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Original Research Article

Coconut Water and Milk Modulate Intravascular Haemolysis and Hepatotoxicity Via Oxido-Inflammatory Mechanism in Rats

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ABSTRACT

Haemolysis and its associated organ pathologies are predicated upon oxidative stress and inflammation, otherwise shown to be mitigated by complement natural products. The antioxidative and anti-inflammatory effects of coconut water (CCW) and milk (CCM) in phenylhydrazine (PHZ)-induced haemolysis and hepatotoxicity in the rat was evaluated, with possible adjunctive effects with dexamethasone (DXM). Anaemic rats were treated with CCW (2 mL/100 g), CCM (2 mL/100 g), DXM (0.01 mg/Kg), CCW+DXM, and CCM+DXM for 14 days post induction. Blood samples were collected for hematological parameters, lipid profiles and liver function tests while oxidative stress markers and inflammatory cytokines were assessed in liver homogenates. PHZ caused significant reduction in concentration of all the haematological parameters, with elevated serum aminotransferases. However, CCW and CCM alone, or in combination with DXM significantly attenuated the PHZ-induced haematotoxocity and hepatoxicity. PHZ's increase LDL and TG with decrease HDL were equally significantly reversed by CCW and CCM. Elevated tissue Malondialdehyde (MDA), Myeloperoxidase (MPO), Tumor Necrosis Factor (TNF-α), Interleukin 1beta (IL-1β) and Nuclear factor-kappa B (NF-kB) in response to PHZ were all mitigated by CCM and CCW, in favour of glutathione peroxidase (GPX), total superoxide dismutase (T-SOD) activities, total antioxidant capacity (T-AOC), Interleukin 10 (IL-10) and Nuclear factor erythroid 2-related factor 2 (NrF2). Histopathology revealed that only combination of CCM or CCW with DXM could distinctively reverse PHZ-induced alterations like macrovesicular steatosis and periportal inflammation. Phenylhydrazine induced toxicity is mitigated by either coconut water and milk or in combination with dexamethasone via oxidative stress reduction and inflammation suppression.

Keywords: coconut, haematotoxicity, hepatotoxicity, inflammation, antioxidants

Introduction

Phenylhydrazine (C6H5NHNH2; PHZ) is a redox derivative of hydrazine with wide applicability in chemical-based industries. Exposure to PHZ can be established via oral, inhalation, and dermal routes[1]. Its use as a pharmacological agent against polycythaemia vera qualifies it as a toxicological agent of haemolysis in healthy animals. Beyond its hematological toxicities, PHZ forms several intermediates such as phenyldiazene and heme adducts such as N-alkyl-protoporphyrin IX which act as free radicals ultimately damaging the liver through oxidative stress. Hence, PHZ has been used in several experimental conditions to induce both hematological and hepatological injuries in oxidative and inflammation-mediated pathophysiology.[2,3]

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The search for a reliable therapeutic alternative for systemic dysfunctions has become a continuum in life science mostly because established pharmacological agents have been plagued not only with high costs but also with undesirable side effects. Most traditions believe in the potencies of natural agents particularly for hematological-related complications. One of the groups of natural agents harnessed for therapeutic purposes in folkloric medicine is diets particularly those that have become staples. Coconut, (*Cocos nucifera*, L) typifies a staple food material extensively harnessed for refreshing and sports purposes.[4] Nutritionally, coconut and its derivatives such as oil, milk and water, are composed of several bioactive components such as polyphenols, enzymes, vitamins, growth-promoting factors, dodecanoic acid, and other saturated fatty acids such as palmitic acid, oleic acid, etc.[5] Other components include macromolecules such as protein, fat and carbohydrates; micromolecules such as calcium (Ca), Iron (Fe), Potassium (K), manganese (Mn), and Phosphorus (P). This, perhaps, is the reason why they have been widely acceptable, investigated, and harnessed in different continents of the world for therapeutic and prophylactic medicinal purposes with proven efficacies. They have been reported to be antioxidative[6,7], cardioprotective[8,9] and anti-inflammatory capacities.[10] In a set of studies from our laboratory which investigated gastric activities of coconut water and milk, we found that the coconut derivatives exhibit gastroprotection, restoring gastric homeostatic condition in gastro-compromised rats via inflammo-suppression,

angiogenesis, mucosa cell proliferation and upregulation of mucus cell population density.[11]

Despite the wide investigation of coconut and its derivatives, not much is known about its possible hepatic effects. Meanwhile, the liver, being the largest visceral organ in the body, is known for many life-dependent hematological and non-hematologically related functions. Detoxification, deamination and hematopoietic functions of the liver have made it further susceptible to xenobiotic-induced damage. In this study, we, therefore, investigated the therapeutic hepato-hematological potencies of coconut water and milk in hematologically and hepatologically compromised rats.

Materials and Methods

Drugs and chemicals

Dexamethasone (DXM) was bought from Sanofi Pharmaceuticals, Bridgewater, NJ, USA. Phenylhydrazine was purchased from Sigma Aldrich®, St Louis, MI, USA. All other laboratory chemicals and reagents used are of analytical grade, and procured from the UK.

