatation, congestion, dilatation, and hemolysis. Invasion of inflammatory cells was determined in some areas round the central vein. There have been clear infections within the liver tissue where multivitamins caused severe sphaecelus of cells infiltrating the vasculature and growth of the blood pockets and accumulation of liver cells. The results obtained in our research are in agreement with several histological studies on white albino rats where administration of high dose of multivitamins for 2 months resulted in many adverse effects on the liver tissues.<sup>28,29</sup>

The results showed a protective potential of white honey against liver damage. It is possible that NO level increased in the liver tissue due to the rich NO content of honey, which is supported by our finding of unchanged NOS activity in liver tissue. Increased NO levels in the obstructive jaundice plus honey group may contribute to the protective effect possibly through the elimination of toxic free radicals by NO. Furthermore, research has shown that cellular damage may result from aerobic stress in tissues. Thus, it has been confirmed that increase in multivitamins causes aerobic stress and generates reactive oxygen

species by reducing antioxidants within the tissues, resulting in cellular injury-associated degree chemical reaction of proteins and fats. Treatment with multivitamins causes depletion of glutathione in tissues.<sup>26</sup> Other study has shown that multivitamin administration to albino rats caused pathology of the mitochondria, accompanied by oxidative stress and a rise in ADP levels within the liver. Raw honey contains antioxidants which are referred to as flavonoids and phenoplast compounds. Antioxidants facilitate the safeguard of individual's body from cellular damage. Free radicals contribute to aging method and may increase the risk of chronic conditions like studies.<sup>28-30</sup>

cancer and other related diseases. Studies from reliable sources show that polyphenols in raw natural honey, significantly might play a role in disease prevention. All of these work together to provide a synergy to treat the cytotoxic effect, scavenging and eliminating free radicals.<sup>27</sup> Recently, some changes are presented in Table 2. It has been found that honey leads to increased levels of total protein and albumin in biological fluids and reduces liver enzymes, such as AST, ALT and ALP in blood,<sup>16</sup> thereby creating a significant improvement in liver tissues. Thus, our results demonstrate protective effect of white honey against hepatotoxicity, and this is consistent with previous

**Table 1:** Relative changes in body weight and liver index in albino rats with hepatic fibrosis induced by multivitamin-toxicity

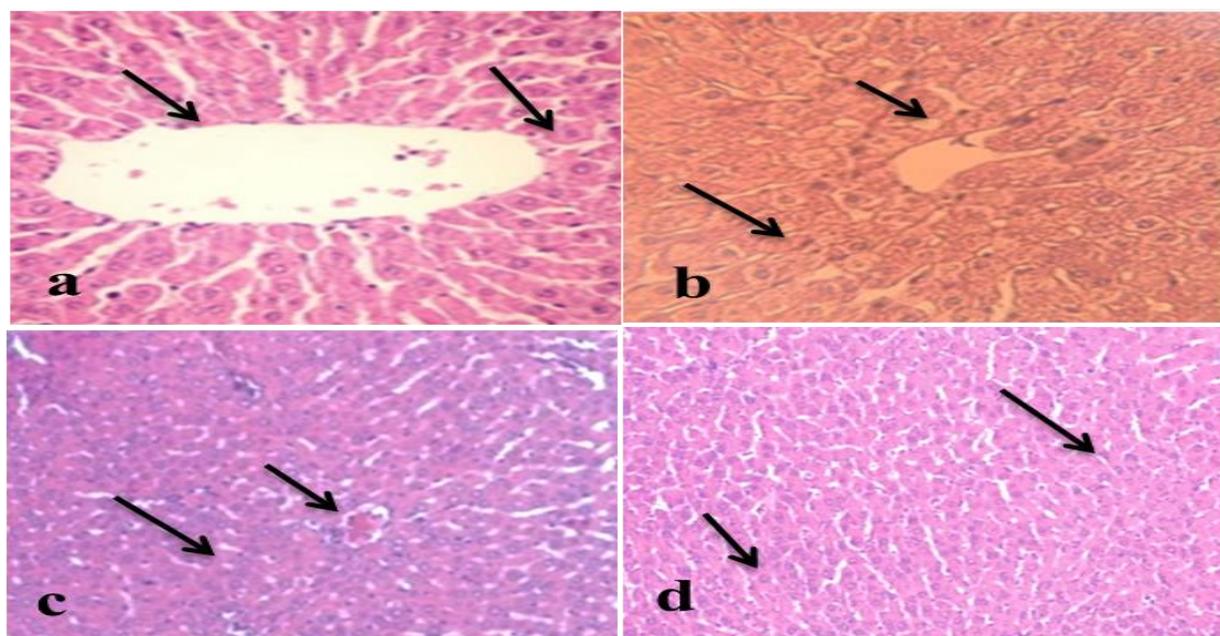
Parameter	Group 1	Group 2	Group 3	Group 4	p-value
Body weight (kg)	18.7±0.1	18.7 ± 0.2	23.3±0.2	20.5±0.1	0.001
Liver index	1.78 ± 0.22	0.99 ± 0.65	0.86 ± 0.22	1.09 ± 0.17	0.001

Group 1: Control group with water; Group 2: Control group with white honey; Group 3: Treatment group with multivitamins; Group 4: Treatment group with multivitamins, followed by white honey.

**Table 2:** Relative changes in liver function test parameters in albino rats with hepatic changes induced by multivitamin-toxicity

Parameter	Group 1	Group 2	Group 3	Group 4
Total protein	6.18 ± 0.31	6.04 ± 0.19	7.09 ± 1.26	6.65 ± 0.22
AST	120.88 ± 5.16	119 ± 1.2	158 ± 3.46	133.49 ± 1.92
ALT	29 ± 1.22	28.21 ± 1.18	42.81 ± 2.33	35.74 ± 0.59
Nitric oxide	0.28 ± 0.08	0.265 ± 0.011	0.39 ± 0.14	0.22 ± 0.04

Group 1: Control group with water; Group 2: Control group with white honey; Group 3: Treatment group with multivitamins; Group 4: Treatment group with multivitamins, followed by white honey.



**Figure 1:** Relative changes in histological structures of liver tissues in albino rats with hepatic fibrosis induced by multivitamin-toxicity. **a** and **b**: Tissue from normal control group (Normal structure of the liver tissues with normal central vein surrounded with ribbons of hepatic cells, intertwined and covalent); **c**: Tissue from treatment group with multivitamins (showed congestion and bleeding in central venous with death of most liver cells [arrow], invasion of inflammatory cells, infiltrations around the central vein, bleeding and enlargement of the hepatic sinuses [Arrows]); **d**: Tissue from treatment group with multivitamins, followed by white honey (showed healing of damage area of hepatocytes and becoming less necrotic and fibrosis); (H and E stain; x400)

## Conclusion

The findings from this study indicated that white honey supplementation had a beneficial effect in the treatment of hepatotoxicity. White honey decreased the levels of serum marker enzymes induced by high dose of multivitamins which caused hyper hepatocellular toxicity. It is hereby recommended that white honey could be used in the management of hepatotoxicity caused by multivitamins.

## Conflict of Interest

The authors declared no conflict of interest.

## Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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