Research Journal of Environmental Toxicology

ISSN 1819-3420

Academic Journals Inc.

www.academicjournals.com

OPEN ACCESS Research Journal of Environmental Toxicology

ISSN 1819-3420
DOI: 10.3923/rjet.2018.24.33

Research Article
Synergistic and Ameliorative Effect of Honey and Coconut Water on Crude Oil Induced Toxicity in Rats

[1]Adebola Olayemi Akintola, [2]Busuyi David Kehinde, [2]Joshua Olugbemiga Fakunle and [3]Ayodeji Folorunsho Ajayi

[1]Department of Science Laboratory Technology, Faculty of Pure and Applied Sciences, Ladoke Akintola University of Technology, Ogbomoso, Nigeria
[2]Department of Biochemistry, Faculty of Basic Medical Sciences, Ladoke Akintola University of Technology, Ogbomoso, Nigeria
[3]Department of Physiology, Ladoke Akintola University of Technology, P.M.B. 4000, Ogbomoso, Nigeria

Abstract

Background and Objective: Crude oil as a major pollutant in oil-producing area requires diverse means of control to its toxicity; particularly on the liver. One strategy is to treat with natural products which are cost effective and non-toxic. This study investigated the ameliorative effect of co-administration of honey and coconut water against toxicity induced by crude oil. **Materials and Methods:** Thirty five male Wister rats were grouped into five groups treated for 11 days after which enzymes, antioxidant, oxidative stress and histopathology of the liver was assessed. **Results:** A significant ($p<0.05$) elevation in the serum activities of alanine aminotransferase, aspartate amino-transferase and alkaline phosphatase in the crude oil (Cr) only treated rats was observed. Consequently, treatment with both honey and coconut water (Cr+Hy+Cw) significantly reduced the enzyme activities than treatment with honey (Hy) and coconut water (Cw) separately. The Cr significantly decreased the concentration of liver's reduced glutathione (GSH), catalase and superoxide dismutase (SOD) activities and significantly increased malondialdehyde (MDA) concentrations. However, the activities of these enzymes: GSH, catalase, SOD increased, while MDA reduced in the group treated with both Hy and Cw than those animals treated with Hy and Cw separately. The Hy and Cw reversed histological damages caused by crude oil administration on the liver. **Conclusion:** The findings showed that honey and coconut water synergistically reversed hepatic damage induced by crude oil in rats via its antioxidant and minerals contents. Administration of honey and coconut water together at a dosage of 5 g kg^{-1} b.wt. and 10 mL kg^{-1} b.wt., respectively daily is recommended.

Key words: Coconut water, crude oil, hepatotoxicity, honey, oxidative stress, synergistic

Citation: Adebola Olayemi Akintola, Busuyi David Kehinde, Joshua Olugbemiga Fakunle and Ayodeji Folorunsho Ajayi, 2018. Synergistic and ameliorative effect of honey and coconut water on crude oil induced toxicity in rats. Res. J. Environ. Toxicol., 12: 24-33.

Corresponding Author: Ayodeji Folorunsho Ajayi, Department of Physiology, Ladoke Akintola University of Technology, P.M.B. 4000, Ogbomoso, Nigeria
Tel: +2348033834495

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The main occupation of people living in oil producing areas of Nigeria includes fishing and farming[1], usually in land used for farming activities, contaminated wastes from petroleum or diesel and accidental spills of crude oil at some drilling sites constitute exposure risks for farmers, public, livestock and wildlife[2]. The hydrocarbons from petroleum can eventually get into man and animals through eating of contaminated food or bio concentration through the food chain[3]. In the Niger delta region of Nigeria, about 50% of oil spills take place as a result of rusting of oil pipelines, 28% to vandalism and 21% due to activities involve in oil production. About 1% of oil spills as a result of engineering drills, failure to adequately control oil wells, breakdown of machines, incompetency in loading and offloading oil vessels, storage and transportation[4].

Crude oil toxicity has attracted much attention in the recent years. It has been shown to affect vital organs of the body and causes serious membrane disruption by the passage of petroleum components through the plasma membrane of the cells[5]. The mis-use of crude oil by individuals due to their belief that it can repel witches, when applied topically or orally to the afflicted individuals have been reported, while some developing countries in Africa relied on crude oil for (unorthodox) treatment of ailments such as convulsion and diarrhoea[6]. The deleterious effects of crude oil have also been reported to include pneumonitis, renal failure, in coordination and conjunctivitis[7]. Findings by Aslani *et al.*[8] showed stools with blood, coughing, constipation, affected fertility and sudden death in female goats exposed to west Texas intermediate crude oil. While chronic exposure of animals to crude oil generates signs and symptoms of central nervous system toxicity[9]. The composition of the crude oil is responsible for the multiple types of toxic effects ranging from acute lethal toxicity to sub-lethal chronic toxicity depending on the method of exposure, dosage and the fragility of the organism exposed. Some components of crude oil have the potential to bioaccumulation within susceptible organisms and cause genotoxicity and during oxidation in the cells, petroleum hydrocarbon produced free radicals or activates metabolites that react with membrane lipids and triggers lipid peroxidation action which can result to membrane damage[10]. These suggested that antioxidants may be important in reversing the toxic effects of crude oil.