Collection and Preparation of Materials

Fresh coconuts (*Cocos nucifera*) of twelve (12) months' maturity used for this study were obtained from the Okada market, 6.7331° N, 5.3913° E, Ovia North-East Local Government Area, Edo State between February and May, 2022 and authenticated at the University Herbarium Ado-Ekiti (UHAE) where a voucher specimen is deposited; UHAE 2023054. The coconuts were dehusked and prepared carefully to get the liquid endosperm. A stock solution of coconut milk was made by dissolving a grated mass of fresh coconut (50 g) in distilled water (500 ml) and heated (65°C). The oil layer was scooped and re-boiled (30mins). The crude milk formed was then allowed to cool at normal room temperature. Two milliliters of coconut water or milk per 100 g body weight was administered orally.[11]

Experimental Animals

A total of 35 male Wistar albino rats (weighing 150-180 g) were obtained from the Central Animal Facility of the Federal University, Oye-Ekiti, Nigeria. Studies on animal experimentation were done following the Current Animal Care Regulations and Standards approved by the Institute for Laboratory Animal Research (ILAR, 1996) and protocols (FUOYE/CHS/AEC/2022/14) approved by the Animal Ethics Committee of the College of Medicine, Federal University, Oye-Ekiti, Nigeria.

Induction of Haemolysis and Experimental Design

Phenylhydrazine was administered to induce haemolysis in rats following an oral administration at a dose of 20mg/kg body weight in 1 ml of distilled water for 4 days to achieve lower haematological concentration and haemoglobin (HGB) levels of PCV ≤ 30% and Hb ≤10 g/dl respectively. [12] Upon confirmation of development of anaemia, rats were randomly distributed into six experimental groups viz: Normal saline (NS), CCW (2 mL/100 g), CCM (2 mL/100 g) and DXM (0.01 mg/Kg) arranged in groups 2-5 respectively. Group 6 and 7 anemic rats received combined treatment of CCW+DXM, and CCM+DXM. Group 1 received no phenylhydrazine treatment. Blood samples were then collected by terminal bleeding from the heart following cervical dislocation for hematological parameters, lipid profiles and liver function tests after 14 days administration of CCM and CCW post anaemia induction. Oxidative stress markers, inflammatory cytokines, NF-kB and NrF2 were equally assessed in liver homogenates.

The phenylhydrazine-induced haemolysis, biochemical changes, and oxidative stress and inflammation parameters were assessed to evaluate the modulatory potentials of coconut water and milk on the haematopoietic and hepatic function in the treated rats.

Haematology

Full blood counts were determined using a fully automatic Haematology Analyser (YNH7021-China).

Biochemical Analysis

Serum triglyceride levels (TG) and liver function biomarkers; Alanine transaminase (ALT), Aspartase transaminase (AST) and Alkaline phosphatase (ALP) were estimated using commercial kits obtained from Randox Laboratories Ltd. (Crumlin, UK). Highdensity lipoprotein (HDL) was estimated according to the method of Swanson et al.[13] while serum low-density lipoprotein (LDL) was determined following the precipitation method described by Wieland and Seidel.[14]

Oxidative stress and inflammatory cytokines assay

Following sacrifice and blood collection, the whole liver was immediately fetched, cut in between right and left lobe. The right lobe, in turn, sliced into small pieces, washed with PBS and prepared to spin for 10 minutes in a homogenizer. The hepatic glutathione peroxidase (GPX) and total superoxide dismutase (T-SOD) activities, malondialdehyde (MDA) concentration, and total antioxidant capacity (T-AOC) level were analyzed in homogenized samples with the aid of their respective kits (Elabscience, USA) in compliance with the manufacturer's instructions. Tumor necrosis factorα (TNFα), interleukin1β (IL-1β), interleukin-10 (IL-10), Nuclear factor-kappa B (NF-kB) and Nuclear factor erythroid 2-related factor 2 (NrF2) levels were also analyzed with an ELISA kit (ElabScience, USA) following the manufacturer's protocol.

Histopathology

The left lobe of the liver samples taken after sacrifice were preserved in 10% formalin solution for 24 h and washed with 70% ethanol. Tissues were then prepared by automatic tissue processor and embedded in paraffin blocks. The paraffin blocks were sectioned at 6-micrometer, distributed onto glass slides and then dried. Slides were observed under a light microscope after being stained with hematoxylin and eosin (H&E) and cover-slipped. Three independent histopathologists assessed the level of damage and graded accordingly using a modified semiquantitative scale: normal = 0, mild = <25%, moderate = 25–50% and severe = >50% of observed area of view.[15]

Statistical Analysis

Data were presented as Mean±standard error of the mean (SEM) and analyzed by oneway analysis of variance (ANOVA) using Graphpad prism (version 5.0), followed by Bonferroni post hoc test. Statistical differences were reported significant at $^{*}p < 0.05$.

