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Research Article

Protective Effect of *Curcuma Longa-Honey* and *Allium Sativum-Zingiber Officinale*-Honey Combinations on Hematological Parameters in Rats Induced with Paracetamol Toxicity

Oladele Habeeb Temitope[1], Abubakar Fatimah Aluko[1], Oladosu Micheal Abimbola[2], Moses Adondua Abah[3]*, Nnanyereugo Chinelo Emmanuella[4], Ekele Jiata Ugwah[5], Taiwo Adeolu Dele-Osibanjo[6], Kevin Emmanuel Amuchechukwu[7], Shinggu Paula Paul[8], Adebayo Biola Rasheedat[9], Aliyu Najeeb Olamilekan[10], Ajala Lawal Rafiat Ayanbukola[11], Ifeanyi Favour Chidindu[3] and Ekele Angel Ojimaojo[3]

[1]*Department of Biochemistry, Faculty of Life Sciences, University of Ilorin, Ilorin, Nigeria*

[2]*Department of Biochemistry, Faculty of Basic Medical Sciences, University of Lagos, Akoka, Nigeria*

[3]*Department of Biochemistry, Faculty of Biosciences, Federal University Wukari, Nigeria*

[4]*Department of Pharmaceutics and Pharmaceutical Technology, Faculty of Pharmaceutical, Sciences, University of Port-Harcourt, Choba, Nigeria*

[5]*Biological and Environmental Sciences, Liverpool John Moores University, Byrom Street, Liverpool, L3 3AF United Kingdom*

[6]*Department of Biochemistry, College of Agricultural and Natural Sciences, University of Nebraska-Lincoln, Lincoln NE, USA*

[7]*Department of Pharmacology and Toxicology, Faculty of Pharmaceutical Sciences, University of Nigeria, Nsukka, Nigeria*

[8]*Department of Microbiology, Faculty of Biosciences, Federal University Wukari, Nigeria*

[9]*Department of Biochemistry, Faculty of Basic Medica-l Sciences, University of Lagos, Nigeria*

[10]*Department of Medical Biochemistry, College of Health Sciences, Nile University of Nigeria, FCT, Nigeria.*

[11]*Dept of Medical Biochemistry, Nile University of Nigeria, F.C.T, Nigeria*

***Corresponding author:** Moses Adondua Abah, Department of Biochemistry, Faculty of Biosciences, Federal University Wukari, Nigeria.

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Abstract

Over time, advancements in the medical sector led to the gradual replacement of natural medicines with synthetic alternatives, because the former also had some negative effects. Even though synthetic medications benefit mankind when used appropriately, many medications have severe side effects. This study evaluated the protective effects of *Curcuma longa*-honey and *Allium sativum-Zingiber officinale*-honey combinations on the hematological parameters of rats induced with paracetamol. Twenty rats with an average weight of 144.61±7.35g, which were randomly assigned into four groups with five rats in each group were used for the experiment. Group A was left uninduced and untreated, and received distilled water only, serving as the control. Group B was left untreated, Group C was pretreated with 200mg/kg of *Curcuma longa*-honey, and Group D was pretreated with 200mg/kg of *Allium sativum-Zingiber officinale*-honey. After the 14day treatment, the rats were induced with hepatotoxicity using paracetamol, three hours after the last administration. Forty-eight hours after induction, the rats were sacrificed, and blood samples were collected for biochem-

ical analysis of hematological parameters using the SYSMEX automated hematological analyzer. The groups pretreated with 200mg/kg of *Curcuma longa*-honey (Group C) and 200mg/kg of *Allium sativum-Zingiber officinale*-honey (Group D) showed significant increase in the WBC levels, however, the control group administered distilled water showed no significant alteration in the hematological parameters in comparison to the treatment groups. The trend observed across the treatment groups analysed shows that Curcuma longa-honey and *Allium sativum-Zingiber officinale*-honey combinations possess immunomodulatory properties.

Keywords: Honey, Rats, Paracetamol, Toxicity, Blood

Abbreviations: WBC: White Blood Cell, NEU: Neutrophil, LYMPH: Lymphocyte, EOS: Eosinophil, MON: Monocyte, RBC: Red Blood Cell, HB: Hemoglobin, PCV: Packed Cell Volume, MCV: Mean Cell Volume, MCH: Mean Cell Hemoglobin, MCHC: Mean Cell Hemoglobin Concentration, NAC: N-acetylcysteine, ALF: Acute Liver Failure.