Honey is a natural substance produced by honey bees, which is widely used both for nutritional and medicinal purposes[11-13]. Research in the last few years provided authentic pieces of evidence in support of the antioxidant, anti-microbial and wound healing properties of honey[14,15]. Previous *in vitro* studies on antioxidant properties of honey showed that Tualang honey had the highest phenolics and flavonoids contents as well as the best free radical scavenging properties when compared to other Malaysian honey[16,17], There is also, the presence of many phenolic acids, such as gallic, syringic, benzoic, trans-cinnamic, P-coumaric acids and flavonoid compounds like catechin and kaempferol in honey[16]. The significance of honey in wound treatments has been documented since ancient times, however, this healing properties is due to its antioxidant and antibacterial activities, with the ability to maintaining a moist wound condition and high viscosity that provides a protective barrier on the wound, which prevents microbial infection and promotes healing[18]. Its immunological and anti-inflammatory effects are also important for wound repair[19,20]. Recent study has shown that honey can exert anti-proliferative effect against cancer cells[21]. The anticancer properties can involve different processes such as apoptosis of cancer cells through depolarization of mitochondrial membrane, cyclooxygenase-2 inhibition by various constituents, the release of cytotoxic hydrogen peroxide and lastly the scavenging of reactive oxygen species (ROS) which have been linked with its phytochemical constituents[22].

Coconut water a natural nutrition beverage considered as a functional nutraceutical. It contains several biologically active components that possess; cardioprotective, hepatoprotective, hypolipidemic and antihypertensive properties in experimental animals[23-28]. More so, coconut water is the most nutritious whole some beverage in all coconut producing countries, having a host of yet scientifically unproven traditional uses in cultures all over the world. Reports from Africa support the position that about 85% of the world's population rely on coconut fruit in traditional medicine. It could prevent and reverse high blood pressure, up-regulate antioxidant status and improved insulin sensitivity[27]. It is also used to control irregular or painful menstruation and when taken during pregnancy it gives the unborn babies strength and vitality, while, It is also used to boost semen quality and induce libido[29]. Coconut water apart from containing numerous antioxidant compounds that have the ability to scavenge free radicals in the body, it also contains sugar, lipids, vitamins, minerals, amino acids, nitrogenous compounds, organic acids, enzymes and phytohormones[30,31].

Since crude oil have severe toxicity on vital organs of the body the liver inclusive and for the fact that orthodox medicine focuses more on the treatment of acute toxicity from accidental consumption, therefore, it becomes imperative to explore the potentials of natural products such as honey and coconut water in reversing the damage to the liver as a result of chronic exposure to crude oil. This study, therefore, investigated the ameliorative and synergetic effects of honey and coconut water on crude oil induced toxicity in rats.

MATERIALS AND METHODS

Materials: Bonny light crude oil was obtained from the Department of Petroleum Resources (DPR), Nigerian National Petroleum Corporation (NNPC), Port Harcourt. A polyfloral wild honey produced in Ola-Osun Farm Ilesha (Goshen honey) Osun state, Nigeria was used in this study, while coconut was procured at Sabo market Ogbomoso, Oyo State, Nigeria. The coconuts were broken and the liquid content was collected into a beaker and filtered with Whatman filter paper and refrigerated for use.

Animal management: A total of 35 male Wistar rats weighing between 200-250 g were used for this study. The animals were allowed to acclimatize to the laboratory condition (temperature 25°C and environmental 12 h light-dark cycle) for 2 weeks before the experiment. The animals were housed in well-ventilated standard plastic cages and fed with standard diet (pelletized growers mash) obtained from Bovajay Feed mill at Orita Naira in Ogbomoso and they were given water *ad libitum* (during the acclimatization and experimental period). Animals were subjected to humane care and all procedures were conducted in accordance to the guiding principles on research involving animals recommended by the declaration of Helsinki and the guiding concepts in the care and use of experimental animals[32].

Experimental design: The rats used for this study were randomly divided into five groups of 7 rats per group. Group A: Served as normal control untreated rats and were given distilled water. Group B: Received crude oil only (4 mL kg^{-1} b.wt.). Group C: Received crude oil (4 mL kg^{-1} b.wt.) and 5 g kg^{-1} body weight of honey orally. Group D: Received crude oil (4 mL kg^{-1} b.wt.) and 10 mL kg^{-1} body weight of coconut water orally. Group E: Received crude oil (4 mL kg^{-1} b.wt.) and were treated with 5 g kg^{-1} body weight of honey and 10 mL kg^{-1} body weight of coconut water, respectively. The animals were treated for 11 days after which they were sacrificed by cervical dislocation.

Duration and year of study: The exact duration and year of study are 14 days of acclimatization and 11 days of treatment which makes a total of 25 days, from December 2nd to December 27th, 2017.

Grading of used chemicals: All chemical used are SIGMA products and of Analytical Grade, while the kits used are RANDOX kits from UK.

Preparation of serum and tissue homogenate: Blood was drawn from the apexes of the heart by needle and syringe and then put into a plain bottle before harvesting the liver. Each clotted blood sample was centrifuged at 3000 revolutions per min for 15 min to obtain the serum. The supernatant was siphoned using micropipette. The liver was quickly removed and rinsed in 1.15% KCl dried and weighed, then liver samples were cut into pieces and homogenized in an equal volume of chilled 10 mM Tris/HCl buffer of pH 7.4 and 0.25 M sucrose solution.

Biochemical analysis: The activities of alanine aminotransferase (ALT), aspartate aminotransferase (AST) were determined in the serum according to the methods of Reitman and Frankel as described by Ochei and Kolhatkar[33]. Plasma alkaline phosphatase (ALP) activity was determined according to Kind and Kind[34]. Serum total proteins concentration was determined by colourimetric Biuret method by Tietz *et al.*[35].

The activity of superoxide dismutase (SOD) was determined by the method of Misra and Fridovich[36]. Lipid peroxidation concentration was determined by the principle of Varshney and Kale[37]. Estimation of lipid peroxidation was based on the reaction of malondialdehyde (MDA) with thiobarbituric acid (TBA) forming an MDA-TBARS adjunct that absorbed strongly at 532 nm. Reduced glutathione (GSH) level in the liver was assayed following the method of Ellman[38] modified by Hissin and Hilf[39]. Catalase activity was determined by the method of Aebi[40].

