Original Research Article

CO-ADMINISTRATION OF GOKO HERBAL CLEANSER®AND PARACETAMOL: A HERB-DRUG INTERACTION STUDY

ABSTRACT

Herbs are known to contain certain phyto-constituents which alter the bioavailability of co-administered drugs resulting to toxicities or diminished efficacy. Therefore, concomitant intake of herbal products with orthodox medicines poses great therapeutic/safety concern. Paracetamol is hydroxylated via CYP2E1 to give the major hepatotoxin, N-acetyl-p-benzoquinone imine (NAPQI). The present study aimed to evaluate the effects of co-administering paracetamol and Goko cleanser®on the liver.The study sought to mimic conventional usage of Goko cleanser®followed by ingestion of paracetamol, a likely scenario given the popularity of both medications. Twenty rats of four groups were used for the experiment. Group one (control group) distilled water only; Group two received paracetamol only (100mg/kg), Group three received Goko cleanser®only (2 mL/kg) and Group four received paracetamol and Goko cleanser®concomitantly for 3 days. Blood and liver samples were harvested after animal sacrifice and subjected to biochemical and histopathological analysis respectively. Results obtained revealed a significant decrease ($P<0.05$) in the levels of the liver enzymes (ALT, AST, and GGT) in the Goko cleanser®-only group compared to control. There was also a significant decrease in AST in paracetamol + Goko cleanser®group. There was no significant change in ALT, ALP and GGT levels in the paracetamol + Goko cleanser®groups. Similar results were obtained from the histology examination, which showed normal liver tissues with normal hepatocytes, sinusoids and central vein. The significant reductions in the liver enzymes by observed in theGoko cleanser®-only group indicated potential hepato-protective effects which could be due to the presence of flavonoids and other phenolic antioxidants.The short-term co-administration of paracetamol and Goko cleanser®produced no liver toxicity. However, this is subject to further studies using pharmacokinetic analysis and also, a long-term study is strongly advocated.

Keywords: paracetamol; Goko cleanser®, herb-drug interaction, hepatotoxicity

INTRODUCTION

Phytochemicals in foods and herbs have been proven to be beneficial to human health(Cecilia N Amadi and Mgbahurike 2018; Rodríguez-Fragoso et al. 2011; Cecilia Nwadiuto Amadi and Nwachukwu 2020). However, the increase in the use of traditional medicines (herbal supplements and natural products) in recent times has come with growing concerns related to the safety and toxicity profile (Cecilia Amadi and Orisakwe 2018). Phytochemicals can influence the therapeutic efficacy and adverse effects of drugs by affecting their absorption and metabolism via interaction with drug transporters and metabolizing enzyme systems(Rodríguez-Fragoso et al. 2011; Cecilia Nwadiuto Amadi and Aghalibe 2019).

Co-morbidities of certain diseases has favoured polypharmacy thence posing an increased risk of drug interaction(C Amadi and Barileela 2018). This has resulted in the rise in the utilization of herbal medicines in combination with conventional drugs(Ekor 2013). The concomitant intake of polyherbal medicines with orthodox drugs raises huge concerns about herb-drug interactions and patient safety, especially as the pharmacokinetic and pharmacodynamic mechanisms of these herbal medicines are unknown(Francis et al. 2018). Certain herbal and dietary supplements may produce potentially dangerous food-drug interaction as they can alter the pharmacokinetics and/or pharmacodynamics of certain medications (Jin and Han 2010). This could be due the presence of adulterants or phytochemicals which could interfere with these processes(Rodríguez-Fragoso et al. 2011; CN Amadi et al. 2012). Such Food-drug interaction may be beneficial or detrimental. Interference (induction/inhibition) with cytochrome P450 isozymes and phase II conjugation enzymes or uptake and influx transport proteins by phytochemicals is the major mechanisms of drug/herb/nutrient interactions(Rodríguez-Fragoso et al. 2011).

