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Immuno-modulation and anti-oxidative roles of olive oil and bee honey following administration of crude oil to Wistar rats

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ABSTRACT

The immuno-modulation and anti-oxidative roles of olive oil and bee honey following administration of crude oil to Wistar rats was investigated. Forty-eight male Wistar rats (145-342g body weight) were divided into six groups of unequal numbers. The rats in group I served as the control and were orally gavaged 10ml/kg body weight of distilled water; group II rats were administered 2ml/kg body weight of olive oil; group III rats were administered 14ml/kg body weight of Bee honey; group IV was orally gavaged 3ml/kg body weight of crude oil; group V were administered the dose as mentioned above of olive oil and 3ml/kg body weight of crude oil; group VI was gavaged the dose as mentioned earlier of Bee honey and 3ml/kg body weight of crude oil for 21days. The results revealed that crude oil markedly induced oxidative stress by significantly ($p<0.05$) reducing activities of superoxide dismutase (SOD) and catalase activity (CAT) while significantly ($p<0.05$) increasing malonydialdehyde (MDA) level when compared to control, olive oil and bee honey groups. Co-administration of olive oil and bee honey with crude oil to groups V and VI respectively significantly elevated SOD and CAT activities while significantly ($p<0.05$) reducing MDA levels when compared with control, olive oil and bee honey groups. This study suggest that crude oil significantly ($p<0.05$) increased immunoglobulin status (IgA, IgG and IgM) compared with control, olive oil and bee honey groups. These increases were significantly ($p<0.05$) reduced following co-administration of olive oil and bee honey with crude oil to groups V and VI respectively. According to the findings from the present study, the antioxidant properties of olive oil and bee honey can prevent oxidative stress and immunoglobulin status disruption that occur after administration of crude oil.

Keywords: Immuno-modulation, immunoglobulins, oxidative stress, lipid peroxidation, crude petroleum.

1. INTRODUCTION

Crude petroleum is a complex hydrocarbon which is composed majorly of carbon and hydrogen with little amount of oxygen, nitrogen and sulfur. Crude petroleum is known to contain several amounts of carbon atoms ranging from five to twenty carbon atoms with, some of these carbon atoms being straight chains while others are branched (Zhang et al., 2014). Dangers associated with frequent exposure of humans to crude petroleum and various petroleum products have proven to be very significant in causing several distortions in physiological processes (Odo et al., 2012). Humans can be exposed to crude petroleum pollution either during exploration, pipeline leakage, transportation, illegal refining or storage (Ufot et al., 2018). Crude petroleum can be ingested through eating of contaminated food and water as well as dermal exposure at sites of pollution or spillage (Adindu et al., 2023).

The persistent abuse of crude petroleum by humans for its unverified therapeutic property has increased the number of people prone to cell damage and distortion in physiological processes due to its toxicity. The disastrous effects of crude petroleum toxicity have been reported to include; renal failure, pneumonitis, inflammation of the liver, weight reduction, alteration in hematological parameters (Adebola et al., 2018). This toxicity can either be acute or chronic, depending on the method of exposure, dosage and the state of the immune system (Adebola et al., 2018). Exposure to crude petroleum by whatever means can disrupt functions of tissues and vital organs to induce oxidative stress, peroxidation reaction to trigger inflammation causing severe inflammatory disease conditions, which might include distorted immune status.

Free radicals produced by this process can activate harmful metabolites which can react with membrane lipids to trigger lipid peroxidation, this can then activate neutrophils, lymphocytes, and platelets amplifying the process to cause membrane damage (Odo et al., 2012). This is indicated by the massive presence of the pro-inflammatory cytokines (IL-6), tumor necrosis factor alpha (TNF-α) and C-reactive proteins (CRP), which can readily predict the risk of organ damages (Ita et al., 2016). Bee honey is a natural occurring organic substance which is produced from the nectar of flowers and it is the only insect derived natural product which serves nutritional, cosmetic, therapeutic and industrial values due to its significant proportion of antioxidants (Fatimah et al., 2013). Bee honey has been accepted generally as food and medicine over the years despite traditions, civilization and religious beliefs in both ancient and modern era (Zafar et al., 2020).

Studies suggest that Bee honey can exert several therapeutic effects such as antioxidants, anti- inflammatory, antibacterial anti-diabetic, protective activities in the respiratory, gastrointestinal, cardiovascular and nervous systems (Noori et al., 2014). Polyphenols and phenolic acids are found in abundance in honey with variation in concentration according to the geographical and climatic condition where the Bee honey was obtained (Akan and Garip, 2011). The popularity of olive oil has increased tremendously due to its organoleptic characteristics and its associated beneficial health effects (Oliveras-lopez et al., 2008). Olive oil is known to have both nutritional and medicinal components. There has been significant increase in its consumption due to its antioxidant, anti-microbial and anti-inflammatory properties which is important in treatment and prevention of several diseases in humans. Studies have shown that the consumption of olive oil is capable of reducing the risk of malignancy such as breast cancer, ovarian cancer, stomach cancer and colon cancer (Mokhtari et al., 2017).