Tissue processing: The liver samples were harvested after sacrificing the animals and the tissues were allowed to fix in 10% normal saline for 48 h, tissues were processed using an automated tissue processor (LECAYP1020), embedded in paraffin wax and section of 5 m thickness were cut using a rotary microtome. The sections were stained by haematoxylin and eosin (H and E) method for light microscopic examination. Photomicrograph of stained sections was taken with the aid of a light camera fitted microscope[41].

Statistical analysis: Data were analyzed using Graph pad prism 6. The results were expressed as the Mean±standard error of the Mean (Mean±SEM). Values were compared using one-way analysis of variance (ANOVA) followed by student t-test. The p-value <0.05 was accepted to be statistically significant and insignificant at p>0.05.

RESULTS

It was revealed in Fig. 1 that, a significant decrease in total protein concentration in the group administered with 4 mL kg^{-1} body weight dose of crude oil (Group B) when compare with the control group (Group A) at p<0.05, there was a significant increase in total protein concentration in Group C when treated with 5 g kg^{-1} body weight of honey when compared with Group B. Also the total protein concentration of Group D increased when treated with coconut water but it was not significant. However, an insignificant increase was observed in Group E after treatment with both coconut water and honey.

The results of Table 1 showed a significantly (p<0.05) higher activities of hepatic biomarker enzymes: Alanine aminotransferase, Aspartate aminotransferase and Alkaline phosphatase in the group administered with crude oil only (Group B) when compared with control group (Group A). However, treatment with honey (Group C), coconut water (Group D) and also honey plus coconut water (Group E) significantly (p<0.05) reduced the activities of hepatic biomarkers enzyme when compared with the untreated Group (Group B).

The results of Table 2 showed that the activities of catalase and SOD significantly decreased in Group B when compared with the Control Group. However, treatment with honey and coconut water separately increased catalase activities and SOD significantly in Group C and D, respectively while the activities of catalase and SOD further increased significantly in Group E treated with both honey and coconut water when compared with Group B.

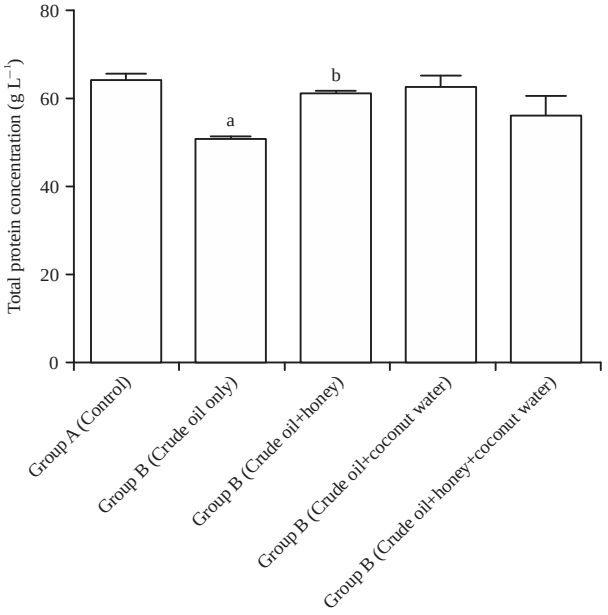

Fig. 1: Effect of honey and coconut water on total protein concentration in the serum of rat with crude oil induced hepatotoxicity

Values are expressed as Mean±SEM of total protein concentration in Wistar rats using student t-test. 'a' represents statistical significance when other groups are compared with Group A (Control) at p<0.05, 'b' represent statistical significance when other groups are compared with Group B (Crude oil only) at p<0.05

Table 1: Effect of honey and coconut water on the activities of Alanine aminotransferase (ALT), Aspartate aminotransferase (AST) and Alkaline phosphatase (ALP) in the serum of rat with crude oil induced hepatotoxicity

Variables	Group A (Control)	Group B (Crd only)	Group C (Crd+honey)	Group D (Crd+Cw)	Group E (Crd+honey+Cw)
ALT (μ L^{-1})	23.48±2.50	43.28±2.43	33.72±1.89	36.40±1.5^{abc}	28.95±2.17^{b}
AST (μ L^{-1})	73.66±2.62	121.40±2.44^{a}	98.56±2.45^{abc}	108.40±4.06^{abc}	92.22±2.04^{ab}
ALP (μ L^{-1})	19.89±2.80	52.06±11.31^{a}	22.55±1.80^{b}	29.56±4.09^{ab}	26.64±1.80^{ab}

Crd: Crude oil, Cw: Coconut water. Values are expressed as Mean±SEM of ALT, AST, ALP activities in Wistar rats using student t-test. 'a' represent statistical significance when other groups are compared with Group A (Control) at p<0.05, 'b' represent statistical significance when other groups are compared with Group B (Crude oil only) at p<0.05, while 'c' represents statistical significance when other groups are compared with Group E (Crude oil+honey+coconut water) at p<0.05

Table 2: Effect of honey and coconut water on catalase and superoxide dismutase activities of liver tissue homogenate of rats with crude oil induced hepatotoxicity

Variables	Group A (Control)	Group B (Crd only)	Group C (Crd+honey)	Group D (Crd+Cw)	Group E (Crd+honey+Cw)
Catalase activity (μmol min^{-1})	8.82±0.85	3.05±0.85^{a}	5.47±0.68^{a,b}	4.52±0.25^{a}	6.79±1.40^{b}
SOD activity (μmol min^{-1})	69.73±2.39	12.13±1.21^{a}	55.97±3.41^{abc}	24.97±4.93^{abc}	56.19±4.34^{ab}

Crd: Crude oil, Cw: Coconut water. Values are expressed as Mean±SEM of Catalase and SOD activities in Wistar rats using student t-test. 'a' represents statistical significance when other groups are compared with Group A (Control) at p<0.05, 'b' represent statistical significance when other groups are compared with Group B (Crude oil only) at p<0.05, while 'c' represents statistical significance when other groups are compared with Group E (Crude oil+Honey+coconut water) at p<0.05

