

Anti-proliferative and Chemotherapeutics Effects of Nigerian Honey and Its Polyphenols on Cancer cells

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Background of the research

Cancer is one of the major deadly diseases affecting humans around the world, Nigeria inclusive. Liver cancer also known as hepatocellular carcinoma (HCC) is the fifth most common cancer in males and the seventh most common cancer in females and the third leading cause of cancer death globally [1]. Limited treatment for high-risk liver cancer and poor prognosis was recorded, in addition to its resistance, recurrence, progression and invasion. Resistance of liver cancer to conventional therapies added to severe side effects prompted the search for a novel therapeutic approach based on natural products such as the Nigerian honey. Honey and its components were documented to have significant anti-cancer activity against many types of cancer including liver cancer in both *in vivo* and *in vitro* studies. However, the mechanism of anti-cancer activity of honey as a chemopreventive and therapeutic agent has not been completely elucidated, and to our knowledge, there is no study carried out to investigate the effects of Nigerian honey on cancer cells. It is in view of this that this project aims to pre-clinically evaluate the molecular mechanism of anti-cancer activity of the Nigerian honey on liver cancer cells through the use of molecular techniques. A full understanding of cellular and molecular mechanisms elicits by honey in killing cancer cells may lead to the use

37 of the honey or its polyphenols at clinical level for effective treatment of many types of human
38 cancer in the future.

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42 **Statement of the problem**

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44 Cancer is a generic term for a large group of diseases that can affect any part of the body and
45 is one of the dreadful diseases among the chronic disorders. Cancer is a leading cause of death
46 worldwide: it accounted for 7.4 million deaths (around 13% of all deaths). Lung, stomach,
47 colorectal, breast and liver cancer cause the most cancer deaths each year over the world
48 including Nigeria [3]. Fortunately, more than 30% of cancer deaths can be prevented by the
49 use of natural foods such as honey [4]. Thus, this project seeks to investigate the inhibitory and
50 anti-proliferative effect of Nigerian honey against liver cancer.

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54 **Objectives of the research**

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56 Our main aim is to investigate and compare the effects of Nigerian honey on serum level of
57 blood parameters, cancer biomarkers, glucocorticoid, glucocorticoid liver cell receptors, and
58 level of CYP-450 in both cancerous rats with or without chemotherapy, and liver cell *in vitro*
59 through the following specific objectives: -

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- 61 1) To investigate and compare the effects of Nigerian honey in different dosage on tumour
62 necrosis factor- α (TNF- α), serum corticosteroid binding globulin (CBG), Serum α -
63 fetoprotein (AFP), homocysteine, α 2-macroglobulin (α 2M), alanine aminotransferase
64 (ALT), aspartate aminotransferase (AST) in cancerous rats with or without
65 chemotherapy treatment.

- 66 2) To investigate and compare the effects of Nigerian honey in different dosage on blood
67 glucose and lipid profile (TG, HDL, LDL, Cholesterol) in cancerous rats with or
68 without chemotherapy treatment.
- 69 3) To investigate and compare the effects of Nigerian honey in different dosage on liver
70 cells in cancerous rats with or without chemotherapy and liver cell culture.
- 71 4) To find out possible linkages between known anti-cancer agents and the Nigerian
72 honey.
- 73 5) To examine the expression of selected apoptosis-related genes using quantitative RT-
74 PCR in both the honey treated, and untreated cancer cells in comparison with normal
75 cell lines (noncancerous).
- 76 6) To investigate apoptosis-inducing effects of Nigerian honey on the cancer cells

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79 **Research questions**

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81 What are the cellular and molecular mechanisms elicited by Nigerian honey in destroying
82 hepatocellular carcinoma (HCC) *in vitro* and *in vivo*?

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85 **Literature review**

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87 Cancer trends are showing upward trends in many developing countries and a mixed pattern in
88 developed countries [1]. According to WHO, the cancer burden could reach 24 million cases
89 per year worldwide, with 17 million cases occurring in developing countries including Nigeria
90 by 2050. Death from cancer is expected to increase to 104% worldwide by 2020. The mainstay
91 of cancer treatment is by using chemotherapy and radiotherapy which themselves are toxic to
92 many viable cells of the body. Honey has recently been reported to have a potential anti-cancer
93 activity. It contains various kinds of phytochemicals with high phenolic and flavonoid content,
94 which contribute to its high antioxidant activity [2]. The phenolic contents of honey have been

95 reported to have anti-leukemic activity against different types of leukemic cell lines [5]. Its
96 anti-cancer activity has been proven against various cancer cell lines and tissues, such as
97 breasts, colorectal, renal, prostate, endometrial, cervical and oral cancer.