Acetaminophen-induced hepatotoxicity is due to the reduced concentration of glutathione as a result of conjugation with N-acetyl-p-benzoquinone imine (NAPQI)(Hinson et al. 2010)a metabolite of acetaminophen metabolism via cytochrome P450 enzyme in the liver(Karbownik et al. 2018). However, the concentration of NAPQI produced at therapeutic doses are quite little to result in an adverse reaction but when overdosed, it's concentration increases which results in the depletion of glutathione resulting in hepatotoxicity and necrosis(Karbownik et al. 2018; Tan et al. 2008; Hinson et al. 2010). In view of this, an overdose of acetaminophen results to hepatotoxicity amongst other side effects(Ewing et al. 2019).

Goko cleanser is an herbal mixture claimed to be effective for various kinds of disease conditions suchas, diabetes, infertility, blood clots, hepatitis, erectly dysfunction. Its contents include: *Vernonia amygdalina*, *Cajanuscajan*, *Zingiberofficinale*, *Allium sativum*, *Saccharumofficinarum* and caramel(Nnamdi 2018). A recent study has identified nephrotoxic effect of Goko cleanser in wistar rats(Nnamdi 2018; Onyejike et al. 2018). This present study aims to evaluate the effect of a co-administration of Goko cleanser® and paracetamol on the histology and biochemical parameters of the liver.

MATERIALS AND METHODS

Materials

Chemicals/Reagents/plant materials

Distilled water, methylated spirit (JHD, China), Xylene, Diethyl ether(JHD GuagdongGuanghuaSci-Tech.co.Ltd. Shantou, Guandong, China), 10%formalin, Gokocleanser® (Kayfahdherbaceuticals), Paracetamol 500mg tablets BP (Panadol® from GlaxoSmithKline). All other solvents and chemicals were of analytical grade.

Apparatus/Equipment

Weighing balance (Ohaus, Advanturer), centrifuge (Techmel&Tecgmel USA), measuring cylinder, syringes, latex gloves, cotton wool, tissue paper, heparinized tubes, 5mL sample bottles, surgical blade, refrigerator, cotton wool, cannula.

Source of herbal formulation

The herbal formulation under study was obtained in August 2020, from Choba open market, Port Harcourt, Rivers State. The herbal formulation was manufactured by Kayfahdherbaceuticals. Exclusively for : Purity biz.com FCT Abuja, Nigeria.

Animals

This study was done using an experimental design previously described by (Ewing et al. 2019) with little modifications. Twenty (20) healthy adult male rats (152.6 ± 20.6) 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 to.

Method
Animal experiment

The dosage for Goko cleanser® herbal mixture was chosen using its prescription label. The drugs were administered to the rats in the test group orally using an oral cannula. Animals were randomly assigned to four groups (1-4) of five animals each. Group I received 0.3mL of distilled water orally daily for 3 days (control group). The group II animals received paracetamol orally at a dosage of 100 mg/kg daily for 3 days. Group III animals received Goko cleanser® orally at a dosage of 2 mL/kg daily for 3days. Group IV animals received both paracetamol and Goko cleanser® orally at a dosage of 100 mg/kg and 2 mL/kg respectively concurrently daily for 3 days. With this experimental setup, we sought to mimic conventional/therapeutic usage of paracetamol and Goko cleanser®. Animals were then fasted overnight on the third day of treatment and sacrificed under ether anaesthesia on the fourth day.

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).

Histopathology results

Liver sections were fixed in 10 % formalin for 6-12 hours. They were processed and examined for histological changes at the college of health sciences Pathology Facility. For light microscopy examination, the formalin fixed tissues (liver) were dehydrated through ascending grades of alcohol, cleared in three changes of xylene, and were embedded in paraffin. Serial sections, each of 4-micron thickness, were cut and stained with H and E as per standard protocol. Stained

sections were morphologically evaluated, and the pictures of the slides were taken for comparison.

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.

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

RESULTS OF BIOCHEMICAL ANALYSIS

In this study, enzyme values were all within normal ranges. The rats treated with Goko cleanser® alone showed a significant ($p<0.05$) decrease in the levels of liver function markers, ALT, AST, and GGT enzymes as compared to the control group, paracetamol-only group and the Goko®-paracetamol group. However, there was a mild increase in the level of ALP in the Goko cleanser®-only and the paracetamol/Goko cleanser® groups when compared with the control. However, this increase was not statistically significant. Similarly, the was a non-significant increase in ALP, ALT and GGT in the Goko®-paracetamol interaction group compared to the control group. These results are shown in Figures 1 to 4.