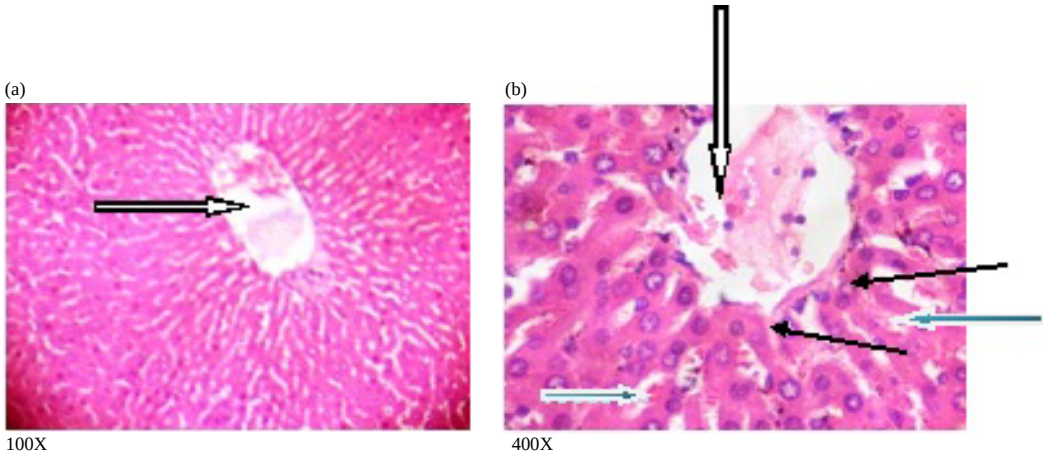

Plate 1(a-b): Photomicrography of Group A (Control) [H and E stain 100X and 400X]. White arrow
Pointing to the central venules, Slender arrow: Pointing to the sinusoids and Blue arrow: Pointing to the hepatocytes

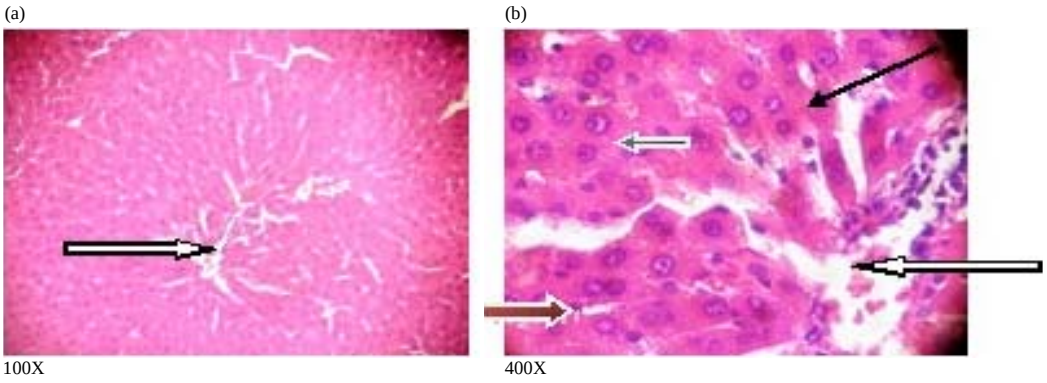

Plate 2(a-b): Photomicrography of Group B (Treated with crude oil only) H and E stain at (a) 100X and (b) 400X
White arrow: Pointing to portal tract, Slender arrow: Pointing to the sinusoids, Blue arrow: Pointing to the hepatocytes and Red arrow: Pointing to cytoplasmic fat infiltration, vacuolation and necrotic cells

Table 3: Effects of honey and Coconut water on MDA and GSH concentrations in the liver tissue homogenate of rats with crude oil induced hepatotoxicity

Variables	Group A (Control)	Group B (Crd only)	Group C (Crd+honey)	Group D (Crd+Cw)	Group E (Crd+honey+Cw)
MDA (mol g^{-1} tissue)	32.40±0.43	65.98±2.41^{a}	53.40±3.52^{ab}	52.90±7.55^{a}	52.43±1.52^{ab}
GSH (µmol g^{-1} tissue)	7.52±0.56	1.63±0.18^{a}	5.61±0.44^{abc}	2.37±0.20^{abc}	4.06±0.29^{ab}

Crd: Crude oil, Cw: Coconut water. Values are expressed as Mean±SEM of MDA and GSH activities in Wistar rats using student t-test.'a' represents statistical significance when compared with Group A (Control) at $p<0.05$; 'b' represent statistical significance when compared with Group B (Crude oil only) at $p<0.05$, while 'c' represents statistical significance when compared with Group E (Crude oil+Honey+Coconut water) at $p<0.05$

The results of the Table 3 revealed a significant $p<0.05$ increase in MDA and significant decrease in GSH concentration in Group B administered with crude oil when compared with Control Group A. Treatment with honey and coconut water separately decrease the MDA concentration and increase the GSH concentration significantly by $p<0.05$ in Groups C and D when compared with Group B. However, administration of both honey and coconut water further decreased the MDA concentration and increased the GSH concentration in Group E when compared with Group B.

