

Original Research Article

Honey attenuates Phenylhydrazine-induced Hematotoxicity and Oxidative Stress in male Wistar rats

Abstract

Honey is a sweet, viscous food substance produced by bees using nectar from flowers. Due to its complex chemical composition, it has been widely used in traditional medicine for its therapeutic properties. The present study evaluated the protective role of honey in attenuating phenylhydrazine (PHZ)-induced toxicity in male Wistar rats. Twenty (20) male Wistar rats with a weight range of 200-250g were used for the study. They were allocated into four (4) groups consisting of five (5) rats each. In the first phase of the experiment, animals in group I (control) received distilled water while animals in groups II, III and IV received 2ml of 15, 30 and 60% honey respectively by oral gavage. In the second phase, haemotoxicity and oxidative stress were induced by intraperitoneal injection of phenylhydrazine (PHZ) at 50 mg/kg to all twenty (20) animals, daily for two (days). The animals continued to receive distilled water and honey as in phase one. Blood collected from animals was analyzed for haematological and oxidative stress parameters following standard laboratory procedures. Results from the present study show that significantly increased packed cell volume, haemoglobin concentration, red blood cell, total white blood cell and neutrophil counts among the experimental groups compared to the control ($p < 0.05$). Also, superoxide dismutase, catalase and glutathione levels increased among the honey-supplemented experimental groups compared while MDA levels reduced compared to the control ($P < 0.05$). The study concludes that oral supplementation of honey may have protected against phenylhydrazine-induced toxicity evidenced by increased packed cell volume, red blood cell, white blood cell and neutrophile counts, catalase and superoxide dismutase as well reduced malondialdehyde. The present evidence suggests that honey could attenuate haematotoxicity and oxidative stress caused by phenylhydrazine

Keywords: phenylhydrazine, honey, haematological parameters, haematoxicity, oxidative stress

INTRODUCTION

As defined by the Codex Alimentarius Commission, honey is a natural sweet substance created by honey bees (*Apis mellifera*) through the collection and transformation of nectar from plants or the secretions and excretions of plant-sucking insects on living parts of plants [1, 2]. The transformed substance is then stored, dehydrated, and left to mature in honeycomb cells. Honey production by bees is believed to serve as a source of food during times of scarcity or adverse weather conditions [3]. Honey has been utilized as both a food and medicinal product for centuries. It is a multifaceted mixture that exhibits significant variations in composition and traits as a result of its geographical and botanical sources. The main characteristics of honey are influenced by the type of nectar that bees forage, which ultimately depends on the floral origin

[4, 5]. Natural honey is a complex mixture of over 180 substances, primarily composed of carbohydrates, mostly fructose, glucose, maltose, fructose and sucrose, making up about 80-85% of its content. Additionally, honey contains approximately 15-17% water, 0.1-0.4% protein, and 0.2% ash, as well as minor amounts of amino acids, enzymes, vitamins, and phenolic antioxidants [1, 3, 6]. Although the major constituents of honey remain consistent across different samples, the precise chemical composition and physical characteristics of natural honey are determined by the specific plant species from which the bees gather nectar and the environmental factors during production, such as weather, humidity, nectar conditions, and honey extraction and storage [7-10]. The unique nutritional and medicinal properties of honey come from the diverse range of substances it contains, which makes it a highly sought-after product.

Honey has been a valuable component of traditional medicine for centuries. Nonetheless, its use in modern medicine is restricted due to insufficient scientific evidence to support its benefits [11]. Some of the medicinal uses include soothing sore throats and coughs, healing wounds and burns, providing relief for digestive issues such as diarrhoea and stomach ulcers, boosting energy levels and athletic performance, treating skin conditions such as acne, eczema, and psoriasis, reducing inflammation and promoting overall health, enhancing sleep and reducing anxiety, supporting the immune system and combating infections, acting as a natural preservative due to its antibacterial properties and promoting oral health and preventing tooth decay [5, 11-13]. Documented research evidence has shown that honey has antidiabetic [14, 15], antioxidant [16, 17], wound healing [18, 19], anti-inflammatory [20, 21], immuno-modulatory [22, 23], antibacterial [24, 25], anti-cancer [26, 27], anti-ulcer [28, 29] and anti-hyperlipidemic and anti-obesity [30, 31] activities. To date, no research shows the impact of honey on haematotoxicity. Consequently, this study intends to fill this gap in knowledge by investigating the potential of honey to alleviate phenylhydrazine-induced haematotoxicity and oxidative stress using Wistar rat models.