Results and Discussion

Pathogenesis of intravascular hemolysis involves inflammation alongside cellular events in the blood and blood vessels.[16] Due to the previously reported antioxidative and anti-inflammatory potentials of coconut owing to the presence of some organic and inorganic compounds[17,18], its actions during haematotoxicity and hepatotoxicity were investigated.

Haematological Indices:

Phenylhydrazine lowered ($P<0.05$) Red blood cell (RBC), Packed Cell Volume (PCV), White blood cell (WBC), Lymphocytes (LYM) and Platelet (PLT) by 51.5%, 50.0%, 52.0% and 46.0% and 50.5% respectively, when compared with the control (Figure 1, 3). Similarly, a significant reduction of Haemoglobin (Hb) content was recorded by 53.1% (Figure 2). However, coconut water, milk and dexamethasone administration elevated the levels of RBC, Hb, PCV, WBC, LYM and PLT significantly ($p<0.05$). Meanwhile, co-administration of either coconut water, or milk with dexamethasone yielded a significant elevation when compared with either coconut water or milk (Figure 1, 2, 3). In addition, percentage neutrophils which were increased upon phenylhydrazine administration, reduced significantly with either coconut water and milk, or in combination with dexamethasone (Figure 3). Red blood cells are destroyed faster during intravascular hemolysis[19], and consequently, some other hematological indices like Packed Cell Volume (PCV), and Haemoglobin (Hb) decrease.[20] One of the known mechanisms of this effect, especially the phenylhydrazine-induced haemolysis, is the initiation of oxidative stress within the erythrocytes which results in the oxidation of oxyhemoglobin causing the formation of methemoglobin.[21] Although,

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the half-life of PHZ stands at 3-4 hours, it is not clear whether this has a relationship with its toxic effects on the red cells. In this study, PCV, RBC, and Hb were markedly decreased which lends credence to the findings of previous workers on phenylhydrazine-induced haematotoxicity.[22] However, the values were elevated when treated with Dexamethasone or co-administered with coconut milk or water probably by scavenging the released Reactive Oxygen Species (ROS) present in the blood, blood vessels, and affected tissues like the liver. We believe that the observed elevation may also be connected with increased erythropoiesis as dexamethasone have the demonstrated such potential as reported by previous workers.[22] Neutrophils which account for more than half of the circulating white blood cells are part of the first-line immunity responder to inflammation[23] and must be tightly controlled for the normal functioning of the body.[24]

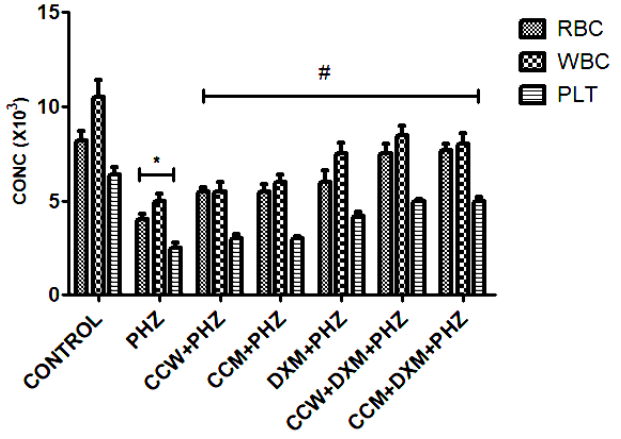

Figure 1: Effect of Coconut water and Milk on blood cell counts in normal and phenylhydrazine-treated male Wistar rats. Results are expressed as mean ± SEM, n = 5. *P < 0.05 when compared to control normal saline group. #P < 0.05 when compared to control phenylhydrazine group. RBC, Red blood cell; WBC, White Blood cell; PLT, Platelet; PHZ, phenylhydrazine; CCW, Coconut water; CCM, Coconut milk; DXM, dexamethasone.

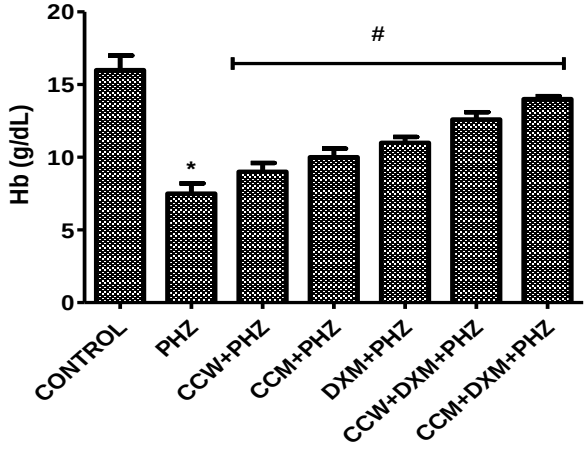

Figure 2: Effect of Coconut water and Milk on haemoglobin concentration in normal and phenylhydrazine-treated male Wistar rats. Results are expressed as mean ± SEM, n = 5. *P < 0.05 when compared to control normal saline group. #P < 0.05 when compared to control phenylhydrazine group. RBC, Red blood cell; WBC, White Blood cell; PLT, Platelet; PHZ, phenylhydrazine; CCW, Coconut water; CCM, Coconut milk; DXM, dexamethasone.