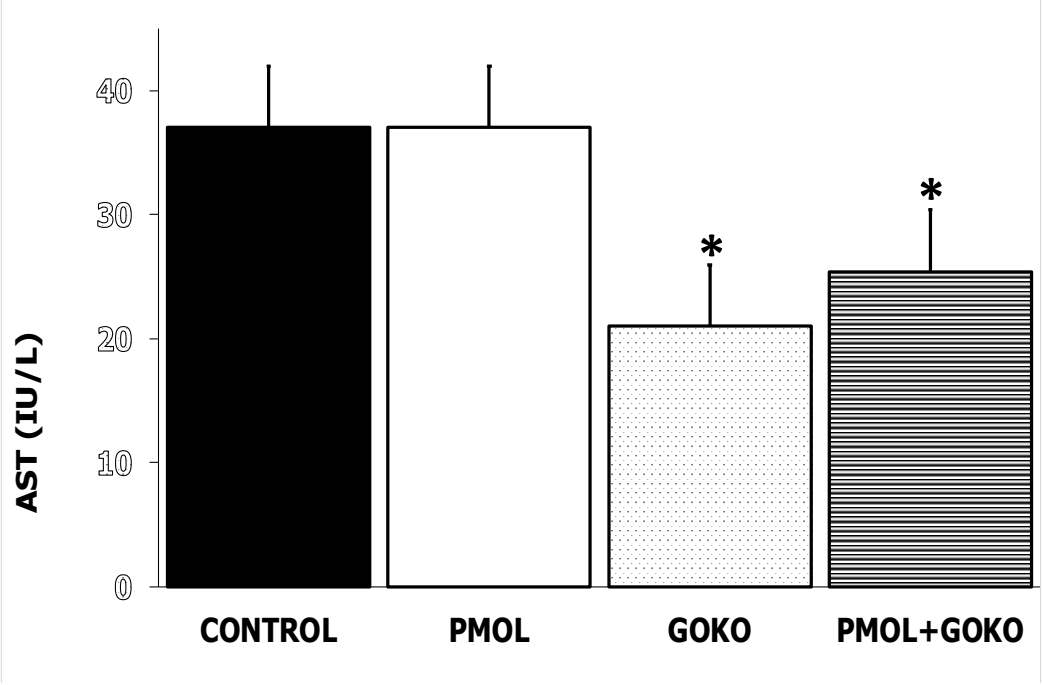

Figure 1: Effect of Paracetamol + Goko® interaction on AST; n=5, values are expressed as mean±SEM. * P< 0.05 compared to control group

