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The Protective Effect of Natural Honey against Acetamenophen-Induced Acute Pancreatitis

[1] Etu Esther Ifeyinwa, [2] Chukwu Chibuike Victor, [3] Huru Cyril Adams, [4] Onyinye Favour Onwe, [5] Ezeh Chibuike Emmanuel, [6] Eze Peter Chinweuba, [7] Umeh Kizito Chikelu, [8] Wanger Jaclyn Tachia, [9] Johnson Tosin Patience, [10] Shittu Favour Olamide, [11] Okike Chinonso Michae, [12] Justice Chibuikem Olelewe, [13] Elee Chimbuoyim Charles, [1] [4] Awe Boluwatife Samuel, [15] Chukwueze Prince Obinna, [16] Okagu Bonaventure Chukwuemeka, [17] Aniodo Rita Chi Dimma, [18] Chukwu Chigozie David, [19] Okoh ifechukwude Lisa

[1, 4, 5, 6, 7, 11, 16] Department of Medical Laboratory Science, University of Nigeria Nsukka, Enugu State, Nigeria
[2, 12, 13, 15] Department of Medical Laboratory Science, Ebonyi State University Abakaliki, Ebonyi State, Nigeria
[3] Department of Biochemistry University of Jos, Plateau State, Nigeria
[8] Erismann Institute of Public Health, The N.A Semashko Public Health and Healthcare Department, I.M Sechenov First Moscow State Medical University
[9] Department Transport Management Technology, Federal University of Technology Akure, Ondo State, Nigeria
[10] Department of Medical Laboratory Science, Babcock University, Ilishan- Remo, Ogun State, Nigeria
[14] Department of Anatomy, Olabisi Onabanjo University, Ogun State, Nigeria
[17] Department of Medical Laboratory Science, Enugu State University of Science and Technology, Enugu State, Nigeria
[18] Department of Medical Laboratory Science, David Umahi federal School of Medical Sciences Uburu, Ebonyi State, Nigeria
[19] Department of Botany, Delta State University, Abraka, Delta State, Nigeria

Corresponding Author: **Chukwu Chibuike Victor**

Abstract

The therapeutic properties of natural honey is important in the protection against acute and chronic diseases. The aim of this work was to study the protective effect of natural honey against acetamenophen-induced acute pancreatitis. A total of 20 male albino wistar rats were divided into four groups. Group. A animals served as control group and were not given any treatment. Group B animals received a single dose of acetamenophen (750 mg/kg, i.p) before treatment with administration of natural honey (15ml/kg) for 7 consecutive days. Group C animals received a single dose of acetamenophen (750mg/kg, i.p) before treatment with N-acetylcystein (600mg/kg, oral) for 7 consecutive days. Group D received a single dose of acetamenophen (750mg/kg, i.p) alone. Pancreatic enzyme (amylase and lipase) tests were done and the results revealed that single dose administration of acetamenophen (750mg/kg, i.p) showed significant drug-induced acute pancreatic injury ($P< 0.01$) when compared to the negative control (group D). However, treatment with natural honey (15ml/kg) for 7 c0nsecutive days demonstrated substantial pancreatic protective effects than N-acetylcysteine (600mg/kg, oral). Therefore, it could be concluded that honey via its antioxidant properties has the ability to protect against acetamenophen-induced acute pancreatitis.

Keywords: Natural Honey, Acute Pancreatitis, Acetamenophen. N-Acetylcystein, Amylase, Lipase

1. Introduction

Honey is a well-known and historically important sweet food which possesses immense antimicrobial properties. Numerous varieties of honey are present in nature, and all of these honey varieties contain certain key ingredients, which confer upon them various antimicrobial properties. These antimicrobial key ingredients include polyphenolic compounds, hydrogen peroxide, methylglyoxal, and bee-defensin among several others. Honey is nowadays used extensively in modern medicine as potent antibiotic for the treatment of surface wounds and burns [1, 2]. Honey has a pH of almost 3.6 and water activity of 0.5 and

0.62. It has high fructose level (honey is 25% sweeter than table sugar). Other phytochemical constituents of honey are flavonoids, polyphenols which act as antioxidants. Evidence indicates that honey can exert several health benefits including respiratory, gastrointestinal, cardiovascular, and nervous system protective effects [2].