It is also known to prevent other diseases affecting cardiovascular, respiratory, and blood. Efficient immune system is crucial to maintaining healthy living against invading foreign substances. One arm of the immune system involved the production of antibodies by the B-type of lymphocytes in response to invading foreign substances to the body such as viruses, bacteria, and toxins. In such situation, the antibodies can activate antigen-antibody complexes resulting in antigen elimination and protection of the body of the individual. Therefore, consumption of foods and substances rich in anti-oxidants properties can be useful in mitigation against crude petroleum and its toxic effects on the account of generating oxidant radicals. Bee honey and olive oil are very rich anti-oxidants with several components that are capable of protecting and preventing the pathogenesis of severe inflammation and as such this study focuses on the immune-modulation and anti-oxidative roles of olive oil and bee honey following administration of crude oil to Wistar rats.

2. MATERIALS AND METHODS

Chemicals

Crude oil was obtained from NNPC Port Harcourt, Nigeria through a written application. The bee honey was purchased from Obanliku Local Government Area in Cross Rivers State, Nigeria. The olive oil was purchased from De Choice Supermarket located on Oron Road, Uyo, Akwa Ibom State. Every other chemical used for this study was of analytical grade.

Experimental Animals

Male Wistar rats were obtained from the Animal House of the Faculty of Basic Medical Sciences of University of Uyo, Nigeria and were kept in clean wooden cages with saw dust placed as beddings in a ventilated part of the Animal House. They were fed with vital feeds and water *ad libitum*. The animals were acclimatized for a period of one week.

Experimental Design and Drug Administration on the Animals.

A total number of forty-eight male Wistar rats were divided randomly into six groups. Group I served as the control and was administered 10ml/kg body weight of distilled water orally. Group II was administered 2 ml/kg body weight of olive oil orally. This dose was calculated as 20% of its lethal dose (LD50). Group III was administered 14 ml/kg body weight of bee honey, which was also calculated as 20% of its lethal dose (LD50). Group IV was administered 3 ml/kg body weight of crude oil, being 20% of its lethal dose. Group V was supplemented with 2 ml/kg of olive oil in addition to 3 ml/kg of crude oil. Group VI was supplemented with 14 ml/kg of bee honey in addition to 3 ml/kg of crude oil. In each of the groups, the doses administered were based on the weight of the rats recorded. The volume calculated in milliliter was administered daily for 21 days. The procedure involving the animal and care was conducted with the approval and guidelines by the Research and Ethical Committee of the Faculty of Basic Medical Sciences of University of Uyo.

Collection of Blood Samples for Analysis

At the end of 21 days of administration, the rats were anesthetized by intraperitoneal administration of pentobarbital. Blood was collected by cardiac puncture using a 5 ml sterile syringe and needle. Total volume of blood collected from each rat was divided into two portions. A portion was transferred into plain sample bottles which was allowed to stand for 2 hours to clot and was spun with table top centrifuge at 4000 rpm for 10 minutes. Serum was carefully separated using a Pasteur pipette into clean sample bottles and preserved for biochemical analysis. A second portion was transferred into EDTA bottles for determination of platelet and white blood cell count.

Determination of Immunoglobulin

The immunoglobulin; IgA, IgG and IgM was determined using immunoglobulin assay kit by spectrophotometric method.

Determination of Malondialdehyde (MDA), Superoxide Dimutase (SOD) and Catalase (CAT) Activities

The determination of MDA, SOD and CAT activities were done using the respective assay kit.

Statistical Analysis

The entire statistical analysis was carried out using window SPSS package. Data were analyzed using one way analysis of variance (ANOVA). Results obtained were subjected to test for the least significant difference. Statistical data were expressed as mean ± standard error of the mean. Values of $p<0.05$ was considered significant.

3. RESULTS

Estimation of Immunoglobulin Status

The result of immunoglobulin levels (g/L) obtained after 21 days of administration of distilled water, olive oil, Bee honey, NBLCO, olive oil + NBLCO, Bee honey + NBLCO to groups I, II, III, IV, V and VI respectively are presented in (Table 1). The results indicated that olive oil, Bee honey administration to groups II and III animals respectively did not alter IgA, IgG and IgM significantly when

compared with the control group I. In a similar manner, Bee honey administration to group III animals did not alter IgG and IgM levels significantly when compared with olive oil group II animals, but a significant elevation ($p<0.05$) of IgA level was recorded in group III.