Histological analysis: The liver section of rats in control group (Group A) showed normal architecture of the venule, sinusoids and hepatocytes as shown in Plate 1a and b, while the liver section of Group B rats treated with crude oil only showed normal portal tract and the sinusoids were not infiltrated by inflammatory cells, but the hepatocytes showed severe vesicular nuclei with coarse chromatin granules, moderate cytoplasmic fats infiltration and vacuolation as well as few necrotized liver sells as shown by Plate 2a and b. While rats in Group C (Treated with crude oil and honey) showed

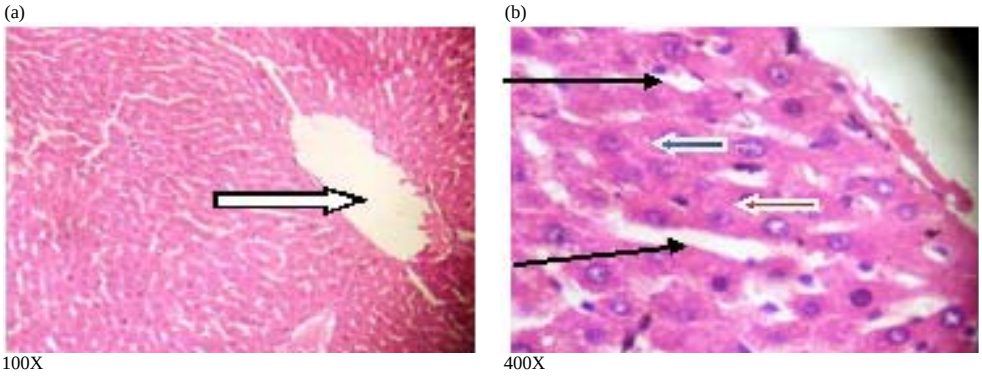

Plate 3(a-b): Photomicrography of Group C (Treated with crude oil and honey) H and E stain at (a) 100X and (b) 400X
White arrow: Pointing to central venules and portal tracts, Slender arrow: Pointing to the sinusoids, Blue arrow: Pointing to liver cells and Red arrow: Pointing to the hepatocytes nuclei

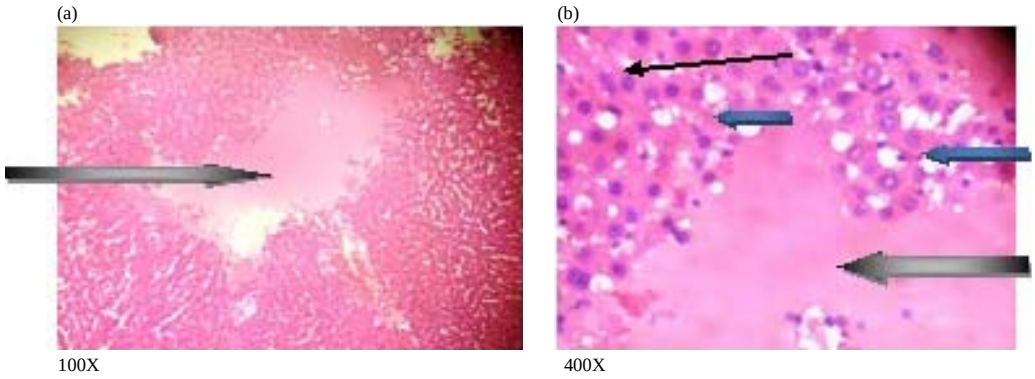

Plate 4(a-b): Photomicrography of Group D (Treated with crude oil and coconut water) H and E stain at (a) 100X and (b) 400X
Black arrow: Pointing to liver parenchyma, Slender arrow: Pointing to the sinusoids, Blue arrow: Pointing to the hepatocyte cytoplasm

moderately normal architecture: The central venules and portal tract were normal and not congested, the sinusoids were normal and not infiltrated some hepatocytes showed few vesicular and hyperchromic nuclei and several liver cells with normal morphology were shown in Plate 3a and b.

The liver section of Group D (rats treated with crude oil and coconut water) Plate 4a and b showed poor architecture: With moderate fluid accumulation seen within the liver parenchyma, the sinusoids were not infiltrated, mild fat infiltration seen within the hepatocytes cytoplasm with mild non-alcoholic steatosis. The liver section of rats in Group E (treated with crude oil, honey and coconut water) Plate 5a and b showed moderately normal architecture: The central venules and portal tract were normal and not congested, the sinusoids were normal and not infiltrated, the hepatocytes showed normal morphology and very few cells with hyperchromic nuclei.

DISCUSSION

Crude oil produces many hazardous compounds which are immunotoxic and carcinogenic to living organisms and also induced variable degenerative changes in the structural integrity of hepatic cells and its enzymes. Previous work in which tablets containing antioxidants such as vitamins, micro-elements and flavonoids were administered improved the oxidative stress associated with petrochemical station workers[42]. Therefore, interventions through supplementation with a combination of honey and coconut water could be a cost-effective way to ameliorate the adverse effects of crude oil exposure and also give more information on one of the mechanisms responsible for ill health from crude oil exposure.

The liver function enzymes; serum alanine aminotransferase (ALT) and serum aspartate aminotransferase (AST) activities assessed the damages done to the liver. It has

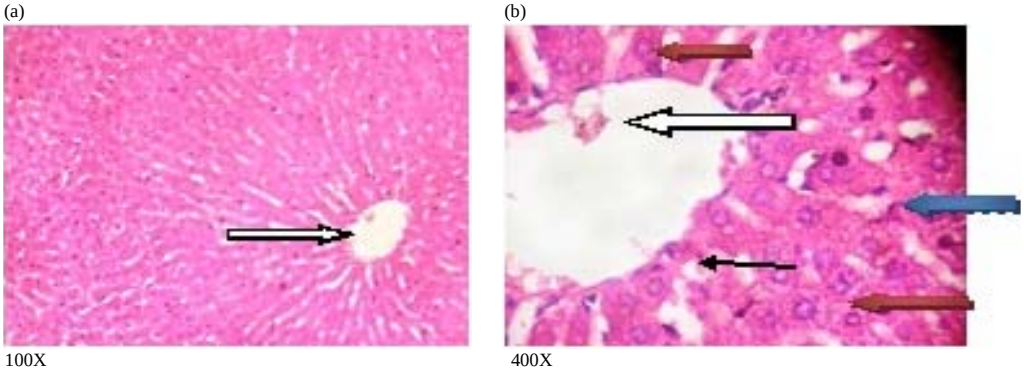

Plate 5(a-b): Photomicrography of Group E (Treated with crude oil, honey and coconut water) H and E stain at (a)100X and (b) 400X