Honey is a natural substance with many medicinal effects such as antibacterial, hepatoprotective, hypoglycemic, reproductive, antihypertensive and antioxidant effects. Studies have shown that honey may ameliorate oxidative stress in the gastrointestinal tract (GIT), liver, pancreas, kidney, reproductive organs and plasma/serum [3]. Because Oxidative stress plays an important role in the pathogenesis of acute pancreatitis, It is reasonable to surmise that antioxidants would play a protective role in ameliorating the deleterious effects of pancreatitis. Recent research data suggest that honey, administered alone or in combination with conventional therapy, might be a novel antioxidant in the management of acute and chronic diseases commonly associated with oxidative stress such as acute pancreatitis [4].

The pancreas is an organ of the digestive system and endocrine system of vertebrates. In humans, it is located in the abdomen behind the stomach and functions as a gland. The pancreas is a mixed or heterocrine gland, i.e. it has both an endocrine and a digestive exocrine function. 99% part of pancreas is exocrine and 1% part is endocrine. As an endocrine gland, it functions mostly to regulate blood sugar levels, secreting the hormones insulin, glucagon, somatostatin, and pancreatic polypeptide. Structurally speaking, the human pancreas consists of two organs in one: an exocrine gland and an endocrine gland. The exocrine gland is made up of pancreatic acinar cells and duct cells that produce digestive enzymes and sodium bicarbonate, respectively. The primary function of the exocrine pancreas is to secrete the digestive enzymes responsible for normal digestion and absorption of daily foodstuffs, and finally assimilation of nutrients into our body. The endocrine gland, meanwhile, is made up of five types of secretory islet cells and secretes peptide hormones for the maintenance of glucose homeostasis. The pancreatic secretory functions are finely regulated by neurocrine, endocrine, and paracrine as well as intracrine mechanisms. In view of this fact, inappropriate activation or inactivation of the pathways mediating the pancreas's fine regulatory mechanisms has considerable impacts on health and disease [5].

Inflammation of the pancreas is known as pancreatitis, with common causes including chronic alcohol use and gallstones and less likely by drug use. Pancreatitis occurs when digestive enzymes become activated while still in the pancrease, irritating the cells of the pancreas and causing inflammation [6].

Acute pancreatitis is the acute inflammation of the pancreas of varying severity, characterized clinically by abdominal pain and elevated levels of pancreatic enzymes in the blood.Numerous risk factors are known to induce acute pancreatitis, particularly gallstones and chronic alcohol abuse. Drug-induced acute pancreatitis accounts for <2% of cases; however, the incidence may have been underestimated; mild pancreatitis may be subclinical and severe and fulminant attacks may not have been clearly confirmed because of an unidentified history or complex interrelationships with other, more common causes of morbidity. Diagnosing drug-induced acute pancreatitis can be challenging; the causes being a lack of clear biological evidence and mechanisms, for example, inadequate criteria for diagnosing acute pancreatitis, failure to preclude common causes, lack of specific clinical appearances of drug reaction, and mixed effects of combined drugs or prescriptions [6, 7]. Amylase and lipase tests are used to detect pancreatitis. The tests measure the amount of these enzymes circulating in the blood stream. These enzymes are typically checked when a patient has symptoms of pancreatitis or other pancreatic disorder [8].

Acetaminophen (paracetamol, *N*-acetyl-p-aminophenol) is the most commonly used analgesic antipyretic. Because of its wide availability and use as a component in hundreds of over-the-counter and prescription medications, acetaminophen has remained one of the leading drugs of intentional and unintentional overdose worldwide. Acetaminophen poisoning is associated with an increased risk of acute pancreatitis. Acute pancreatitis is among other, less-common extrahepatic manifestations of acetaminophen poisoning for which few reports exist [7]. The aim of this study is to investigate the protective effect of natural honey against acetaminophen induced pancreatitis in male wistar rats. This study provides a natural and cheap remedy against the toxic effect of acetaminophen overdose on the pancreas.

2. Materials and Methods

2.1 Plant Materials

Fresh natural honey used for the study was obtained from Nsukka, a town in Enugu, Enugu state, Nigeria.

2.2 Chemicals and Reagents

Chemical: N-acetyl cysteine (NAC) and Acetaminophen injection were purchased from Alpha Pharmaceutics, Enugu, Nigeria. Amylase and Lipase kits were purchased from Randox Laboratory Ltd, UK.