Introduction

Herbal treatments and traditional medicines have long been used to treat a wide range of illnesses in many countries, both developed and developing [1]. Native American midwives, healers, and herbalists, who were primarily women, treated a wide range of illnesses using just herbal remedies or spices [2,3]. Over time, advancements in the medical sector led to the gradual replacement of natural medicines with synthetic alternatives, because the former also had some negative effect s. Even though synthetic medications benefit mankind when used appropriately, many medications have severe side effects [4]. Paracetamol (acetaminophen) is one of the most widely used medicines worldwide and is readily available without prescription in most countries. It is listed on the World Health Organizations' (WHO) Essential Medicines List [5]. It is recommended as a first-line treatment for most cases of pain and fever and is safe to use in children as young as one-month old as well as women who are pregnant. It comes in a variety of forms and strengths including oral tablets, capsules, and liquid formulations as well as rectal suppositories [6]. Compared to other analgesics accessible without a prescription, paracetamol has a relatively narrow therapeutic index [7]. Paracetamol is one of the most used drugs as an analgesic and antipyretic, it is a structural analog of phenacetin, which was withdrawn due to concerns for nephrotoxicity [8]. Toxicity can occur following dosing errors, accidental exploratory ingestions in children, and deliberate self-harm overdose [9]. Paracetamol can cause severe hepatotoxicity with as little as 10g (or 200 mg/kg for patients under 50 kg) in an acute overdose. Repeated supratherapeutic ingestion can cause toxicity in doses only slightly above the maximum daily therapeutic dose [10]. N-acetylcysteine (NAC) is an effective antidote to paracetamol; however, despite this, paracetamol toxicity is still the leading cause of Acute Liver Failure (ALF) in most developed countries. Severe cases may require liver transplantation or result in death [11].

Turmeric (*Curcuma longa*) is renowned for its potent anti-inflammatory and antioxidant properties [12]. Curcumin, the active component of Curcuma longa, has been shown to mitigate liver damage by scavenging free radicals and enhancing the activity of endogenous antioxidant enzymes [13,14]. Honey, with its rich composition of phenoli c compounds, also exhibits significant antioxidant and anti-inflammatory effects, which can contribute to hepatoprotection [15,16]. Similarly, garlic (*Allium sativum*) and ginger (*Zingiber officinale*) are widely recognized for their medicinal properties. *Allium sativum* contains allicin, which has been demonstrated to protect against oxidative stress and reduce liver inflammation [17]. *Zingiber officinale* contains bioactive compounds such as gingerols and shogaols, which also exhibit antioxidant and anti-inflammatory activities that can protect against liver damage [18,19].

Despite the promising hepatoprotective properties of these natural substances, there is limited research on the combined effects of *Curcuma longa* and *Allium sativum/Zingiber officinale* with honey in the context of paracetamol-induced liver toxicity. Given the potential for synergistic effects, it is imperative to investigate whether these combinations can provide enhanced protection against liver damage. Therefore, this research aimed at addressing the lack of comprehensive data on the protective effect of *Curcuma longa* and *Allium sativum/Zingiber officinale* with honey combinations on hematological parameters following paracetamol-induced toxicity. By assessing the protective effects of these natural remedies through detailed hematological examination, this research seeks to identify effective and accessible alternatives for managing paracetamol-induced hepatotoxicity, thereby contributing to the development of safer therapeutic strategies for liver protection.

Materials and Method

Drugs and Reagents Used

Paracetamol tablets, a product of May & Baker, Nigeria Plc., 3/5 Sapara Street, Industrial Estate, P.M.B. 21049, Ikeja, Lagos, were obtained from a reputable Pharmacy in Ilorin, Kwara State, Nigeria (Batch Number – A231749). The remaining reagents utilized were of analytical quality, prepared using distilled water, and stored in airtight reagent bottles.