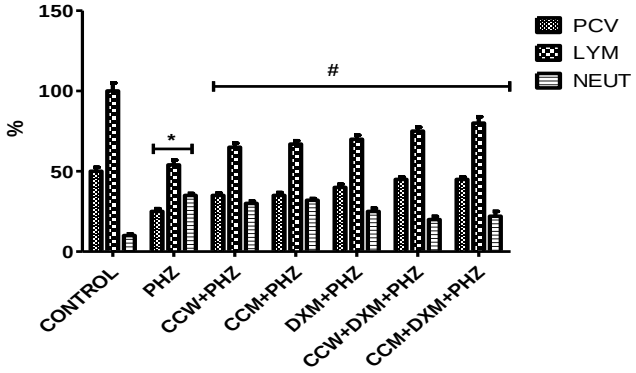

Figure 3: Effect of Coconut water and Milk on packed cell volume, %age Lymphocytes and Neutrophils in normal and phenylhydrazine-treated male Wistar rats. Results are expressed as mean ± SEM, n = 5. *P < 0.05 when compared to control normal saline group. #P < 0.05 when compared to control phenylhydrazine group. PCV, Packed cell volume; LYM, Lymphocyte; NEUT, Neutrophils; PHZ, phenylhydrazine; CCW, Coconut water; CCM, Coconut milk; DXM, dexamethasone

Table 1: Semi-quantitative grading of hepatic cytoarchitectural derangement in PHZ-induced toxicity upon treatment with Coconut water, milk and dexamethasone

Groups	% Damage
CONTROL	0.00
PHZ	65.50 ± 7.5*
CCW+PHZ	50.00 ± 5.0#
CCM+PHZ	50.50 ± 5.5#
DXM+PHZ	30.50 ± 2.5#
CCW+DXM+PHZ	25.50 ± 7.5#
CCM+DXM+PHZ	20.50 ± 5.5#

Results are expressed as mean ± SEM, n = 5. *P < 0.05 when compared to control normal saline group. #P < 0.05 when compared to control phenylhydrazine group. PHZ, phenylhydrazine; CCW, Coconut water; CCM, Coconut milk; DXM, dexamethasone.

Elevated levels of circulating Neutrophils suggest the existence of inflammation as it mediates the activation of nuclear factor-kappa B (NF-κB)[25], and lymphocytes regulate the release of inflammatory mediators that stimulates inflammation[23]. The low platelet count observed in the untreated animals is associated with liver injury[26,27] thereby suppressing the release of growth factors and other anti-inflammatory mediators. Strikingly, the level of neutrophils circulating in the blood was considerably lowered and that of WBC, Lymphocytes, platelets, and packed cell volume (PCV) increased in all the treatment groups. Although, the level of observed reversal was dependent on the product of the coconut administered with or without Dexamethasone with the most efficient being coconut milk with DXM.

Lipid Profiles:
Phenylhydrazine produced elevated serum total triglyceride level by 27.3% when compared with control group (Figure 5), which dropped significantly towards normal in the treated rats ($p<0.05$). Low density lipoprotein (LDL) level was increased in the phenylhydrazine treated rats with high density lipoprotein (HDL) level decrease by 33.3% and

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35.0% respectively. Phenylhydrazine+CCW+DXM offered a 19% and 41%, while Phenylhydrazine+CCM+DXM afforded 20% and 45% amelioration to LDL and HDL respectively (Figure 5). Irregularities in lipid profiles have been associated with the pathogenesis of several liver diseases due to the involvement of the liver in the metabolism of lipids and lipoproteins.[28,29] High-Density Lipoprotein (HDL) is one of the many biomarkers of liver functions that are always lowered in response to some conditions e.g. anemia, arterial hypotension, etc.[30,31] as evidenced in untreated group with hepatotoxicity. Moreover, the Low-Density Lipoprotein (LDL) and Triglycerides (TG) increased significantly indicating the compromised state of the liver. The presence of oxidants and pro-inflammatory cytokines probably interfered with lipids metabolism. Nevertheless, the administration of coconut water/milk with or without DXM alleviated this toxicity.

