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RESEARCH ARTICLE

HEPATOTOXICITY EFFECTS OF PARACETAMOL-DENIK CLEANSER® CO-ADMINISTRATION

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Abstract

Aim and Objective: The concurrent use of herbal products with orthodox medicine is on the rise with the risk of herb-drug interaction that could be beneficial or harmful to the body. Paracetamol (acetaminophen) is an anti-pyretic and analgesic drug metabolized by CYP2E1 to give the major hepatotoxin, N-acetyl-p-benzoquinone imine (NAPQI). Denik cleanser® is an oral herbal preparation from plants part of *Occimum grattissimum*, *Colocynthis citrullus*, *Khaya ivorensi*. The aim of this study was to evaluate the hepatotoxic effects triggered by Denik® and paracetamol co-administration in rats. The study sought to mimic conventional usage of Denik cleanser® followed by ingestion of paracetamol, a likely scenario given the popularity of both compounds.

Method: Twenty animals were randomly assigned to four groups, the first group (control) received 0.3 mL distilled water, 2nd group received paracetamol 100 mg/kg, 3rd group received Denik cleanser® 2 mL/kg while the 4th group received both paracetamol and Denik cleanser® at 100 mg/kg and 2 mL/kg daily for 3 days after which biochemical and histological analysis were carried out.

Results: From histological analysis revealed that rats that received Denik®-only and Denik®/paracetamol (concomitantly) showed markedly distorted liver architecture compared to control indicating toxicity. Similarly, the biochemical analysis results showed a significant ($p < 0.05$) increase in AST and ALT for the Denik®/paracetamol group compared to the control group indicating a hepatotoxic event. A non-significant increase in ALP and GGT were also observed in the Denik cleanser® + paracetamol group. Enhanced metabolism of paracetamol by Denik cleanser® to NAPQI (hepatotoxic metabolite) is indicated as possible mechanism.

Conclusion: The results of this study demonstrated the *in vivo* potential for a herb/drug interaction involving paracetamol and Denik cleanser® resulting in liver injury. Therefore, caution is strongly advised against its casual, and non-medically supervised usage.

Keywords: Denik cleanser®, herb-drug interaction, hepatotoxicity, paracetamol.

INTRODUCTION

Evidences have shown that nutrients in foods, fruits, vegetables, and herbal supplements can affect the therapeutic activities of drugs by affecting their bioavailability through drug - food interactions. This can occur via interaction with the drug transporters, or drug-metabolizing enzyme systems, by formation of complexes, or modification in gastric emptying/pH¹. Increased use of herbal products and wide spread poly-pharmacy have enhanced the chances of drug interactions and adverse drug reactions (ADR)². Disease co-morbidity is one major reason for co-

administration of certain drugs with herbs, leading to increased risk of hepatotoxic effects of drugs. For instance, pre-existing liver disease has been identified as an essential risk factor for hepatotoxicity of certain drugs³. This has been observed in patients with viral hepatitis and tuberculosis co-infections who develop liver injuries due to co-administration of antiviral and anti-tuberculosis drugs⁴.

The presence of impurities and other hepatotoxins are also risk factors for hepatotoxicity of herbal preparations^{3,5}. Some herbs may affect drugs metabolized by cytochrome P450 enzymes and thereby impair the activity of cellular drug transporters and

glucuronidation pathways⁶. It has been shown that certain herbs have dose-dependent inhibition effect on CYP3A4 and reduces expression of P-glycoprotein, leading to drug accumulation and increased hepatotoxicity of co-administered drugs^{4,7,8}. Often, co-medication with herbs and synthetic drugs occur, predisposing drug-herb interaction at the hepatic cytochrome P450 (CYP) system^{8,9,10}.