White arrow: Pointing to central venules and portal tract, Slender arrow: Pointing to the sinusoids, Blue arrow: Pointing to the hepatocytes

been established that increase in the activities of these enzymes reflects major permeability or rupture of hepatic cells[43]. The statistically higher activities of these enzymes in Group B (Crude oil only) showed that exposure to crude oil damaged the liver, while the treatment with both honey and coconut water reduced the activities of the liver markers than when treated with honey only and coconut water separately. The increase in liver enzymes activities may be as a result of leakages due to liver damage from the generation of free radicals by hydrocarbons present in the crude oil[44], while antioxidant content of honey and coconut water ameliorated the effect of the free radicals, the result obtained is in agreement with previous work of Achuba and Nwokogba[44] in which administration of honey ameliorates the effect of gasoline and kerosene on the kidney and liver.

The exposure to crude oil reduced serum total protein; this can also be as a result of the activities of hydrocarbons and their metabolic products which may enhance the production of reactive oxygen species which lead to cellular damage through protein oxidation[45]. Serum protein concentration increased following treatment with both honey and coconut water, then treatment with honey only increased it further, while coconut water treatment only significantly increased the total protein content of serum of exposed rats. This result may be due to the antioxidant properties of coconut water which protect against oxidative damage as reported on human serum albumin by Zhang *et al.*[46].

Oxidative stress is important in the pathogenesis of different diseases of the liver such as hepatitis, non-alcoholic fatty liver, fibrosis, cirrhosis and hepatocellular carcinoma, Therefore; assessment of endogenous and exogenous antioxidant and enzymes that control free radicals can be important in development and progression of liver diseases and antioxidant therapies may be considered a supportive therapy for the conventional treatment[47]. Lipid peroxidation is the oxidative breakdown of polyunsaturated fatty acids. It is widely accepted as a general mechanism for cellular injury and death. It has been implicated in toxicity associated with drugs[48]. Malondialdehyde (MDA) increased in liver tissue homogenate after exposure to crude oil which confirms the induction of oxidative stress by crude oil exposure, but treatment with a combination of honey and coconut water reduced MDA level significantly in this study. While reduced glutathione decreased following exposure to crude oil but treatment with both honey and coconut water significantly increased the concentration of GSH in liver tissue homogenate. The GSH is a major non-protein in living tissue that plays a crucial role in the coordination of body's antioxidant defence process, a decline in GSH content of the liver can lead to its damage by H_2O_2 and OH, because Glutathione peroxidase catalyses the destruction of H_2O_2 and lipid hydroperoxides by reacting with reduced glutathione (GSH) to form glutathione disulphide and then reduce the production of hydro-peroxide[47]. The implication of this study is that lipid peroxidation and oxidative stress elicited by crude oil is nullified due to the effect of antioxidants in honey and coconut water. This observation is in agreement with hepatoprotective and antioxidant effects of indigenous plants on petroleum product intoxicated rats reported by Ujowundu *et al*[49].

Superoxide dismutase (SOD) is an antioxidant enzyme that catalyses the consumption of superoxide anion (0^{2-}) which peroxidises cell membrane. It is widely distributed in the mammalian tissues[50]. The activity of SOD in the cell is, therefore, a predictor of oxidative status. The lower the SOD activity observed in the crude oil only administered rats in this

study leads to the excess availability of superoxide (O_2^-) and hydrogen peroxide in a biological system which consequently leads to the generation of hydroxyl radicals resulting in the initiation and propagation of lipid peroxidation[51]. While the activities of SOD in rats treated with combination of honey and coconut water increased even more than the groups that were treated with honey and coconut water separately suggesting that co-administration of both has free radical scavenging activity which could synergistically exert a beneficial effect against pathological alterations caused by the presence of superoxide hydroxyl radicals (O_2^- and OH^-). The same trend of the result was obtained for catalase. These observations are consistent with the findings of previous studies[50,52,45].

The histological study confirmed all the results obtained above, the histological section of Group B (crude oil induced rats) reflected enlarged central vein periportal fatty infiltrated with focal necrosis of hepatocytes and enlarged sinusoids with fatty infiltration. However, actions of antioxidant and vitamins present in both honey and coconut water administered to the animals reversed the crude oil toxicity observed.

CONCLUSION

This present investigation showed that honey and coconut water when administered together synergistically reversed hepatic damage induced by crude oil in rats through the antioxidant, vitamins and minerals contents of this combination. It is therefore, recommended that petrochemical station workers and people exposed to crude oil pollution can take honey and coconut water together to prevent hepatotoxicity associated with crude oil exposure at a dosage of 5 g kg^{-1} b.wt. and 10 mL kg^{-1} b.wt., respectively daily.

SIGNIFICANCE STATEMENT

This study discover that the co-administration of honey and coconut water at a dosage of 5 g kg^{-1} b.wt. and 10 mL kg^{-1} b.wt., respectively daily could be beneficial for people exposed to crude oil toxicity, This study will help other researchers to uncover the critical areas of prevention of crude oil toxicity through the use of more than one natural products that many researchers were not able to explore. Thus a new theory on synergistic property of co-administration of honey and coconut water may be arrived at for the development of new agents against crude oil toxicity.