Liver Functions Parameters:

Phenylhydrazine caused increase (P<0.05) in serum ALT, AST and ALP levels by 147%, 172.5%, 117% respectively, when compared with control normal saline group (Figure 4). Phenylhydrazine+CCW, Phenylhydrazine+CCM and Phenylhydrazine+Dexamethasone, however, lowered ALT levels by (20.0%, 21.0% and 32%), AST (32.8%, 33.0%, 37.5%), ALP (31.4%, 32.5% and 35.0%) respectively, in rats. In addition, the co-administration of either coconut water or milk with dexamethasone offered a significantly lower of the hepatic enzymes when compared with dexamethasone alone. The liver's integrity can further be checked by the Liver Function Tests (LFTs) which include alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP). The elevation in the serum level of some of these liver enzymes can also stimulate inflammation.[32] While ALP is known to be non-liver specific, further studies need to be carried out in order to explore other liver enzymes. PHZ distorted the cytoarchitecture of the liver in correlation with the elevated liver enzymes (AST, ALP, and ALT) further confirming hepatic damage (Table 1). PHZ caused visible macrovesicular steatosis, periportal inflammation with relatively wide central vein and the sinusoidal inlet into the central venule. Nevertheless, coconut derivatives administered to animals enhanced recovery from this defect which is largely due to several nutrients and micronutrients acting positively on the cells,[33] and the co-administration with dexamethasone offered significant protection. Reduction in the initial rise of these enzymes was observed in all the treated groups with the most efficient being the animals administered with CCM+DXM. Many studies have also reported the hepatoprotective potential of *Cocos nucifera*.[6,34]

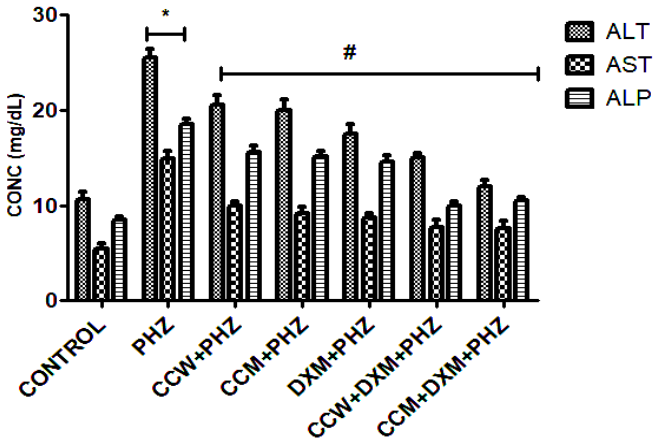

Figure 4: Effect of Coconut water and Milk on liver function in normal and phenylhydrazine-treated male Wistar rats. Results are expressed as mean ± SEM, n = 5. *P < 0.05 when compared to control normal saline group. #P < 0.05 when compared to control phenylhydrazine group. ALT, alanine aminotransferase; AST, aspartate aminotransferase;ALP indicates alkaline phosphatase; PHZ, phenylhydrazine; CCW, Coconut water; CCM, Coconut milk; DXM, dexamethasone.

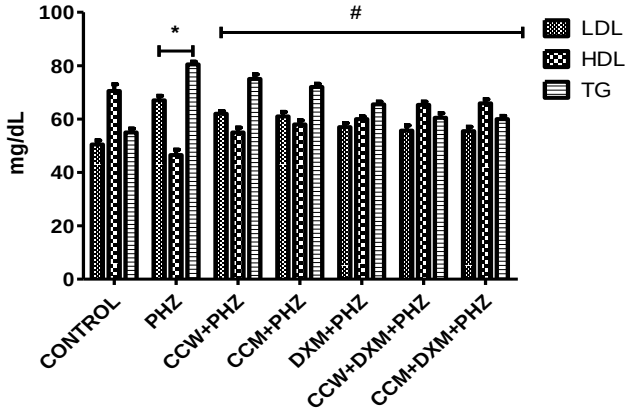

Figure 5: Liver profile in normal and phenylhydrazine-intoxicated rats upon treatment with Coconut water and Milk. Results are expressed as mean ± SEM, n = 5. *P < 0.05 when compared to control normal saline group. #P < 0.05 when compared to control phenylhydrazine group. LDL, Low density lipoprotein; HDL, High density lipoprotein; TG, Triglyceride; PHZ, phenylhydrazine; CCW, Coconut water; CCM, Coconut milk; DXM, dexamethasone.

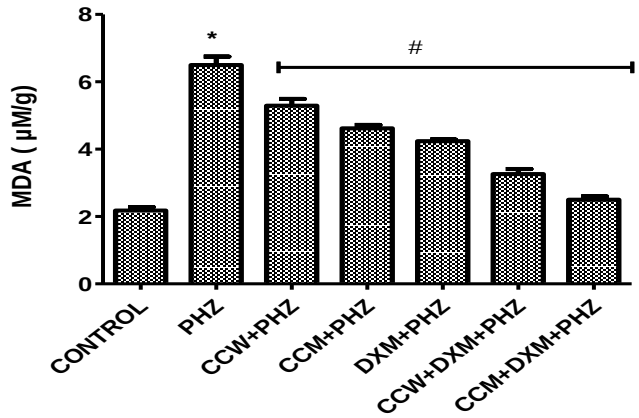

Figure 6: Lipid peroxidation in normal and phenylhydrazine-intoxicated rats upon treatment with Coconut water and Milk. Results are expressed as mean ± SEM, n = 5. *P < 0.05 when compared to control normal saline group. #P < 0.05 when compared to control phenylhydrazine group. LDL, Low density lipoprotein; HDL, High density lipoprotein; TG, Triglyceride; PHZ, phenylhydrazine; CCW, Coconut water; CCM, Coconut milk; DXM, dexamethasone.