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100 **Theoretical framework**

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1. Identification of major constituent of the Nigerian honey

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2. Induction and treatment of liver cancer in rats

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3. Isolation and determination of genetic changes resulting from honey treatment of liver

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cancer

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4. Comparing honey with a conventional drug in liver cancer treatment

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5. Toxicological evaluation of Nigerian honey for possible drug development

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109 System biology are valuable frameworks for understanding the regulatory processes in cancer.

110 Thus, investigating the cellular and molecular regulatory mechanisms elicited by honey in killing

111 cancer cells, and understanding the underlying pathways can lead to the use of the honey or its

112 polyphenols at clinical level for effective treatment of many types of human cancer in the future

113 and ultimately prevent cancer development.

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116 **Research methodology**

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118 *Animal Experiment:* This study will be done by a randomized study and will be conducted at

119 Bauchi State University, Gadau (BASUG). The study protocol will be reviewed for design as

120 well as ethical and sensitive issues and will be applied from the BASUG Animal Research

121 Ethics Committee before study initiation.

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123 *Standardization and Phytochemistry of Honey*; Nigerian Honey to be subjected to chemical analysis
124 of its constituent and it will be standardized to cover a guaranteed quantity of a certain
125 constituent to be used during the study.

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127 *Induction of Cancer*: All 82 rats will be induced with cancer by intraperitoneal injection of
128 200mg/kg Diethyl Nitrosamine (DEN) dissolved in corn oil and then will be followed by a
129 recovery of 2 weeks on food, which will be mixed with 2-Acetylaminofluorene (0.02% AAF)
130 as promoter of hepatocarcinogenesis.

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132 *Chemotherapy*: A single dose of Doxorubicin HCl (30mg/kg body weight of rats) will be
133 injected intraperitoneally.

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135 *In vitro study*: Glucocorticoid receptors of HepaRG™ Cell cultures as well as CYP450 of the
136 cells will be analyzed using MRM analysis on an AB SCIEX Triple Quad™ or QTRAP®
137 system.

138 *Serum analysis*: GPX, AST, ALT, FBS, TC, TG, LDL, & HDL, Total antioxidant status (TAS),
139 and HbA1C will be analyzed using Cobas autoanalyzer.

140 *Total glucocorticoid receptors*: Fluorescent in situ hybridization (FISH) will be used to analyze
141 glucocorticoid receptor RNA activity by QuantiGene® ViewRNA ISH Tissue 2-Plex Assay
142 kit (Affymetrix® Inc, USA).

143 *CYP450 Protein Assay*: The CYP450 Protein Assay will be done using an LC/mass
144 spectrometry workflow to enable direct measurement of protein expression changes of
145 individual CYP450 isoforms with high specificity, sensitivity, and accuracy.

146 *Statistical analysis:* Data will be expressed as Mean±SEM using Statistical Package for Social
147 Sciences (SPSS V. 21) and R/Bioconductor. Statistical differences between treated and control
148 groups will be determined.

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150 **Expected results**

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152 ***1. Novel theories/New findings/Knowledge***

153 This study would provide invaluable information to better understand the use of Nigerian
154 honey in liver cancer treatment and to formulate a more effective therapy for patients
155 with liver cancer. It is therefore expected that this research studies will yield positive
156 results in cancer prevention and will explore a way of finding treatment measures for
157 hepatocellular carcinoma. In addition, the chemistry of honey will comprehensively be
158 understood.

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160 ***2. Research Publications***

161 The outcome from this study will be published in a high-impact factor peer-reviewed
162 scientific journal. At least, three scientific papers will be produced and published.

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164 ***3. Number of PhD and Masters (by research) Students***

165 This project is expected to train 1 Master and 1 PhD student.

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168 **Innovation**

169 Generally, honey is documented to have played a significant role in cancer chemoprevention
170 and therapeutics. This study could reveal the synergistic effect and positive or negative
171 consequences of Nigerian honey on hepatocellular carcinoma. The results of this study will

172 lead to curing of liver cancer as well as highlight new aspects of liver cancer via glucocorticoids
173 and its cell receptors.

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176 **Reference**

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