2.3 Induction of Acute Pancreatitis

Average weight of the rats was, one hundred and eight gram (108.5gm) and a calculated dosage of 90mg was required to induce pancreatitis. About 90mg was contained in 0.6ml of 150mg/ml of paracetamol. A single dose of 0.6ml paracetamol (i.e., 750mg/kg body weight) was injected intraperitonally to induce acute pancreatitis in each experimental rat.

2.4 Animals

Twenty (20) adult albino rats, weighing one hundred and eight grams (108.5gm), were obtained from the animal house of the College of Veterinary Medicine, University of Nigeria. The animals were housed in metallic under standard conditions of temperature (22 ± 3^{o}C) and a 12 h light, 12 h dark cycle. The animals were kept under observation for about 14 days before the onset of the experiment for acclimatization. Experimental protocol and handling was according to Institutional guidelines describing the use of rats and in accordance with the American Physiological Society guiding principles for research involving animals and human beings [9].

2.5 Experimental Design

The twenty (20) albino rats were grouped into grouped into (A-D) of five rats each and the received the following treatments which lasted for 7 days:

Group A: (Normal Control): No treatment was given to this group.

Group B: (Test group): received a single dose of acetaminophen (750 mg/kg, i.p) before treatment with oral administration of natural honey (15ml/kg) for 7 consecutive days.
Group C (Positive control): Received a single dose of acetaminophen (750 mg/kg, i.p) before treatment with daily administration of N-acetylcysteine (600 mg/kg, oral) for 7 consecutive days.
Group D (Negative control): Received a single dose of acetaminophen (750 mg/kg, i.p) alone.

2.6 Sacrificing of Animals and Sample Collection
Blood samples for the determination of serum analyses of serum amylase and lipase were taken by cardiac puncture of the left ventricle of the heart under chloroform anesthesia.

2.7 Biochemical Analysis
The serum obtained was used for the analysis of serum amylase and serum lipase. Amylase and lipase activities in the serum were determined using colorimetric detection [10].

2.8 Histopathological Analysis
The paraffin wax embedding method was employed to prepare the removed pancreas Sections of each organ were made at a thickness of 5 microns, and Hematoxylin and Eosin staining technique was used for better general examination of the tissues [11]. An Olympus™ light microscope was used to examine the tissue sections.

2.9 Statistical Analysis
Data analysis was done using GraphPad prism version 7.0 (GraphPad, San Diego, CA, USA). The results of the biochemical assays were reported as mean±SEM (standard error of mean). The level of significance was tested using one-way analysis of variance (ANOVA), followed by the Tukey post hoc analysis. Probability levels less than 0.05 ($p<0.05$) was considered significant.

3. Results
3.1 Biochemical Results
Estimating the blood level of biochemical markers (i.e., pancreatic enzymes) was used to determine the pancreatic functioning: Amylase and Lipase levels (Table 1). The results indicate that single dose administration of (750 mg/kg, i.p) showed significant drug-induced acute pancreatic injury ($P< 0.01$) when compared to negative control. However, treatment with natural honey (15ml/kg) for 7 consecutive days demonstrated substantially pancreatic protective effects than N-acetylcysteine (600 mg/kg, oral).

3.2 Histopathological Results
In Figure 1, the pancreas of the normal control, the natural honey and the N-acetyl cysteine groups appeared morphologically and functionally normal. The hepatocytes showed a well conserved morphology. The pancreas of group D treated with acetaminophen alone showed greatest Interlobular septum dilatation although moderately appearance of pancreatic tissues. The plates showed that natural honey was as protective as the positive control, N-acetyl cysteine.

Table 1: Statistical Comparison of biochemical concentrations of the pancreatic enzymes of treated groups with negative controls Groups after acetaminophen administration

Groups	Amylase (IU/L)	Lipase (IU/L)
A- Normal Control	639 ± 42.7**	121.71 ± 21.2**
B- APAP + NH	657.35 ± 53.7 **	164.13 ± 22.7**
C- APAP + NAC	671.09 ± 48.7 **	167.27 ± 27.9**
D- APAP only	1175 ± 74.8	327.57 ± 38.2

Values are Mean ± SEM. $**P < 0.01$ is significant when (APAP only) is compared with all other groups. ***APAP**: N-acetyl- p-aminophenol, APAP, paracetamol.