Lipid Peroxidation, Antioxidant status and Cytokine level:

Results in Figure 6 showed that PHZ increased (*P*<.05) MDA levels in the liver by 198% when compared with control group. Phenylhydrazine + CCW, PHZ + CCM or PHZ+DXM ameliorated the hepatic MDA by 18.5%, 24.5%, 27.5% respectively when compared with control PHZ group. Meanwhile, DXM combination with CCW or CCM afforded greater amelioration of PHZ-induced lipid peroxidation than either DXM alone. Similarly, Figure 7 showed explicitly that CCW and CCM, alone, or in combination with DXM inhibited TNF-α and IL-1β elaboration in the PHZ-treated rats. Likewise, IL-10 earlier reduced in the PHZ-treated rats were elevated significantly. In comparison with the control group, the hepatic GPX , T-SOD, and the T-AOC activities which were all reduced (p <0.05) in the PHZ group increased upon treatment with CCW, CCM or DXM (Figure 8). Glutathione Peroxidase (GPx), Catalase, and Superoxide Dismutase

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are among the antioxidant systems in the body.[35] Phenylhydrazine depleted the antioxidant levels of the rats as seen in this study, similar to what has been reported previously by other workers.[36] Further, the antioxidative potentials of coconut water and milk[37,38] are demonstrated here as there was an elevation in the earlier depleted Total Superoxide Dismutase (T-SOD), Total Anti-oxidant Concentration (T-AOC), and Glutathione Peroxidase (GPx) levels in the treated groups.

Lipid peroxidation that occurs within the red blood cells (and probably the concerned tissues) has been imputed as one of the many effects of PHZ induction.[39] Experimental animals administered with PHZ had a high measurable malondialdehyde (MDA) which decreased markedly in treated animals. The reduction in lipid peroxidation in these treated groups suggests that the defense system of the body was enhanced by Dexamethasone, coconut derivatives, or both. Thus, the earlier reported potential of coconut water to scavenge free radicals was corroborated in this study.[40]

NF-kB and NrF2 Activation:

Moreover, phenylhydrazine produced an elevated level of NF-kB by 230% when compared with control group (Figure 9), which reduced significantly in all the treated rats. Phenylhydrazine+CCW+DXM and Phenylhydrazine+CCM+DXM produced a 52% and 46% decrease in the elevated NF-kB level observed in the PHZ treated only. Conversely, PHZ caused a 65% decrease in NrF2 level when compared with control group (Figure 10), while the Phenylhydrazine+CCW+DXM and Phenylhydrazine+CCM+DXM groups afforded 100% improvement over the PHZ treated only. Nuclear factor-kappa B (NF-κB), a proinflammatory transcription factor was implicated in the initiation of inflammatory responses with the capacity to activate more than 500 genes linked to inflammation.[41] NF-κB is the central modulator for inflammation in the body[42] and plays a critical role in the inflammatory signaling pathway.[43] There are two (2) major pathways in which NF-κB can be activated—canonical and non-canonical; however, the most studied one is the canonical pathway. Constitutive activation of canonical NF-κB pathway can be facilitated by cell surface receptors (Toll-like receptors, TNF-receptors, IL-1 receptors, etc.) which are the accompaniment of stimulated pro-inflammatory cytokines like Interleukin (IL-1β), and Tumour Necrotic Factor (TNF-α).[44] Overexpression of NF-κB, Interleukin (IL-1β), and Tumour Necrotic Factor (TNF-α) was observed in the PHZ control group of our study. TNF-α is a known initiator of inflammation in the liver.

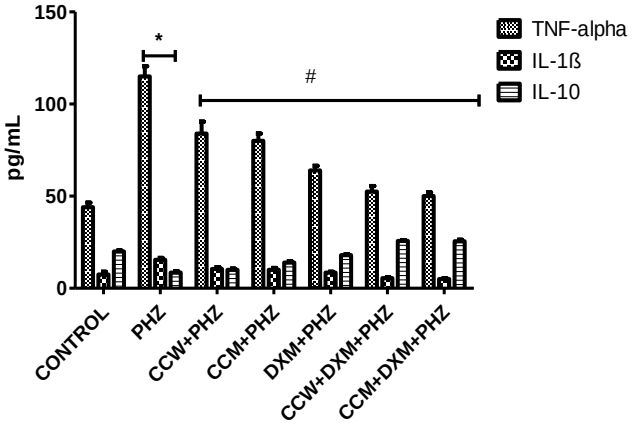

Figure 7: Pro- and anti-inflammatory cytokines level in normal and phenylhydrazine-intoxicated rats upon treatment with Coconut water and Milk. Results are expressed as mean ± SEM, n = 5. *P < 0.05 when compared to control normal saline group. #P < 0.05 when compared to control phenylhydrazine group. TNF-α, Tumor Necrosis Factor-alpha; IL-1β, Interleukin 1beta; IL-10, Interleukin-10; PHZ, phenylhydrazine; CCW, Coconut water; CCM, Coconut milk; DXM, dexamethasone.