Sample Collection

Curcuma longa rhizome was obtained from Ipata market in Ilorin South local government, Kwara state, Nigeria. Zingiber officinale rhizomes and Allium sativum bulbs were purchased from Mandate market in Ilorin West local government, Kwara state, Nigeria around Jan. 2024. The plants were authenticated at the herbarium of the Department of Plant Biology, University of Ilorin, Nigeria.

Pure honey was obtained from a local market in Ilorin South, Kwara State, Nigeria and stored at room temperature.

Experimental Animals

Twenty (20) adult Wistar (albino) rats were obtained from the Animal House of the Department of Biochemistry, Faculty of Life Sciences, University of Ilorin, Ilorin, Nigeria. The rats were housed in well-aerated cages with sawdust as bedding. The rats were clinically healthy and were acclimatized to the standard laboratory conditions for 2 weeks before commencement of the experiment with free access to pellet (Top Feed Pellet Finisher plus) and clean water.

Sample Preparation

The fresh samples were peeled and air-dried in the shade at room temperature (25°C - 30°C). They were then ground into a fine powder and stored in airtight containers. 1gram of the dried Curcuma longa, *Allium sativum* and *Zingiber officinale* powders were weighed and mixed with 10 ml of pure honey in a sample bottle. The mixture was thoroughly mixed and heated at 40°C for 6 hours. Another 10 ml of pure honey was then added, mixed well, and heated again at 40°C for another 6 hours. To prepare the dosage, 6 ml of the Curcuma longa with honey mixture was diluted with 200 ml of distilled water to achieve a concentration of 200 mg/kg body weight. Additionally, a combination of 3 ml each of the Allium sativum with honey and Zingiber officinale with honey mixtures was diluted with 200 ml of distilled water to obtain a dosage of 200 mg/kg body weight.

Induction of Hepatotoxicity

To induce hepatotoxicity, the rats were given 200 mg/kg of paracetamol dissolved in distilled water on the 14th day, three hours after the final treatment. The rats were then monitored for 48 hours to allow the hepatic injury to develop.

Experimental Design

20 Wistar albino rats of average weight of 144.61 ± 7.35g were randomly divided into four groups (n=5 per group), groups A-D with each group containing five rats.

I. Group A: Neither pre-treated nor induced (normal control)

II. Group B: Induced but not treated (negative control)

III. Group C: Administered 200mg/kg bodyweight Curcuma longa-honey + induced.

IV. Group D: Administered 200mg/kg bodyweight *Allium sativum-Zingiber officinale*-honey combinations and induced with paracetamol.

Collection of Blood for Haematological Analysis

The rats were anesthetized using diethyl ether, the neck area was quickly cleared of fur, and the jugular veins exposed to reduce pain and distress during the sacrificing procedure. The rats were placed securely on a dissection board and the area around the jugular vein was sterilized and cleaned to prevent infection. A small incision was made over the jugular vein to expose it, blood was allowed to flow out into a container until the desired volume is obtained. EDTA was used as an anticoagulant to prevent blood clotting after collection.

Determination of Hematological Parameters

The method described by *Ayo, et al.,* [20] was used to estimate the haematological parameters assayed for in this work. The haemoglobin (Hb), Packed Cell Volume (PCV), Red Blood Cell Count (RBC), Mean Corpuscular Haemoglobin (MCH), Mean Corpuscular Haemoglobin Concentration (MCHC), Mean Corpuscular Volume (MCV), Neutrophils (NEU), Lymphocyte (LYMPH), White Blood Cell Count (WBC), and Platelet Count (PLC), were determined using automated haematological analyzer, SYSMEX-K21 (SYSMEX-K21 Corporation, Japan).

Principle: Whole blood samples are used for the analysis of all parameters on the machine. The machine uses cell packs which functions as a detergent and self-rinse system to avoid the introduction of errors and a stromalizer that works on the cells. The stromalizer counts the red cell and lyses them thereby releasing the hemoglobin and estimates its concentration using photometric analysis. The machine assumes that all nucleated cells are white and therefore counts all as white cells into their different forms i.e. lymphocytes and neutrophils but will not differentiate between eosinophils, monocytes, and basophils, and as such, they are recorded as mixed. It lyses the white blood cells based on the size of the nucleus and counts the number of white cells.