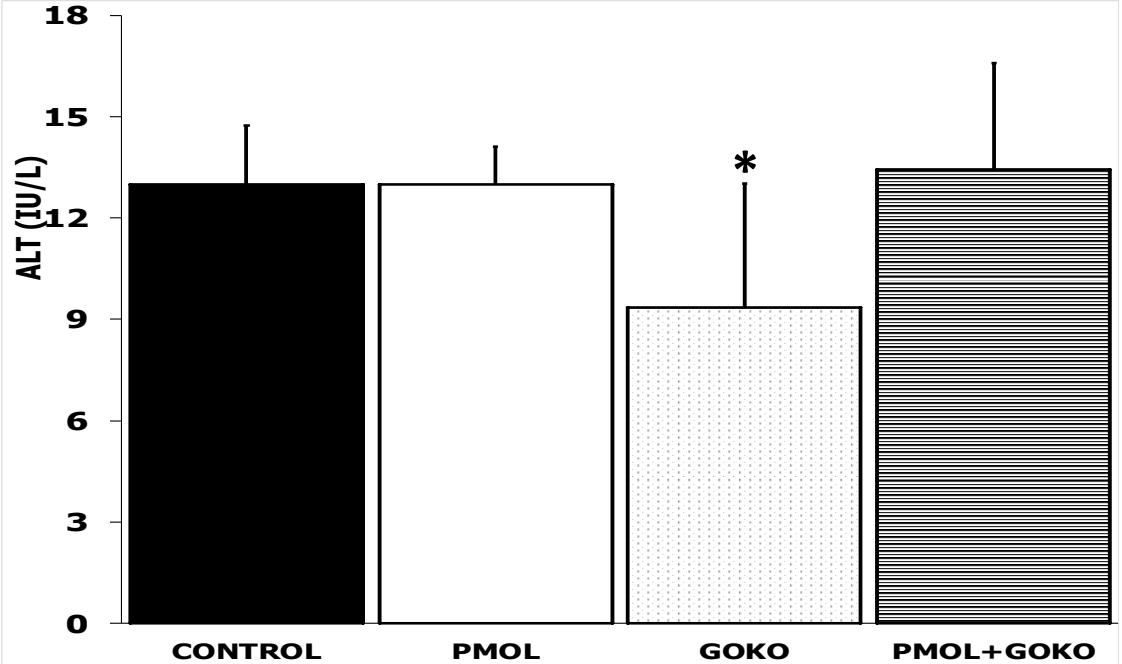

Figure 2: Effect of Paracetamol + Goko® interaction on ALT; n=5, values are expressed as mean±SEM. * P< 0.05 compared to control group

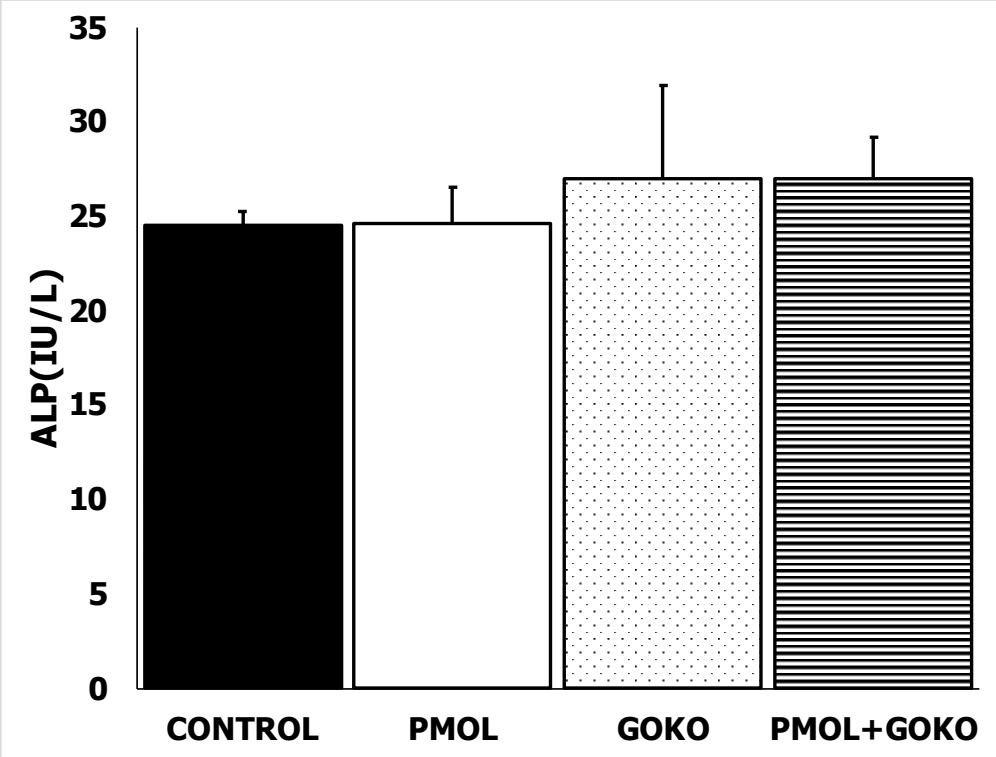

Figure 3: Effect of Paracetamol + Goko® interaction on ALP; n=5, values are expressed as mean±SEM.