MATERIALS AND METHODS

Source and Preparation of Honey

Pure and high-quality honey was acquired from the Department of Forestry at the University of Port Harcourt, Nigeria. The honey was obtained in its unprocessed form and was authenticated to be of good quality. To create the final form of administration, the honey was diluted with distilled water into 15, 30 and 60% honey concentrations.

Research Animals

Twenty (20) male Wistar rats with a weight range of 200-250g were procured from the Animal house of the Department of Human Physiology, University of Port Harcourt. They were allocated into four (4) groups consisting of five (5) rats each. The rats were habituated and kept under controlled laboratory conditions of temperature, humidity, and light for two weeks for acclimatization. All the rats were provided with unrestricted access to commercial pellet food

and water. The experiment was conducted in adherence to the animal experimentation guidelines of the University of Port Harcourt Research Ethics.

Research Design and Procedure

The experiment was conducted in two phases, lasting two (2) weeks and Forty-eight (48) hours respectively. During the first phase, animals in group I (control) received distilled water while animals in groups II, III and IV received 2ml of 15, 30 and 60% honey respectively by oral gavage. In the second phase, haemotoxicity and oxidative stress were induced by intraperitoneal injection of phenylhydrazine (PHZ) at 50 mg/kg to all twenty (20) animals, daily for two (days). The animals continued to receive distilled water and honey as in phase one.

Blood Collection and Laboratory Analysis

The animals underwent cervical dislocation for anaesthesia, following which blood was drawn through cardiac puncture and placed into an EDTA sample bottle for haematological analysis using a haematology auto-analyzer (Automatic Haematology Analyzer, Mindray, China) for the determination of (PCV), haemoglobin concentration (Hb), red blood cell (RBC) and white blood cell (WBC) count, MID, lymphocyte and neutrophil counts, and platelet counts and plain sample bottle for oxidative stress enzymes: malondialdehyde (MDA), catalase (CAT), superoxide dismutase (SOD), glutathione (GSH) by standard laboratory methods [32, 33].

Statistical Analysis

Data from the laboratory investigation data were analyzed using IBM Statistical Product and Service Solutions (SPSS version 25). For each research group, the mean and standard error of the mean were calculated for each parameter. To compare the mean values of study groups II, III, and IV with the control group (Group I), an analysis of variance (ANOVA) was performed, followed by a least significant difference (LSD) posthoc analysis. A p-value of less than 0.05 ($p < 0.05$) was considered statistically significant.

RESULTS

Table 1: Effects of honey on haematological parameters of phenylhydrazine-induced haematotoxicity in Wistar rats

| Parameters | Control PHZ (n=5) | PHZ+ 15% Honey (n=5) | PHZ+ 30% Honey (n=5) | PHZ+ 60% Honey (n=5) |
|---------------------------------|-------------------------|----------------------------|----------------------------|----------------------------|
| PCV (%) | 34.30±3.2 | 42.18±0.97* | 43.40±1.33* | 48.50±2.1* |
| Hb.conc. (g/dl) | 10.70±0.10 | 13.50±0.34* | 13.72±0.41* | 15.68±0.46* |
| RBC ($\times 10^{12}/L$) | 5.41±0.48 | 6.87±0.34* | 7.12±0.10* | 8.03±0.26* |
| MCV (fL) | 63.26±2.20 | 61.37±1.29 | 60.30±1.78 | 60.36±1.75 |
| MCH (pg) | 19.74±0.66 | 19.64±0.45 | 19.06±0.57 | 19.52±0.17 |
| MCHC (g/dL) | 31.28±1.01 | 32.05±0.18 | 31.64±0.69 | 32.48±1.21 |
| WBC ($\times 10^9/L$) | 4.7±0.40 | 5.94±0.48 | 5.94±0.75 | 7.5±0.67* |
| Lymphocytes ($\times 10^9/L$) | 3.66±0.50 | 3.78±0.32 | 4.08±0.47 | 4.92±0.69 |