Administration of NBLCO to group IV animals significantly ($p<0.05$) increased IgA, IgG and IgM levels compared to groups I (control), II (olive oil) and III (Bee honey). The co-administration with olive oil to group V and Bee honey to group VI significantly ($p<0.05$) reduced the levels of the aforementioned immunoglobulins compared to group IV animals that were administered only crude oil. Comparison between group V and group VI did not record any significant alteration.

Estimation of SOD Activity

The result of SOD activity indicated significantly ($p<0.05$) higher activity in group II (olive oil) and group III (Bee honey) when compared with group I (control). Administration of NBLCO to group IV animals indicated significant ($p<0.05$) reduction in SOD activity when compared with groups I (control), II (olive oil) and III (Bee honey). The intervention with olive oil and Bee honey in groups V and VI respectively showed significantly ($p<0.05$) higher activity of SOD activity when compared with groups I (control) and IV (NBLCO), (Figure 1).

Estimation of Catalase Activity

The result showed that the activity of CAT was not altered significantly between groups I, II and III. CAT activity in group IV was significantly ($p<0.05$) lower than groups I, II, and III. The activity of CAT in group V was not significantly different from groups I, II, and III but significantly ($p<0.05$) higher than group IV. The CAT activity in group VI was significantly higher than groups I, II, III, IV and V, (Figure 2).

Estimation of MDA Activity

The result indicated that there was no significant alteration in MDA activity in group II (olive oil) when compared with group I (control). When Bee honey was administered to group III animals, it indicated significant ($p<0.05$) higher levels when compared with groups I (control) and II (olive oil). Administration of NBLCO to group IV animals indicated significant ($p<0.05$) higher levels of MDA activity when compared with groups I (control), II (olive oil) and III (Bee honey). Interestingly, co-administration of olive oil with NBLCO in group V and Bee honey with NBLCO in group VI respectively recorded significant ($p<0.05$) reduction in MDA activity when compared with groups I (control), II (olive oil), III (Bee honey) and IV (NBLCO); (Figure 3).

Table 1 Results of olive oil and bee honey interventions on the immunoglobulins concentration of rats following Nigerian Bonny light crude oil administration.

Groups	IgA (g/L)	IgG (g/L)	IgM (g/L)
I	0.74± 0.01	9.45± 0.09	0.67± 0.01
II	0.67± 0.02	9.68 ± 0.09	0.60± 0.03
III	0.84± 0.01b	10.93 ± 0.05	0.80± 0.01
IV	0.94± 0.06a, b	15.17± 0.66a, b, c	1.30± 0.11a, b, c
V	0.60± 0.00c, d	9.62± 0.12d	0.48± 0.01c, d
VI	0.59± 0.01c, d	10.62 ± 0.36d	0.54± 0.02d

Legend: a= versus group I, b= versus group II, c= versus group III, d= versus group IV, e= versus group V, all at $p<0.05$.