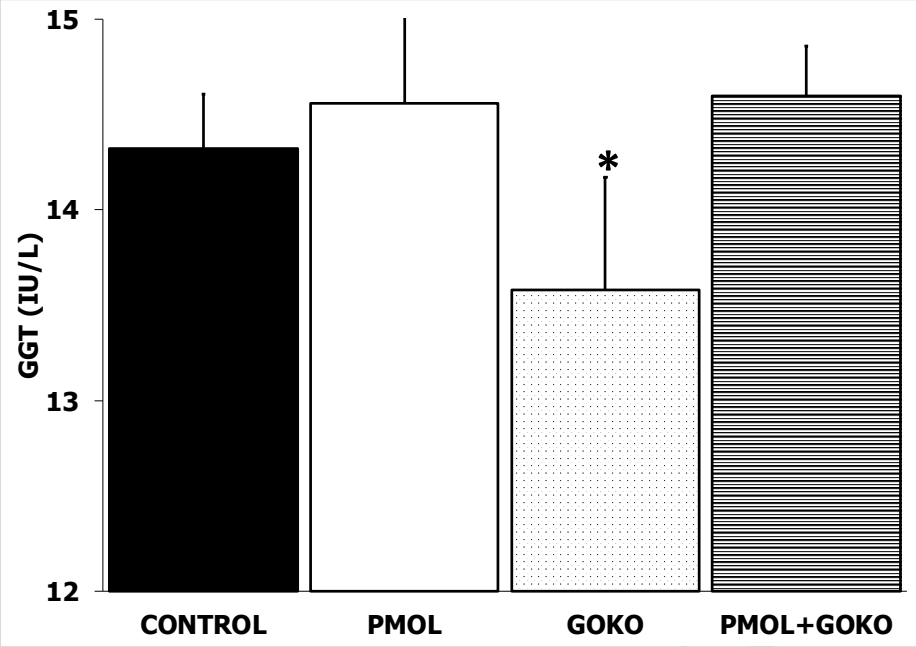

Figure 4: Effect of Paracetamol + Goko® interaction on GGT; n=5, values are expressed as mean±SEM. * P< 0.05 compared to control group

HISTOPATHOLOGY RESULTS

Autopsy at the end of the experiment period revealed no apparent changes in the liver tissues from both control and treated rats in the histopathology analysis. The liver tissues were histologically normal showing normal hepatocytes, sinusoids, central vein and portal triad (portal vein, hepatic artery and bile duct).