Acetaminophen (Paracetamol) is a common antipyretic /analgesic drug which is easily obtained over-the-counter (OTC). It is metabolized by CPY3A4 to N-acetyl-p-benzoquinone imine (NAPQI) in the liver¹¹. NAPQI is produced in little amount at therapeutic doses, but overdosing of paracetamol, leads to increased production of NAPQI^{11,12} with consequent formation of conjugates with glutathione. The depletion of glutathione is the major reason for severe liver damage as it conjugates with NAPQI¹³. This depletion is the main reason for hepatotoxicity and necrosis^{13,14}. Paracetamol effectively relieves pain due to selective inhibition of cyclooxygenase-3 (COX3) in the central nervous system by interrupting the descending serotonergic pathways and to some extent blocking the activity of pain mediators (bradykinin, substance P)¹⁵. Therapeutic doses are safe and well tolerated, but large intake of the drug may cause hepatotoxicity¹⁶. Paracetamol is essentially metabolized in the liver. It is mostly changed by glucuronidation (40-60%) and sulphation (20-46%). Pharmacological inactive metabolites are formed, while less than 10% is oxidized to a toxic metabolite, i.e. N-acetyl-p-modulate the P-gp activity¹¹.

Herb- drug interactions involving paracetamol has been identified in the study by Ewing *et al.*, that investigated interaction between Cannabidiol-rich Cannabis (CBD) extract and paracetamol¹⁷. Data obtained demonstrated a potential for CBD extract/drug interactions resulting to hepatotoxicity¹⁷.

Denik cleanser[®] is a herbal oral preparation made from a blend of various parts and fruits of medicinal plants such as; *O. grattissimum*, *C. citrullus*, *Khaya ivorensis*. This product is claimed by the producers to possess important therapeutic effects such as antioxidant, fertility, weight loss, and immune boosting effects. It is claimed to be effective against indigestion and also in the reduction of cancer risk, blood purification, enhancing eye and skin health. Given the wide spectrum of pharmacological use of Denik cleanser[®] by the Nigerian populace, a possible co-administration of this herbal drug with paracetamol is inevitable. The aim of this study was to investigate the effect of co-administration of paracetamol and Denik cleanser[®] on the histology and biochemical parameters of the liver.

MATERIALS AND METHODS

Distilled water, methylated spirit (JHD, China), Xylene, Diethyl ether, 10% formalin, Denik cleanser[®] (Kayfahd herbaceuticals, Nigeria), Paracetamol 500mg tablets BP (Panadol[®] from GlaxoSmithKline). All other solvents and chemicals were of analytical grade.

The herbal formulation under study was obtained in August 2020, from a medicine store at Rumuokoro, East West Road, in Port Harcourt, Rivers State, Nigeria. The herbal formulation was manufactured by Kayfahd herbaceuticals, Abuja, Nigeria.

Animals

This study was conducted using an experimental design previously described by Ewing *et al.*,¹⁷ with little modifications. Twenty (20) healthy adult male rats (140±0.25 g) were used for the experiment. They were acclimatized in the animal house of the Department of Experimental Pharmacology and Toxicology, Faculty of Pharmaceutical Sciences, University of Port Harcourt, Nigeria for two weeks and fed *ad libitum* with standard feed (Broiler finisher- Guinea feeds) with free access to water before experiment. They were maintained under standard conditions of humidity and temperature. Animal ethics and proper handling methods were strictly adhered.

Animal experiment

The drugs were administered to the rats in the test group orally using an oral cannula with rubber tubing. Animals were randomly assigned to four groups (1-4) of five animals each. Group I consisted of the control group; animals who were given 0.3 mL of distilled water orally daily for 3 days. The group II animals received paracetamol orally at a dosage of 100 mg/kg daily for 3 days. Group III animals received Denik cleanser[®] orally at a dosage of 2 mL/kg daily for 3 days. Group IV animals received both paracetamol Denik cleanser[®] orally at a dosage of 100 mg/kg and 2 mL/kg respectively concurrently daily for 3 days. Animals were then fasted overnight on the third day of treatment and sacrificed under ether anesthesia on the fourth day. With this experimental setup, we sought to mimic conventional usage of Denik cleanser[®] followed by ingestion of paracetamol, a likely scenario given the popularity of both compounds.