REFERENCES

1. Egborge, A.B.M., 1991. Industrialization and heavy metal pollution in Warri river. 32nd Inaugural Lecture, University of Benin, Benin City, Nigeria, pp: 486.
2. Khan, A.A., R.W. Coppock and M.M. Schuler, 2001. Effects of multiple exposures of small doses of Pembina Cardium crude oil and diesel in rats. Arch. Environ. Contam. Toxicol., 40: 418-424.
3. Jessup, D.A. and F.A. Leighton, 1996. Oil Pollution and Petroleum Toxicity to Wildlife. In: Noninfectious Diseases of Wildlife, Fairbrother, A., L.N. Locke (Eds.). Iowa State University Press, Ames Iowa, pp: 141-156.
4. Nwilo, P.C. and O.T. Badejo, 2008. Impacts of oil spills along the Nigerian coast. Niger Delta Today. https://nigerdeltatoday.wordpress.com/2008/10/24/mpacts-of-oil-spills-along-the-nigerian-coast/
5. Freedman, B., 1989. Environmental Ecology: The Impacts of Pollution and other Stresses on Ecosystem Structure and Function. 2nd Edn., Academic Press, San Diego, California, pp: 159-188.
6. Dede, E.B., N.M. Igboh, O.A. Ayalogu, P.C. Ofojekwu, I.S. Ezenwaka and W.O. Alegbeleye, 2002. Chronic toxicity study of the effect of crude petroleum (Bonny light), kerosine and gasoline on rats using haematological parameters. J. Applied Sci. Environ. Manage., 6: 60-63.
7. Otaigbe, B.E. and A.F. Adesina, 2005. Crude oil poison in a two-year old Nigerian-A case report. Ann. Agrawal's Int. J. Forensic Med. Toxicol., 6: 2-11.
8. Aslani, M.R., A.R. Movassaghi, M. Mohri and M. Vojdani, 2000. Experimental poisoning in goats. Vet. Hum. Toxicol., 42: 354-355.
9. Onuoha, G.C. and F.O. Nwadukwe, 1990. Influence of liquid Petroleum Refinery Effluent on the Hatching success of *C. garippinas* (Africa Mud fish) eggs. Environ. Ecol., 4: 1201-1206.
10. Onwurah, I.N.E. and M.O. Eze, 2000. Superoxide dismutase activity in *Azotobacter vinelandii* in the disposition of environmental toxicants exemplified by Fenton reagent and crude oil. Toxic Substance Mech., 19: 111-123.
11. Gardner, G.R., P.P. Yevich, J.C. Harshbarger and A.R. Malcolm, 1991. Carcinogenicity of Black Rock Harbor sediment to the Eastern Oyster and trophic transfer of Black Rock Harbor carcinogens from the blue mussel to the winter flounder. Environ. Health Perspect., 90: 53-66.
12. Abubakar, M.B., W.Z. Abdullah, S.A. Sulaiman and A.B. Suen, 2012. A review of molecular mechanisms of the anti-leukemic effects of phenolic compounds in honey. Int. J. Mol. Sci., 13: 15054-15073.