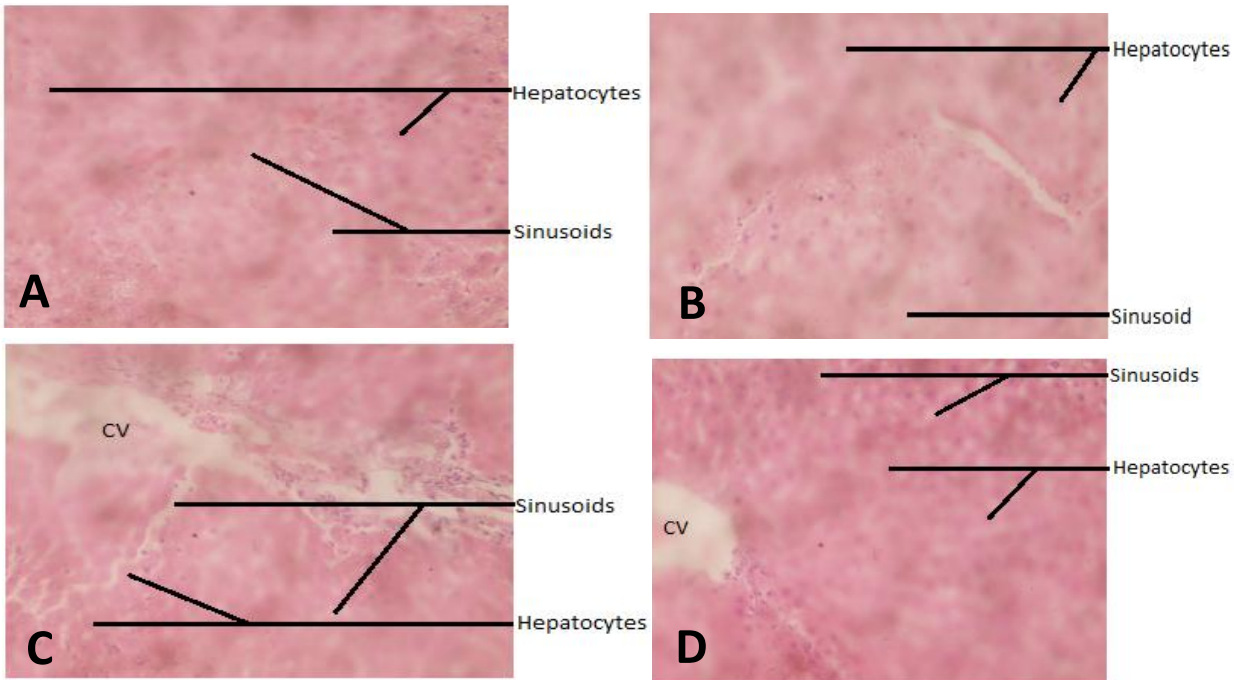

Figure 5:**(A)** Photomicrograph of liver from rats in the control group that received distilled water only. 400x magnification, **(B)** Photomicrograph of liver tissue of rats that received paracetamol only. 400x magnification, **(C)** Photomicrograph of liver from rats that receivedGoko cleanser® only. 400x magnification, **(D)** Photomicrograph of liver from rats that received paracetamol + Goko cleanser®.400x magnification

EFFECT OF PARACETAMOL-GOKO CLEANSER INTERACTION ON RAT BODY WEIGHTS

The body weights of the animals were determined and are shown in Table 1. All animals exhibited increment in body weight at the end of the experiment with significant difference between both control and treated groups.

Table 1: Showing the effects of paracetamol and Goko cleanser® interaction on rat body weights

Groups	Initial Weight (g)	Final Weight (g)	% Body Weight Change
Control	125.6±5.8	138.4 ±8.4	10.2
Paracetamol	134± 1.6	138.8 ± 2.9	3.6
Goko cleanser®.	145.6 ±3.4	151.8 ±4.7	4.1

DISCUSSION

Drug-herb interaction is a matter of concern especially in the developing countries, as these herbal medicines are associated with complications such as liver damage with high incidence of mortalities and morbidities(Cecilia Amadi and Orisakwe 2018).The clinical manifestations could range from asymptomatic cases with abnormal liver function tests to sudden and severe liver failure(Cecilia Amadi and Orisakwe 2018).Herbal remedies have become universally popular in primary healthcare, and some have been mistakenly regarded as safe just because they are a natural source(Cecilia Nwadiuto Amadi and Aghalibe 2019). Nevertheless, these bioactive products from medicinal plants are presumed to be safe without any compromising health effect, and thus widely used as self medication(Vaghasiya et al. 2011). However, there is a lack of proven scientific studies on the toxicity and adverse effect of these remedies(Jothy et al. 2011).

Recent studies have reported that certain herbs have the potential to induce drug metabolizing enzymes and transporters (Mekjaruskul and Sripanidkulchai 2019). For example, aherbal preparation containing *Kaempferiaparviflora* have been shown to induce CYP2E1 which is an enzyme that metabolizes paracetamol. Paracetamol (acetaminophen) is hydroxylated to give the major hepatotoxin, (*N*-acetyl-p-benzoquinone imine; NAPQI)(Ward and Alexander-Williams 1999). In view of this, Mekjaruskul and Sripanidkulchai investigated the combined use of *K. parviflora* extract with CYP2E1 paracetamol. Results obtained from that study provided a possible drug-herb interaction between *K. parviflora*and paracetamol leading to increased metabolism and possible enhanced levels of the metabolite NAPQI(Mekjaruskul and Sripanidkulchai 2019).

Besides the negative effects of drug-herb interaction, some studies have highlighted beneficial effects. For example, certain herbs have been found to possess ameliorative effects against some drug-induced toxicities. In a study by Hamid et al.*Zingiber zerumbet* was documented to have protective activity against paracetamol-induced acute hepatotoxicity in rat model(Hamid et al. 2018). Further to this, *Curcuma longa* extract was shown to protect the liver against carbon tetrachloride-induced liver injury(Samojlik et al. 2012). In addition, some herbs such as *Ginkgo biloba* have shown beneficial synergistic effects that enhances the efficiency of haloperidol(Borse et al. 2019).