Blood sampling and biochemical analysis

Blood samples were collected via cardiac puncture. The blood samples were collected by cardiac puncture and kept at a temperature of 4°C for 6 hours. The blood samples were then centrifuged at 3000 rpm for 10 minutes and used for biochemical analysis. In the present study, the liver function was evaluated with serum levels of glutamyl transferase (GGT), alkaline phosphatase (ALP), alanine amino transferase (ALT) and, aspartate amino transferase (AST) using commercial diagnostic kits (Randox laboratory kit, England).

Ethical issues

The protocol of this study is designed in accordance with the ethical principles of the International Committees for the Protection of Animal Rights Laboratory. This project was approved by Ethics Committee of the University of Port Harcourt, Rivers State, Nigeria (Ref No. UPH/798EC/8/2020).

Histopathological Analysis

Liver sections were fixed in 10 % formalin for 6-12 hours. They were processed and examines for histological changes at the college of health sciences Pathology Facility, Nigeria. Samples of the liver tissue were cut, about 3 mm by 3mm in size, with a sharp knife. Each tissue was then fixed with 10% formalin

for about 6-12 hours and subsequently dehydrated by passing the tissue slowly through ascending grades of absolute alcohol (50%, 70%, 90% and 95%) and lastly in the absolute alcohol (100%), for a period of 1-2 hours in each grade of alcohol. The tissue is cleared using xylene for about 1-2 hours twice. It is then placed in the molten paraffin wax at a constant temperature of 56–60°C in an oven or paraffin bath (changing it twice keeping for about 2 hours each time) for infiltration. The tissue is embedded by placing it in special L-shaped metal blocks filled with molten paraffin wax and after solidification, the L-metal blocks are removed. For microscopic study, extremely thin (5-15 µm) sections of the tissues were cut using a microtome. The tissues were then stained using haematoxylin-eosin, and the stained tissue on the slide is then mounted in Canada balsam under a cover slip and ready for examination. The microscopic examination

was carried out on the livers of both the control and treated groups.

Statistical analysis

Statistical analysis involved use of the Microsoft Excel. Data are expressed as the Mean ± SD. Statistics were performed using one-way Anova and *t*-tests. *p* values less than 5% were considered statistically significant ($p < 0.05$).

RESULTS

The organ-body weight index is recorded in Table 1. There was little reduction in organ-body weight index at $p < 0.05$. The microscopic structure of the liver depicted in Figure 1 to Figure 4 shows no abnormalities in the control and paracetamol-only groups.

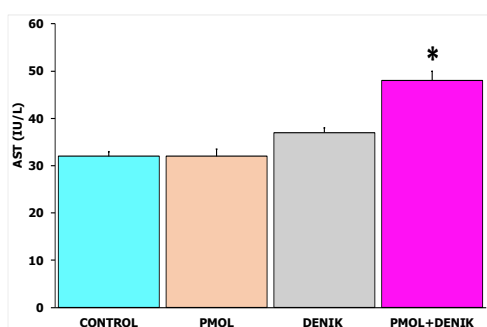


Figure 1: Effect of Paracetamol + Denik® interaction on AST.

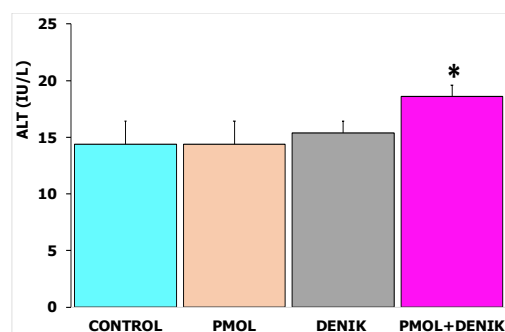


Figure 2: Effect of Paracetamol + Denik® interaction on ALT.

For the Denik®-only and paracetamol + Denik® groups, there was markedly distorted liver tissue showing hepatocytes with microvesicular steatosis and councilman's bodies (arrowed), patent central vein (CV) Results from biochemical analysis (Figure 1-Figure 4) revealed higher levels of liver function markers aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), and gamma-glutamyl transferase (GGT) enzymes in the paracetamol + Denik cleanser® group compared to the control group indicating liver toxicity. The elevation in ALT, AST and GGT in the paracetamol+Denik® group was statistically significant at $p < 0.05$.