Hence, the surge in the level of the expressed TNF-α is an indication that the inflammatory response was stimulated by Phenylhydrazine. Conversely, a member of T-helper cells (Th2) cytokines—IL-10 downturns the activation of NF-κB[42] and as shown in this study. Animals administered with coconut water/milk or co-administration with DXM showed an increase in the expression of IL-10 and a decrease in the expressed TNF-α and IL-1β which suggest that this pathway was engaged in establishing the anti-inflammatory effects. Worthy of note is Nuclear factor erythroid 2-related factor-2 (Nrf2), a transcription factor that controls the body's antioxidant systems and facilitates the production of antioxidants.[45] In this study, Nrf2 was richly expressed in animals treated with coconut derivatives and DXM.

Down-regulation of inflammation was obvious in the groups treated with Dexamethasone, coconut milk, coconut water, or combined. Administration of coconut water/milk undoubtedly activated the oxido-inflammatory signaling pathway by reducing the expression of NF-κB which subsequently up-regulated the expression of Nrf2 in the hepatocytes.

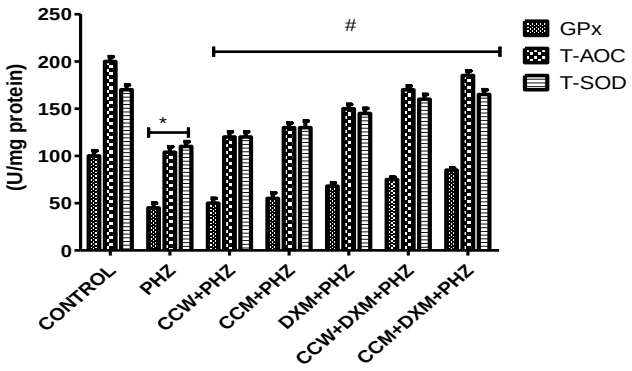

Figure 8: Antioxidant activities in normal and phenylhydrazine-intoxicated rats upon treatment with Coconut water and Milk. Results are expressed as mean ± SEM, n = 5. *P < 0.05 when compared to control normal saline group. #P < 0.05 when compared to control phenylhydrazine group. GPx, Glutathione peroxidase; T-AOC, Total antioxidant concentration; T-SOD, Total superoxide dismutase; PHZ, phenylhydrazine; CCW, Coconut water; CCM, Coconut milk; DXM, dexamethasone.

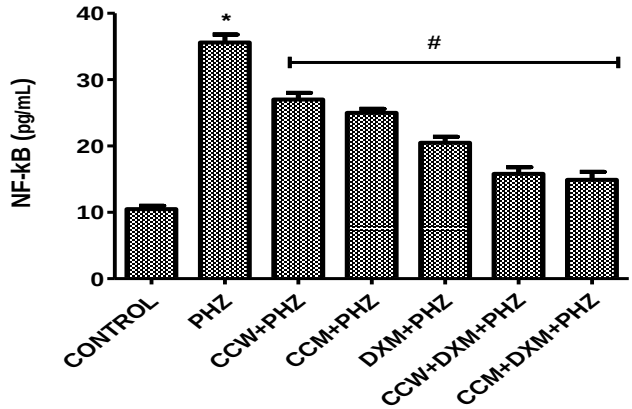

Figure 9: Effect of Coconut water and Milk on Nuclear factor-kappa B (NF-kB) in the liver in normal and phenylhydrazine-treated male Wistar rats. Results are expressed as mean ± SEM, n = 5. *P < 0.05 when compared to control normal saline group. #P < 0.05 when compared to control phenylhydrazine group. PHZ, phenylhydrazine; CCW, Coconut water; CCM, Coconut milk; DXM, dexamethasone.

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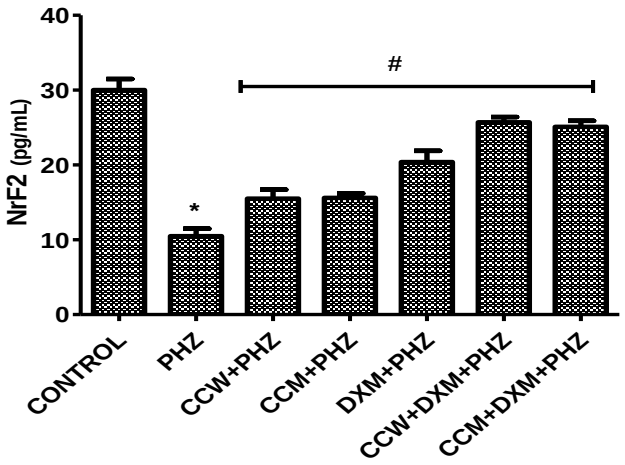

Figure 10: Effect of Coconut water and Milk on Nuclear factor erythroid 2-related factor 2 (NrF2) in the liver in normal and phenylhydrazine-treated male Wistar rats. Results are expressed as mean ± SEM, n = 5. *P < 0.05 when compared to control normal saline group. #P < 0.05 when compared to control phenylhydrazine group. PHZ, phenylhydrazine; CCW, Coconut water; CCM, Coconut milk; DXM, dexamethasone.