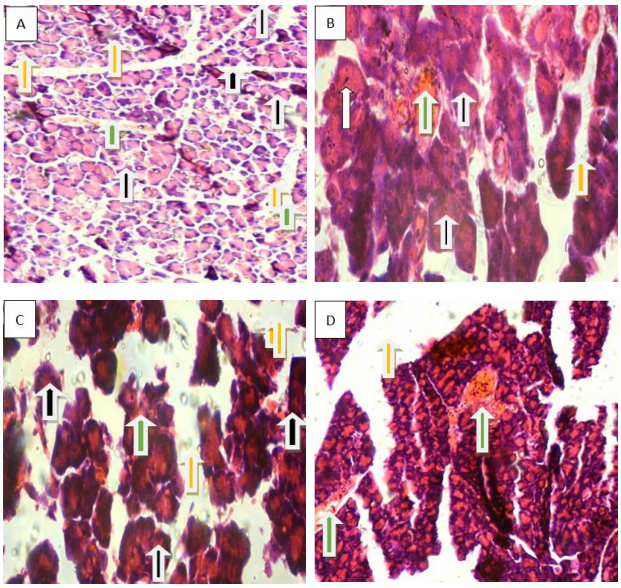

Fig 1: (Plate A) Photomicrograph of the pancreas section from the normal control rat, stained with H and E showing normal pancreatic tissue. Plate B) Photomicrograph of the pancreas section from group 2 Albino rat, stained with H and E showing normal pancreatic tissue. Plate C) photomicrograph of the pancreas section from group 3 Albino rat, stained with H and E showing normal pancreatic tissue. Plate D) Photomicrograph of the pancreas section from group 2 Albino rat, stained with H and E showing normal pancreatic tissue (Magnification of x40)

Keys: Zymogen granules:
Pancreatic duct:
Pancreatic lobule:
Capillary:
Interlobular septum:

4. Discussion
The purpose of this study is to ascertain the protective effect among other properties of natural honey against acetamenophen-induced acute pancreatitis. Acute pancreatitis is an acute inflammation of the pancrease with variable involvement of surrounding tissues and/or distant organs. While alcohol and gallstones account for the most important aetiological factors, the impact of other agents, such as prescribed drugs, is being increasingly appreciated owing to the accumulating evidence in published literature. Drug-induced acute pancreatitis is rare with a reported incidence of 0.1%-2%, but should not be overlooked.

However, drug-induced pancreatitis remains a difficult and challenging diagnosis due to atypical clinical and laboratory tests, which is usually established by excluding other causes. Our study demonstrates the important role of natural honey in the protection against acetaminophen-induced acute pancreatitis in albino wistar rats. The rats were administered with acetamenophen and honey. Amylase and Lipase test were done for different studied groups to assess their status. In this work, the pancreatic enzymes amylase and lipase in group D were significantly elevated after acetamenophen treatment (APAP only) compared to the control group denoting the presence of pancreatic dysfunction. However, in group B and C which involved the administration of natural honey and acetaminophen (APAP+NH), and N-acetylcystein respectively, the level of pancreatic enzyme activity is significantly reduced compared to the control group. This finding indicates the protective effect of natural honey in ameliorating the the deleterious effect of acetamenophen on the pancreas. Similar observation was obtained by Abdel-Moneim *et al* [12].

Acetaminophen is frequently used for pain and fever in the clinic and is easily obtained from any pharmacy without prescription. The diagnosis of acetaminophen-induced pancreatitis was established by excluding common etiologies such as gallstone pancreatitis, hyperlipidemic pancreatitis and ethanol-induced acute pancreatitis and other suspicious drugs that have been reported to cause pancreatitis [13, 14].

5. Conclusion

This study, demonstrated that honey administered against acetamenophen, minimized the toxic effect of the drug. Honey afforded protection against acetamenophen-induced acute pancreatitis. There was a higher level of protection with administration of natural honey as compared to administration of N-acetylcystein which produced little effect. This study proves that natural honey protects the pancrease from the deletrous effect of acetaminophen on the pancrease.

6. Compliance with Ethical Standards

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Disclosure of Conflict of Interest

The author(s) declared no potential conflicts of interest.

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