DISCUSSION

Emerging evidence indicates that certain herbs pose a significant risk for hepatotoxicity^{3,9,10}. Liver damage is associated with cellular necrosis, increase in tissue lipid peroxidation and depletion in the tissue glutathione (GSH) level¹⁰. In addition, serum levels of many biochemical markers like serum glutamic-oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT), triglycerides, cholesterol, bilirubin, alkaline phosphate (ALP) are also elevated with liver damage¹⁸. Therefore, biochemical abnormalities and histological characteristics, in association with clinical presentation, help to define the pattern of a drug or substance-induced liver injury. Paracetamol is metabolized by a

cytochrome P450 enzyme known as CYP2E1¹⁷. Certain herbs have been shown to induce CYP2E1 resulting in enhanced production of *N*-acetyl-para-benzo-quinone imine (NAPQI) which is a hepatotoxin^{17,19}. In a study by Zendulka *et al.*, Cannabidiol from *Cannabis sativa* (commonly used for its anti-seizure activity) was shown to induce drug interactions involving the modulation of various cytochrome P450 enzymes responsible for paracetamol metabolism²⁰. Paracetamol is a common analgesic medication. When paracetamol is administered using its indicated dosage, the risk of adverse effects is low. However, when the therapeutic dosage range is exceeded, paracetamol toxicities emanate¹⁷. The most serious adverse reaction associated with paracetamol is hepatotoxicity attributable to a major metabolite known as *N*-acetyl-p-benzoquinone imine (NAPQI)²¹. Denik cleanser® is an oral herbal preparation obtained from *O. grattissimum*, *C. citrullus*, *Khaya ivorensi*. It is claimed by the manufactures to boost immunity, aid digestion and to treat a wide array of ailments. Generally, with an increase in the utilization of herbal remedies for different conditions, the concomitant administration of herbal medicines with conventional drugs might be inevitable¹⁹ and this has created a need to consider possible herbal-drug interactions. From current study, data obtained revealed no histological changes in the paracetamol-only group (control).

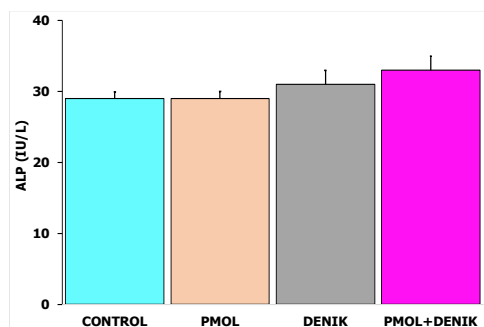


Figure 3: Effect of Paracetamol + Denik® interaction on ALP.

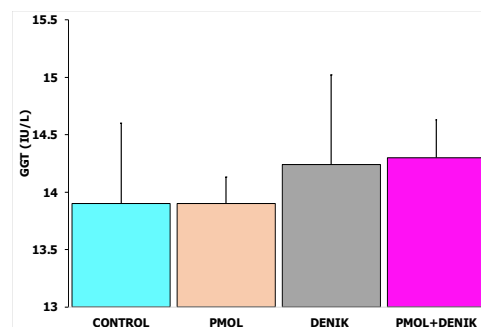


Figure 4: Effect of Paracetamol + Denik® interaction on GGT.

For Denik cleanser®-only and paracetamol-Denik® groups, there were marked distorted liver tissues indicative of hepatotoxicity. This was further supported by the results obtained from the biochemical analysis where elevations in the levels of the liver enzymes AST, ALT, ALP, and GGT were observed. The rise in AST and ALT levels in the paracetamol-Denik® group was statistically significant at $p < 0.05$ indicative of toxic

events in the liver on co-administration. For the Denik®-only groups, there was increase in the levels of AST, ALT, ALP and GGT, however, this was not statistically significant. Paracetamol metabolism to the reactive metabolite (*N*-acetyl-*p*-benzoquinone imine; NAPQI) by the CYP P450s, especially CYP2E1, is well recognized for its role in the initiation of toxicity¹⁷.