Statistical Analysis

Data were presented as means of ± Standard Error of the Mean (SEM). All the data obtained were analysed using GraphPad prism v8.0.1by GraphPad Software, 225 Franklin Street, Fl. 26, Boston, MA 02110 and IBM Statistical Package for Social Sciences (SPSS) version 27.0 by SPSS Inc., Chicago, Illinois, USA and the significance of difference between each group were analysed by one-way Analysis of variance (ANOVA) followed by Duncan's multiple range post hoc test. The significant differences between the means were determined at $p < 0.05$ (95% confidence interval).

Results

Effects of *C Longa*-Honey on Selected Haematological Parameters

Statistical analysis indicated a significant increase ($p<0.05$) in WBC counts of the treated paracetamol induced rats when compared to those untreated as indicated by distinct superscripts in Table 1. The Red Blood Cell Counts (RBC) and other erythrocyte hematological parameters (Hemoglobin, Mean Cell Volume, Mean Cell Hemoglobin, Mean Cell Hemoglobin Concentration, and Packed Cell Volume) were not significantly ($p>0.05$) altered as indicated by uniform superscripts across the data in Table 2 (Tables 1-4).

Table 1: White blood cell counts in the blood of rats pretreated with *C. Longa*-honey in paracetamol-induced hepatotoxicity.

Groups	WBC	NEU %	LYMPH %	EOS %	MON %
Normal control	4.00±0.50[b]	31.00±1.00 [a]	67.00±3.00 [a]	2.00±0.00 [a]	2.00±0.00 [a]
Negative control	1.80±0.30[c]	42.00±6.00 [a]	58.00±6.00 [a]	0.00±0.00 [b]	0.00±0.00 [b]
200mg/kg bw CLH	7.85±0.05 [a]	30.00±10.0 [a]	70.00±10.0 [a]	0.00±0.00 [b]	0.00±0.00 [b]

Note*: *Values are expressed in mean ± standard error of mean (SEM), n=2 animals in a group

CLH = *C. Longa*-honey; WBC = White blood cell, NEU = Neutrophil, LYMPH = Lymphocyte, EOS = Eosinophil; MON = Monocyte.

Table 2: Red blood cell counts in the blood of rats pretreated with *C. Longa*-honey in paracetamol-induced hepatotoxicity.

Groups	RBC $\times 10^{12}$/L	HB (g/dl)	PCV (%)	MCV (fl)	MCH (Pg)	MCHC (g/dl)
Normal control	6.8±0.05 [a]	13.7±0.35 [a]	41.0±1.00 [a]	60.3±0.55 [a]	20.2±0.25 [a]	33.3±0.04 [a]
Negative control	8.9±1.36 [a]	14.0±2.80 [a]	45.5±4.50 [a]	51.8±2.95 [a]	15.7±0.75 [a]	30.5±3.15 [a]
200mg/kg bw CLH	6.9±0.49 [a]	12.1±0.90 [a]	40.0±1.00 [a]	57.4±2.65 [a]	15.6±0.65 [a]	27.7±0.40 [a]

Note*: *Values are expressed in mean ± standard error of mean (SEM), n=2 animals in a group

CLH = *C. Longa*-honey, RBC = Red Blood Cell, HB = Hemoglobin, PCV = Packed Cell Volume, MCV = Mean Cell Volume, MCH = Mean Cell Hemoglobin, MCHC = Mean Cell Hemoglobin Concentration

Table 3: White blood cell counts in the blood of rats pretreated with *A. Sativum-Z. Officinale*-honey in paracetamol-induced hepatotoxicity.

Groups	WBC	NEU %	LYMPH %	EOS %	MON %
Normal control	4.00±0.50[b]	31.00±1.00 [a]	67.00±3.00 [a]	2.00±0.00 [a]	2.00±0.00 [a]
Negative control	1.80±0.30[c]	42.00±6.00 [a]	58.00±6.00 [a]	0.00±0.00 [b]	0.00±0.00 [b]
200mg/kg bw AZH	7.75±0.15[a]	24.50±0.50 [a]	75.50±0.50 [a]	0.00±0.00 [b]	0.00±0.00 [b]

Note*: *Values are expressed in mean ± standard error of mean (SEM), n=2 animals in a group

AZH = *A. sativum-Z. officinale*-honey; WBC = White blood cell, NEU = Neutrophil, LYMPH = Lymphocyte, EOS = Eosinophil; MON = Monocyte.