The reduced expression of NF-κB is predicated on the low circulating inflammatory cells (e.g. Neutrophils, Lymphocytes), inhibition of further release of pro-inflammatory cytokines (TNF-α and IL-1β), increased expression of IL-10 and up-regulation of Nrf2. This pathway resonates with the coconut mechanism of action already reported by several workers.[40,46]

Conclusion

Intravascular hemolysis and associated liver damage induced by phenylhydrazine were overturned by coconut water/milk with or without Dexamethasone. These observations are linked with various pathways with two major highlights—antioxidative and anti-inflammatory paths. Coconut milk had a higher rate of healing than coconut water; likewise their coupling with Dexamethasone. A synergistic effect between coconut water/milk with Dexamethasone is appreciated in the whole study. Derivatives of coconut used, specifically coconut milk, enhanced the antioxidant activities and anti-inflammatory capacity of Dexamethasone to mitigate the toxicity in the liver. Hence, coconut milk (and coconut water) has the potential to be an effective adjunctive in the treatment of haematotoxicity and hepatotoxicity.

Conflict of Interest

The authors declare no conflict of interest.

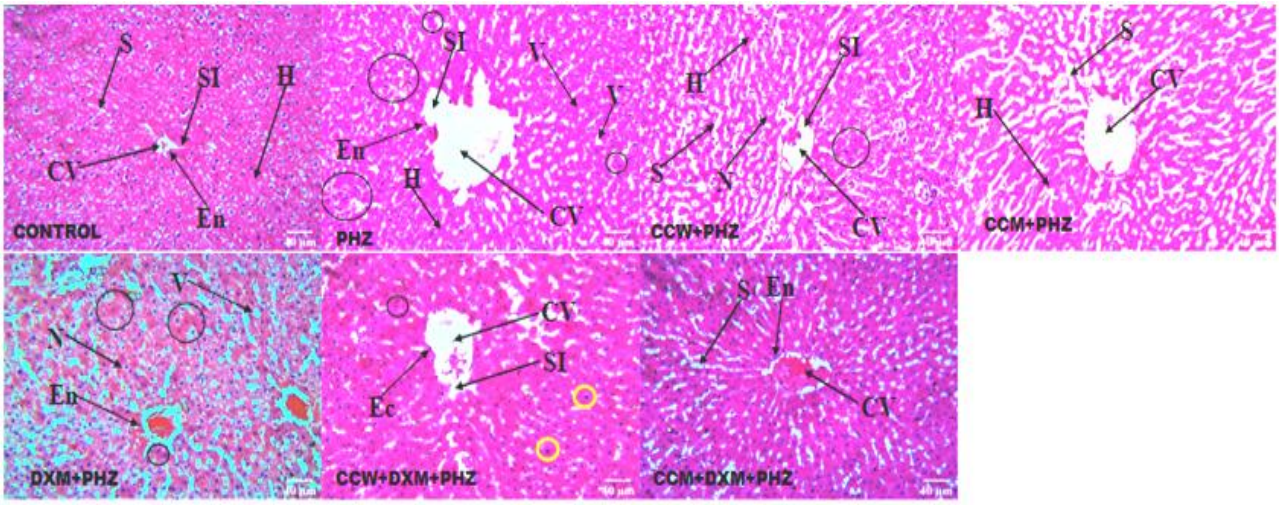

Plate 1: Photomicrograph of phenylhydrazine-intoxicated liver sections treated with Coconut water and milk with or without dexamethasone .

CONTROL: Cords of hepatocytes (pink area) with prominent basophilic nuclei (blue dots) and radiating sinusoid (S). The central venule endothelium (En) is simple squamous with prominent flat nuclei. PHZ: The central vein and the sinusoidal inlet into the central venule are relatively wide. Some nuclei appear dark and shrunken, marked nuclear crowding (large black circle) and small-droplet steatosis (V), binucleated cells are also seen (small black circle). Hepatocytes (H) and sinusoids (S) retain their respective cordlike and radiating appearance. CCW+PHZ: Some nuclei appear dark and shrunken with perinuclear halos (N) and marked nuclear crowding (black cicle). CCM+PHZ: Nucleus is barely seen in this group (H), other features appear normal. DXM+PHZ: Increased eosinophilia, pyknosis (black circle), small-droplet steatosis, central venule congestion, binucleated cells are seen. CCW+DXM+PHZ: focal increased eosinophilia, hyperchromatic nuclei and binucleated cells. CCM+DXM+PHZ: Congested central venule. Other features appear intact.
Scale bars - 40µm, light microscopy, captured field – pericentral, H and E – Hematoxylin and Eosin.

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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