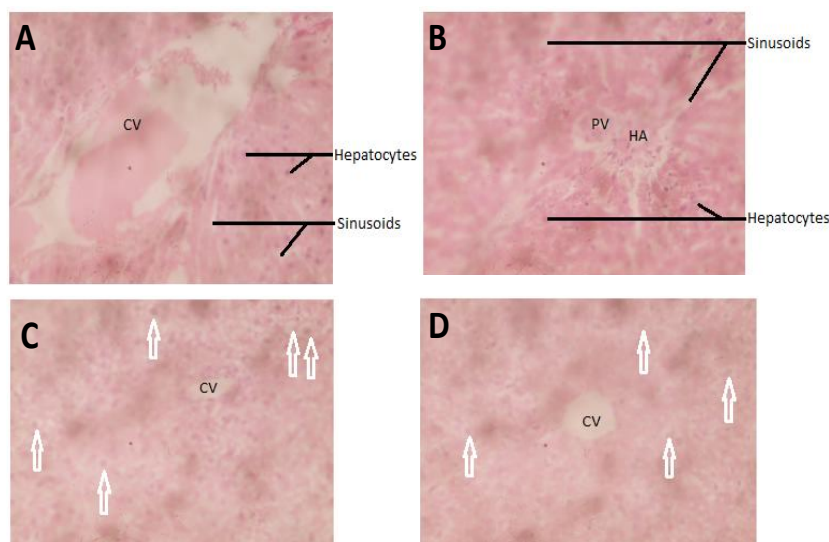


Figure 5: (A). Photomicrograph of liver from rats in the control group (distilled water only).

Histologically normal liver tissues showing normal hepatocytes & sinusoids and central vein (CV); (B). Photomicrograph of liver tissue of rats that received paracetamol only. Histologically normal liver tissues showing; normal hepatocytes & sinusoids and patent central vein (CV); (C). Photomicrograph of liver tissue of rats that received Denik® only. Markedly distorted liver tissue showing hepatocytes with microvesicular steatosis and Councilman's bodies (arrowed), patent central vein (CV); (D). Photomicrograph of liver tissue of rats that received Denik® only. Markedly distorted liver tissue showing hepatocytes with a spectrum of destructions (micro and macrovesicular steatosis and Councilman's bodies (arrowed), patent central vein (CV). (All the images are under 400x magnification).

Table 1: Organ-body weight index (%).

| Groups | Organ-body index |
|-------------------------------|------------------|
| Control | 4.0 ± 0.27 |
| Paracetamol | 3.90 ± 0.45 |
| Denik cleanser® | 3.80 ± 0.05 |
| Paracetamol + Denik cleanser® | 3.70 ± 0.08 |

There was elevations in the levels of liver enzyme biomarkers and abnormalities in the liver architecture in the paracetamol ± Denik® group, we hypothesize that phyto-constituents in Denik cleanser® may have increased metabolism and bioactivation of paracetamol resulting to enhanced production of NAPQI by the co-administered herbal remedy. Results obtained from the

present study indicates a potential hepatotoxic effect of Denik cleanser® when administered alone and when co-administered with paracetamol.

Limitations of the study

The high cost of the animals and the cost of their maintenance limited the number of animals used for the study.

CONCLUSIONS

The results of this study demonstrated the potential for *in vivo* herb/drug interaction involving paracetamol and Denik cleanser[®] resulting in liver injury. Obtained data suggest that Denik cleanser[®] creates a significant drug interaction that could lead to serious adverse health such as hepatotoxicity. However, this is subject to validation via pharmacokinetic study. Nevertheless, caution is strongly advised against its casual, non-medically supervised usage.

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AUTHORS' CONTRIBUTIONS

Obasi CN: research idea, writing, review, and editing, methodology, data curation. **Maagbo LJ:** literature search, formal analysis, data curation, supervision. **Mgbahurike AA:** designed and conducted the bench work. All the authors approved the finished version of the manuscript.

DATA AVAILABILITY

Data will be made available on reasonable request.

CONFLICT OF INTEREST

None to declare.

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