In the present study, the results obtained from biochemical analysis produced a reduction in the levels of liver enzymes in the Goko cleanser®-paracetamol group compared to control. These results are in agreement with the data obtained from histopathological examination, which showed normal liver tissues with normal hepatocytes, sinusoids, central vein and portal triad (portal vein, portal artery and bile duct).Goko cleanser®when administered alone and in combination with paracetamol reduced the levels of ALT, AST and GGT significantly ($P<0.05$) compared to control and paracetamol-only groups. While AST levels were significantly reduced in group that received Goko cleanser® alone and paracetamol + Goko cleanser® concurrently, the AST level in the paracetamol-Goko cleanser®group was higher than the Goko cleanser® -only group suggestive of an elevation accruing from an interaction between paracetamol andGoko cleanser®. Furthermore, increased body weights of the animals across all treatment groups were suggestive of the absence of toxicities from the co-administration of paracetamol and Goko cleanser®.

Taken together, the significant reductions in the liver enzymesbyGoko cleanser®indicated potential hepato-protective effects, this provides some level of support to the claims made by the manufacturer about Goko cleanser®being hepato-protective. The potential hepato-protective

effect of Gokocleanser®could be due to the presence of flavonoids and other phenolic compounds like tannins contained in some of its constituent herbs. Flavonoids and tannins are plant-based antioxidants, which could be associated with reduced risk of occurrence of numerous human disease related to oxidative stress(Mehrandish et al. 2019).Oxidative stress is known to play a foremost role in the progress of liver diseases(Zhu et al. 2012). Earlier studies have highlighted the potential hepato-protective effects of flavonoids and other plant-based antioxidants in herbs. For example, a study by Kim et al. demonstrated that extracts of *Alnus japonica*alleviated the acetaminophen induced hepatic injury in rats (Kim et al. 2004).

CONCLUSION

From the results obtained from this study, no hepatotoxic effects were observed with short-term administration of paracetamol and Goko cleanser®at therapeutic doses in rats. Therefore, we hypothesize that a short-term co-administration of the duo poses no potential health risk, however this is subject to further studies. Specifically, pharmacokinetics studies and long-term co-administration studies are strongly advocated.

REFERENCES

Amadi, C., & Barileela, L. (2018). Effect of pineapple (ananas comosus) and uziza (piper guineense) extracts on fexofenadine bioavailability: possible role of p-glycoprotein (P-GP) and organic anion transporting polypeptides (OATPs). *Int. Res. J. Pharm, 9*(3), 17-21.

Amadi, C., & Orisakwe, O. (2018). Herb-induced liver injuries in developing nations: An update. *Toxics, 6*(2), 24.

Amadi, C., Orisakwe, O., & Roberts, I. (2012). Elemental impurities in registered herbal supplements in Nigeria: a look at mercury, antimony and tin. *Rasayan J Chem, 5*(2), 220-228.

Amadi, C. N., & Aghalibe, P. O. (2019). Evaluation of Drug-diet interaction between Psidium guajava (Guava) fruit and Metoclopramide. *Journal of Drug Delivery and Therapeutics, 9*(2), 144-147.

Amadi, C. N., & Mgbahurike, A. A. (2018). Selected Food/Herb–Drug Interactions: Mechanisms and Clinical Relevance. *American journal of therapeutics, 25*(4), e423-e433.

Amadi, C. N., & Nwachukwu, W. I. (2020). The effects of oral administration of Cola nitida on the pharmacokinetic profile of metoclopramide in rabbits. *BMC Pharmacology and Toxicology, 21*(1), 1-6.

Borse, S. P., Singh, D. P., & Nivsarkar, M. (2019). Understanding the relevance of herb–drug interaction studies with special focus on interplays: a prerequisite for integrative medicine. *Porto biomedical journal, 4*(2).

Ekor, M. (2013). The growing use of herbal medicines: issues relating to adverse reactions and challenges in monitoring safety. Front Pharmacol. 2013; 4: 177.

Ewing, L. E., McGill, M. R., Yee, E. U., Quick, C. M., Skinner, C. M., Kennon-McGill, S., et al. (2019). Paradoxical Patterns of Sinusoidal Obstruction Syndrome-Like Liver Injury in Aged Female CD-1 Mice Triggered by Cannabidiol-Rich Cannabis Extract and Acetaminophen Co-Administration. *Molecules, 24*(12), 2256.