| | | | | |
|--------------------------------|---------------------|---------------------|--------------------|--------------------|
| Neutrophil ($\times 10^9/L$) | 0.84 \pm 0.17 | 1.54 \pm 0.22* | 1.34 \pm 0.19 | 1.56 \pm 0.26* |
| Platelets ($\times 10^9/L$) | 786.60 \pm 190.11 | 850.60 \pm 104.40 | 966.00 \pm 90.87 | 928.80 \pm 31.40 |

Result is given as mean \pm standard error of mean; *significantly different compared to control (p<0.05)

Table 1 depicts the impact of oral administration of honey on haematological parameters in male Wistar rats with phenylhydrazine-induced haematotoxicity. The data show that the mean values of PCV, Hb, and RBC significantly increased in a dose-dependent manner in the experimental groups (II, III, and IV) compared to the control group (p<0.05). However, only Group IV (PHZ+60% Honey) demonstrated a significant increase in WBC count, while Groups II and IV (PHZ+15% Honey & PHZ+60% Honey) showed a significant increase in neutrophil count compared to the control group (p<0.05).

Table 2: Effects of honey on oxidative stress markers on phenylhydrazine-induced oxidative stressed Wistar rats

| Parameters | Control PHZ (n=5) | PHZ+ 15% Honey (n=5) | PHZ+ 30% Honey (n=5) | PHZ+ 60% Honey (n=5) |
|-----------------------------|-------------------------|----------------------------|----------------------------|----------------------------|
| Malondialdehyde (nmol/ml) | 29.39 \pm 1.51 | 22.80 \pm 0.48* | 20.36 \pm 0.67* | 13.12 \pm 1.43* |
| Superoxide dismutase (U/ml) | 88.16 \pm 5.06 | 105.51 \pm 3.74* | 107.17 \pm 3.51* | 112.75 \pm 2.73* |
| Catalase (U/ml) | 9.57 \pm 1.58 | 17.93 \pm 3.41 | 16.96 \pm 1.51 | 33.53 \pm 4.49* |
| Glutathione (mg/dl) | 75.33 \pm 3.15 | 110.09 \pm 3.22* | 99.63 \pm 7.88 | 154.44 \pm 19.91* |

Result is given as mean \pm standard error of mean; *significantly different compared to control (p<0.05)

The effect of oral administration of honey on oxidative stress markers and enzymes on phenylhydrazine-induced oxidative stressed male Wistar rats. The results indicate that there was a significant decrease in MDA among all the experimental groups (II, III & IV) compared to the control (p<0.05). The mean values of SOD increased among all the experimental groups; catalase significantly increased for group IV (PHZ+60% Honey) while glutathione increased for groups II and IV ((PHZ+15% Honey & PHZ+60% Honey).

DISCUSSION

The complex nature of the constituents of honey confers on it many uses in traditional medicine for many centuries. Despite this, its use in modern medicine is limited due to a lack of adequate scientific evidence to substantiate its benefits. The present study examined the protective effect of honey on phenylhydrazine-induced haematological and oxidative stress toxicity using Wistar rat models. Phenylhydrazine (PHZ) is commonly used to induce anaemia and oxidative stress in animal experimental models [34, 35]. It causes haemolysis, which is the destruction of red blood cells by oxidizing haemoglobin, the protein in red blood cells that carries oxygen. PHZ in the bloodstream reacts with haemoglobin to form unstable intermediates form, methemoglobin, a form of haemoglobin which cannot bind to oxygen, hence reducing the oxygen-carrying capacity of red blood cells [36, 37]. Furthermore, the breakdown of unstable intermediates also produces reactive oxygen species (ROS), which can cause oxidative damage to red blood cells and their