Table 4: Red blood cell counts in the blood of rats pretreated with *A. Sativum-Z. Officinale*-honey in paracetamol induced-hepatotoxicity.

Groups	RBC $\times 10^{12}$/L	HB (g/dl)	PCV (%)	MCV (fl)	MCH (Pg)	MCHC (g/dl)
Normal control	6.8±0.05 [a]	13.7±0.35 [a]	41.0±1.00 [a]	60.3±0.55 [a]	20.2±0.25 [a]	33.3±0.04 [a]
Negative control	8.9±1.36 [a]	14.0±2.80 [a]	45.5±4.50 [a]	51.8±2.95 [a]	15.7±0.75 [a]	30.5±3.15 [a]
200mg/kg bw AZH	6.5±0.35 [a]	13.2±0.85 [a]	40.5±2.50 [a]	60.8±0.05 [a]	20.3±0.10 [a]	33.3±0.05 [a]

Note*: *Values are expressed in mean ± standard error of mean (SEM), n=2 animals in a group

AZH = A. Sativum-Z. Officinale-honey, RBC = Red Blood Cell, HB = Hemoglobin, PCV = Packed Cell Volume, MCV = Mean Cell Volume; MCH = Mean Cell Hemoglobin; MCHC = Mean Cell Hemoglobin Concentration

Discussion

Over time, advancements in the medical sector led to the gradual replacement of natural medicines with synthetic alternatives, because the former also had some negative effects [21]. Even though synthetic medications benefit mankind when used appropriately, many medications have severe side effects [22]. The present study evaluated the protective effects of *Curcuma longa*-honey and *Allium sativum-Zingiber officinale*-honey combinations on the hematological parameters of rats induced with paracetamol. The significant increase in WBC counts among the groups, particularly in the *Curcuma longa*-honey and *Zingiber officinale-Allium sativum*-honey groups ($p < 0.05$) suggests that these treatments may enhance immune responses, potentially providing protective effects against liver injury. Increased WBC counts are indicative of an immune reaction, possibly due to the immunomodulatory properties of *Curcuma longa* and *Zingiber officinale*, which have been shown to influ-

ence immune cell activity [23]. The response could also be due to the modulation of inflammatory pathways and oxidative stress reduction, common effects associated with these natural compounds.

Despite the overall increase in WBC, the percentages of neutrophils (NEU%), lymphocytes (LYMPH%), monocytes (MON%), and eosinophils (EOS%) did not significantly differ across the groups ($p > 0.05$). This stability suggests that the *Curcuma longa*-honey and *Zingiber officinale-Allium sativum*-honey treatments did not specifically alter the proportions of these immune cells, maintaining a balanced immune response. Such findings align with previous studies indicating that natural antioxidants can enhance immune function without disrupting the immune cell balance.

The Red Blood Cell Count (RBC), Hemoglobin Levels (HB), Packed Cell Volume (PCV), Mean Cell Volume (MCV), Mean Cell Hemoglobin (MCH), and Mean Cell Hemoglobin Concentration (MCHC) did not show significant differences ($p > 0.05$) among the groups. This consistency suggests that the treatments did not adversely affect erythropoiesis or hemoglobin synthesis, maintaining normal hematological function. This finding is important, as it indicates that while these treatments might modulate immune responses, they do not impair red blood cell parameters, which is crucial for the overall health of the organism.

Conclusion

The findings from this study suggest that *Curcuma longa*-honey and *Zingiber officinale-Allium sativum*-honey combinations may enhance immune response in rats with paracetamol-induced liver injury, as evidenced by increased WBC counts. However, these treatments did not significantly alter the proportions of specific immune cells or affect other hematological parameters, such as RBC and HB levels. This indicates that these natural remedies can potentially support liver health and immune function without adversely impacting overall blood health. These results support the therapeutic potential of these natural products in managing liver injury and suggest avenues for future research to explore their clinical applications.

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Conflicts of Interest

The authors declared that there are no conflicts of interest.

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