Francis, E., Amaeze, O., & Anyika, E. (2018). Ruzu® Herbal Bitters and Glibenclamide Tablets: Dissolution and In Vitro Release Kinetics Studies. *Nigerian Journal of Pharmaceutical Research, 13*(2), 73-82.

Hamid, A., Lee, L. S., Karim, S. R., & Jufri, N. F. (2018). Hepatoprotective effects of zerumbone against paracetamol-induced acute hepatotoxicity in rats. *The Malaysian journal of medical sciences: MJMS, 25*(2), 64.

Hinson, J. A., Roberts, D. W., & James, L. P. (2010). Mechanisms of acetaminophen-induced liver necrosis. In *Adverse drug reactions* (pp. 369-405): Springer.

Jin, M. J., & Han, H. K. (2010). Effect of piperine, a major component of black pepper, on the intestinal absorption of fexofenadine and its implication on food–drug interaction. *Journal of food science, 75*(3), H93-H96.

Jothy, S., Zakaria, Z., Chen, Y., Lau, Y. L., Latha, L. Y., & Sasidharan, S. (2011). Acute oral toxicity of methanolic seed extract of Cassia fistula in mice. *Molecules, 16*(6), 5268-5282.

Karbownik, A., Szałek, E., Sobańska, K., Grabowski, T., Klupczynska, A., Plewa, S., et al. (2018). The concomitant use of lapatinib and paracetamol-the risk of interaction. *Investigational new drugs, 36*(5), 819-827.

Kim, S. T., Kim, J. D., Ahn, S. H., Ahn, G. S., Lee, Y. I., & Jeong, Y. S. (2004). Hepatoprotective and antioxidant effects of Alnus japonica extracts on acetaminophen-induced hepatotoxicity in rats. *Phytotherapy Research, 18*(12), 971-975.

Mehrandish, R., Rahimian, A., & Shahriary, A. (2019). Heavy metals detoxification: A review of herbal compounds for chelation therapy in heavy metals toxicity. *Journal of Herbmed Pharmacology, 8*(2), 69-77.

Mekjaruskul, C., & Sripanidkulchai, B. (2019). In vivo effect of Kaempferia parviflora extract on pharmacokinetics of acetaminophen. *Drug and chemical toxicology*, 1-7.

Nnamdi, O. D. (2018). Biochemical Effects of Goko Cleanser Herbal Mixture on the Kidney of Adult Female Wistar Rats. *International Invention of Scientific Journal, 2*(04), 117-129.

Onyejike, D. N., Aladeyelu, S. O., Onyejike, I. M., & Nwankwo, O. K. (2018). Histopathological of Goko Cleanser®(Herbal Mixture) on the kidney of adult female Wistar rats. *Int J Innov Res Adv Studies. 2018a, 5*(6), 254-262.

Rodríguez-Fragoso, L., Martínez-Arismendi, J. L., Orozco-Bustos, D., Reyes-Esparza, J., Torres, E., & Burchiel, S. W. (2011). Potential risks resulting from fruit/vegetable–drug interactions: effects on drug-metabolizing enzymes and drug transporters. *Journal of food science, 76*(4), R112-R124.

Samojlik, I., Đaković-Švajcer, K., Božin, B., & Mikov, M. Herb-drug interactions: the influence of essential oil of caraway (Carum carvi L.) on the pharmacokinetics of paracetamol. In *BMC Pharmacology and Toxicology, 2012* (Vol. 13, pp. 1-1, Vol. 1): BioMed Central

Tan, S. C., New, L. S., & Chan, E. C. (2008). Prevention of acetaminophen (APAP)-induced hepatotoxicity by leflunomide via inhibition of APAP biotransformation to N-acetyl-p-benzoquinone imine. *Toxicology letters, 180*(3), 174-181.

Vaghasiya, Y., Shukla, V., & Chanda, S. (2011). Acute oral toxicity study of Pluchea arguta boiss extract in mice. *J Pharmacol Toxicol, 6*(2), 113-123.

Ward, B., & Alexander-Williams, J. M. (1999). Paracetamol revisited: a review of the pharmacokinetics and pharmacodynamics. *Acute pain, 2*(3), 139-149.

Zhu, R., Wang, Y., Zhang, L., & Guo, Q. (2012). Oxidative stress and liver disease. *Hepatology Research, 42*(8), 741-749.