membranes. This damage ultimately leads to the rupture of red blood cells and the release of haemoglobin into the bloodstream, resulting in haemolysis. These free radicals can initiate a redox cycle in which they can interact with oxygen to form superoxide anion, hydrogen peroxide, and other ROS [38-40]. These ROS can cause oxidative damage to cellular macromolecules such as lipids, proteins, and DNA, leading to cellular dysfunction and damage [40, 41]. PHZ-induced oxidative stress can also lead to the depletion of endogenous antioxidant enzymes which are involved in scavenging ROS [42, 43].

Effects on haematological parameters

Data from the present study show that oral administration of honey caused a significant increase in PCV, Hb, RBC, WBC and neutrophil among the experimental groups compared to the untreated control ($p < 0.05$) (Table 1). This effect was seen to be dose-dependent. It is possible that the two-week administration of honey before PHZ-induced toxicity may have boosted haematopoiesis as honey is known to contain several mineral nutrients such as iron and vitamins [3, 6, 44] required for the production of blood. Honey has been shown to enhance RBC, WBC, and PCV [45, 46] and to reverse Aluminum-induced anaemia [47]. Red blood cells (RBCs) are highly specialized cells that play a critical role in transporting oxygen to the body's tissues and organs. However, their lack of a nucleus and continuous exposure to oxygen makes them susceptible to damage from free radicals. This oxidative stress can cause changes in RBC structure and mechanical stability, ultimately resulting in cellular damage [48]. The antioxidant nature of honey may have prevented PHZ-induced-haemolytic anaemia through its antioxidant properties. Honey administration may have prevented damage to cells and tissues including red blood by donating an electron to ROS released by PHZ and stabilizing the membrane structure. White blood cells, also known as leukocytes, play a crucial role in the immune system by aiding the body in combating infections and other foreign substances [49-51]. The increased WBC and neutrophil levels may be due to the ability of honey to enhance hematopoiesis and boost immunity. Honey is a good source of iron, zinc and vitamin C which are essential for the production of white blood cells and other immune cells [11, 46].

Effect on Oxidative stress parameters

This study shows that honey supplementation significantly reduced MDA among all the experimental groups compared to the control ($p < 0.05$) (Table 2). The result also shows that SOD, catalase and glutathione were increased in some of the experimental groups compared to the control ($p < 0.05$). Oxidative stress results when the level of harmful reactive oxygen species (ROS) surpasses the body's antioxidant defence. PHZ triggers the production of excessive ROS, disrupting the balance between oxidation and antioxidant defence. This imbalance results in lipid peroxidation (LPO) and eventual oxidative harm to proteins and DNA [42, 43]. MDA is the main result of the peroxidation of polyunsaturated fatty acids, and its presence indicates the level of injury in cells or tissues. The elevated levels of MDA detected in animals exposed to PHZ is an indication that PHZ induced tissue and cellular injury. The significantly reduced MDA following honey supplementation MDA is attributable to the antioxidant activity of honey to mitigate cellular and tissue injury [16, 17, 52]. Similarly, the increase in the SOD, catalase and glutathione which are antioxidant enzymes lays credence to the potent antioxidant activity of honey. Antioxidants have the capacity to scavenge free radicals and harmful oxygen-derived

species such as hydroxyl radicals, singlet oxygen, and hydrogen peroxide. This ability enables them to prevent or alleviate damage to cells and the negative impacts of diseases caused by oxidative stress [52-54]. The present evidence suggests that honey enhanced activity of antioxidant enzymes which possibly protected the Wistar rats from PHZ-induced oxidative stress.

CONCLUSION

Based on the available data from the present study. It appears that oral supplementation of honey protected against phenylhydrazine-induced toxicity evidenced by increased packed cell volume, red blood cell, white blood cell and neutrophil counts, catalase and superoxide dismutase as well reduced malondialdehyde. The present evidence suggests that honey could attenuate haematotoxicity and oxidative stress caused by phenylhydrazine.

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