13. Othman, N.H., 2012. Honey and cancer: Sustainable inverse relationship particularly for developing nations-a review. Evidence-Based Complement. Alter. Med., Vol. 2012. 10.1155/2012/410406.
14. Tan, H.T., R.A. Rahman, S.H. Gan, A.S. Halim, S.A. Hassan, S.A. Sulaiman and B.S. Kirnpal-Kaur, 2009. The antibacterial properties of Malaysian tualang honey against wound and enteric microorganisms in comparison to manuka honey. BMC Complement. Altern. Med., Vol. 9. 10.1186/1472-6882-9-34.
15. Erejuwa, O.O., S.A. Sulaiman, M.S. Ab Wahab, K.N. Sirajudeen, S. Salleh and S. Gurtu, 2012. Honey supplementation in spontaneously hypertensive rats elicits antihypertensive effect via amelioration of renal oxidative stress. Oxidative Med. Cell. Longev., Vol. 2012. 10.1155/2012/374037.
16. Khalil, M.I., M. Mahaneem, S.M.S. Jamalullail, N. Alam and S.A. Sulaiman, 2011. Evaluation of radical scavenging activity and colour intensity of nine Malaysian honeys of different origin. J. ApiProd. ApiMed. Sci., 3: 4-11.
17. Moniruzzaman, M., S.A. Sulaiman, M.I. Khalil and S.H. Gan, 2013. Evaluation of physicochemical and antioxidant properties of sourwood and other Malaysian honeys: A comparison with Manuka honey. Chem. Central J., Vol. 7. 10.1186/1752-153X-7-138.
18. Ahmad, A., R.A. Khan and M.A. Mesaik, 2009. Anti inflammatory effect of natural honey on bovine thrombin-induced oxidative burst in phagocytes. Phytother. Res., 23: 801-808.
19. Tonks, A.J., R.A. Cooper, K.P. Jones, S. Blair, J. Parton and A. Tonks, 2003. Honey stimulates inflammatory cytokine production from monocytes. Cytokine, 21: 242-247.
20. Mandal, M.D. and S. Mandal, 2011. Honey: Its medicinal property and antibacterial activity. Asian Pac. J. Trop. Biomed., 1: 154-160.
21. Fukuda, M., K. Kobayashi, Y. Hirono, M. Miyagawa and T. Ishida *et al*., 2011. Jungle honey enhances immune function and antitumor activity. Evidence-Based Complement. Alter. Med., Vol. 2011. 10.1093/ecam/nen086.
22. Forbes-Hernandez, T.Y., F. Giampieri, M. Gasparrini, L. Mazzoni, J.L. Quiles, J.M. Alvarez-Suarez and M. Battino, 2014. The effects of bioactive compounds from plant foods on mitochondrial function: A focus on apoptotic mechanisms. Food Chem. Toxicol., 68: 154-182.
23. Anurag, P. and T. Rajamohan, 2003. Beneficial effects of tender coconut water against isoproterenol induced toxicity on heart mitochondrial activities in rats. Indian J. Biochem. Biophys., 40: 278-280.
24. Loki, A.L. and T. Rajamohan, 2003. Hepatoprotective and antioxidant effect of tender coconut water on carbon tetrachloride induced liver injury in rats. Indian J. Biochem. Biophys., 40: 354-357.
25. Sandhya, V.G. and T. Rajamohan, 2006. Beneficial effects of coconut water feeding on lipid metabolism in cholesterol-fed rats. J. Med. Food, 9: 400-407.
26. Sandhya, V.G. and T. Rajamohan, 2008. Comparative evaluation of the hypolipidemic effects of coconut water and lovastatin in rats fed fat-cholesterol enriched diet. Food Chem. Toxicol., 46: 3586-3592.
27. Bhagya, D., L. Prema and T. Rajamohan, 2012. Therapeutic effects of tender coconut water on oxidative stress in fructose fed insulin resistant hypertensive rats. Asian Pac. J. Trop. Med., 5: 270-276.
28. Prathapan, A. and T. Rajamohan, 2011. Antioxidant and antithrombotic activity of tender coconut water in experimental myocardial infarction. J. Food Biochem., 35: 1501-1507.
29. Sofowora, A., 1993. Medicinal Plants and Traditional Medicine in Africa. Spectrum Books Ltd., Ibadan, pp: 191-289.
30. Yong, J.W.H., L. Ge, Y.F. Ng and S.N. Tan, 2009. The chemical composition and biological properties of coconut (*Cocos nucifera* L.) water. Molecules, 14: 5144-5164.
31. Da Fonseca, A.M., A.M.C. Bizerra, J.S.N. de Souza, F.J.Q. Monte and M.D.C.F. de Oliveira *et al*., 2009. Constituents and antioxidant activity of two varieties of coconut water (*Cocos nucifera* L.). Rev. Bras. Farmacogn., 19: 193-198.
32. APS., 2002. Guiding principles for research involving animals and human beings. Am. J. Physiol.-Regul. Integr. Comp. Physiol., R283: R281-R283.
33. Ochei, J. and A. Kolhatkar, 2000. Medical Laboratory Science: Theory and Practice. 2nd Edn., Tata McGraw-Hill Publishing Co. Ltd., New Delhi, pp: 331-349.
34. Kind, P.R. and E.J. King, 1954. Estimation of plasma phosphatase by determination of hydrolysed phenol with amino-antipyrine. J. Clin. Pathol., 7: 322-326.
35. Tietz, N.W., E.L. Prude and O. Sirgard-Anderson, 1994. Tietz Textbook of Clinical Chemistry. 2nd Edn., W.B. Saunders Company, London, ISBN: 10-0721656102, pp: 1354-1374.
36. Misra, H.P. and I. Fridovich, 1972. The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. J. Biol. Chem., 247: 3170-3175.
37. Varshney, R. and R.K. Kale, 1990. Effects of calmodulin antagonists on radiation-induced lipid peroxidation in microsomes. Int. J. Radiat. Biol., 58: 733-743.
38. Ellman, G.L., 1959. Tissue sulfhydryl groups. Arch. Biochem. Biophys., 82: 70-77.
39. Hissin, P.J. and R. Hilf, 1976. A fluorometric method for determination of oxidized and reduced glutathione in tissues. Anal. Biochem., 74: 214-226.
40. Aebi, H.E., 1983. Catalase. In: Methods of Enzymatic Analysis, Bergmeyer, H.U., J. Bergmeyer and M. Grassl (Ed.). 3rd Edn., Verlag Chemie, Weinhem, pp: 273-286.

41. Carleton, H., 1967. Carleton Histology Technology. 4th Edn., Oxford University Press, USA., pp: 33.
42. Georgieva, T., A. Michailova, T. Panev and T. Popov, 2002. Possibilities to control the health risk of petrochemical workers. Int. Arch. Occup. Environ. Health, 75: 21-26.
43. Kaplan, M.M., 1979. Understanding Serum Enzymes Tests in Clinical Liver Diseases. In: Problems in Liver Diseases. Davidson, C.S. (Ed.). Stratton Intercontinental Medical Book, New York, pp: 79-85.
44. Achuba, F.I. and C.C. Nwokogba, 2015. Effects of honey supplementation on hydrocarbon-induced kidney and liver damage in wistar albino rats. Biokemistri, 27: 50-55.
45. Altenburger, R., M. Nendza and G. Schuurmann, 2003. Mixture toxicity and its modeling by quantitative structure activity relationships. Environ. Toxicol. Chem., 22: 1900-1915.
46. Zhang, Y., Y. Zheng, K. Duan and Q. Gui, 2016. Preparation, antioxidant activity and protective effect of coconut testa oil extraction on oxidative damage to human serum albumin. Int. J. Food Sci. Technol., 51: 946-953.
47. Arauz, J., E. Ramos-Tovar and P. Muriel, 2016. Redox state and methods to evaluate oxidative stress in liver damage: From bench to bedside. Ann. Hepatol., 15: 160-173.
48. Marklund, S.L., 1992. Regulation by cytokines of extracellular superoxide dismutase and other superoxide dismutase isoenzymes in fibroblasts. J. Biol. Chem., 267: 6696-6701.
49. Ujowundu, C.O., F.N. Kalu, C.U. Igwe, N.C. Agha and K.O. Igwe, 2011. Biochemical studies on the amelioration of petroleum product intoxication with indigenous plants. Int. J. Biochem. Res. Rev., 2: 87-97.
50. Aebi, H., 1984. Catalase *in vitro*. Methods Enzymol., 105: 121-126.
51. Kumthekar, M.M. and S.S. Katyare, 1992. Altered kinetic attributes of Na^++K^+-ATPase activity in kidney, brain and erythrocyte membranes in alloxan-diabetic rats. Indian J. Exp. Biol., 30: 26-32.
52. Achuba, F.I. and S.A. Osakwe, 2003. Petroleum-induced free radical toxicity in African catfish (*Clarias gariepinus*). Fish Physiol. Biochem., 29: 97-103.