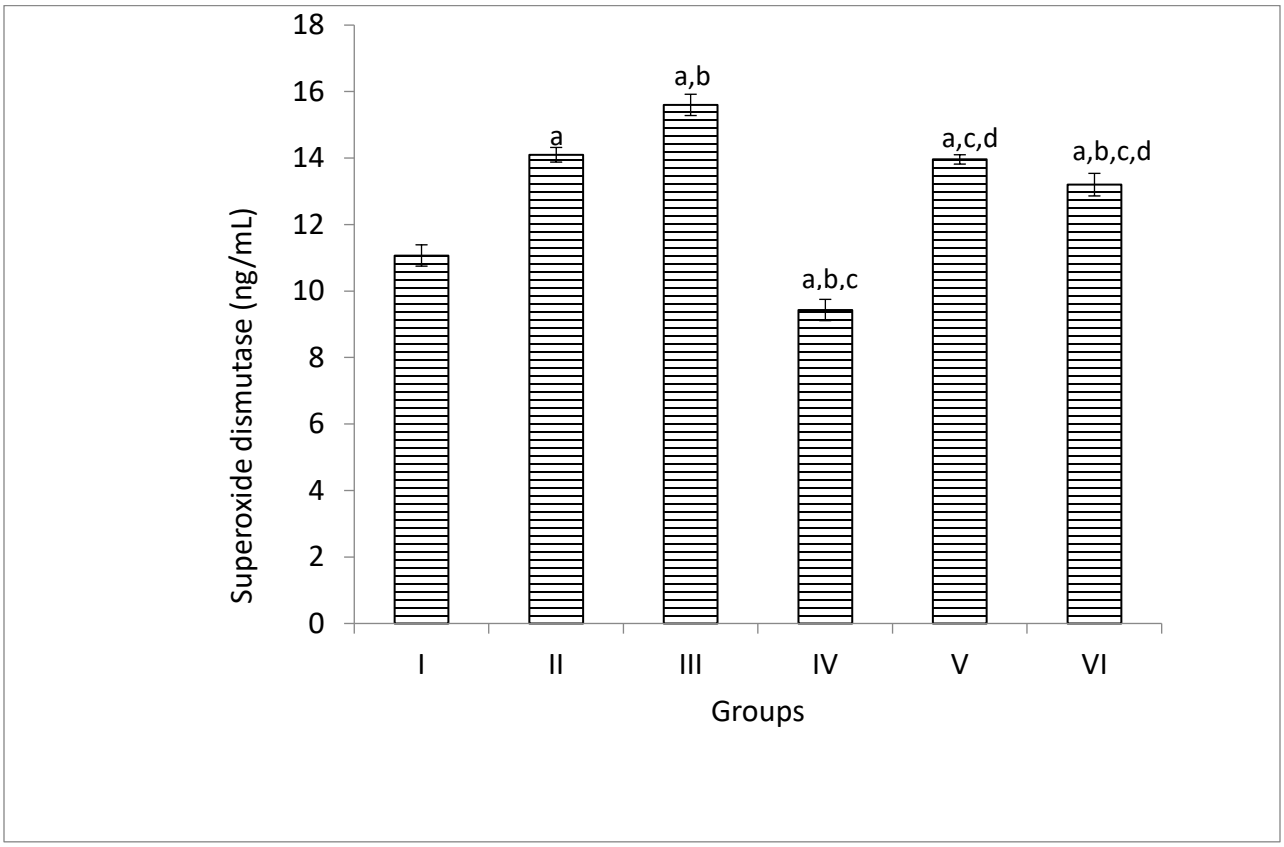

Figure 1 The superoxide dismutase (SOD) activity in the six groups presented as mean ± SEM. a = versus I, b = versus II, c = versus III, d = versus IV at $p<0.05$

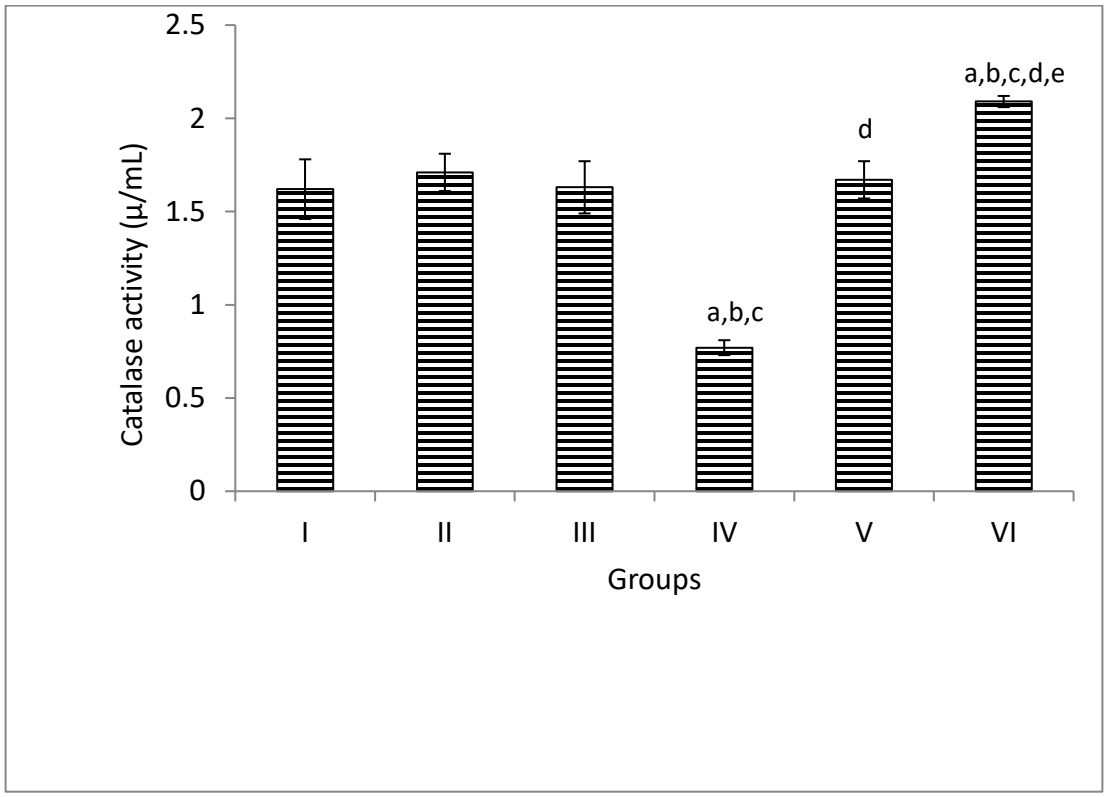

Figure 2 The catalase (CAT) activity in the six groups presented as mean ± SEM. a = versus I, b = versus II, c = versus III, d = versus IV and e = versus V at $p<0.05$

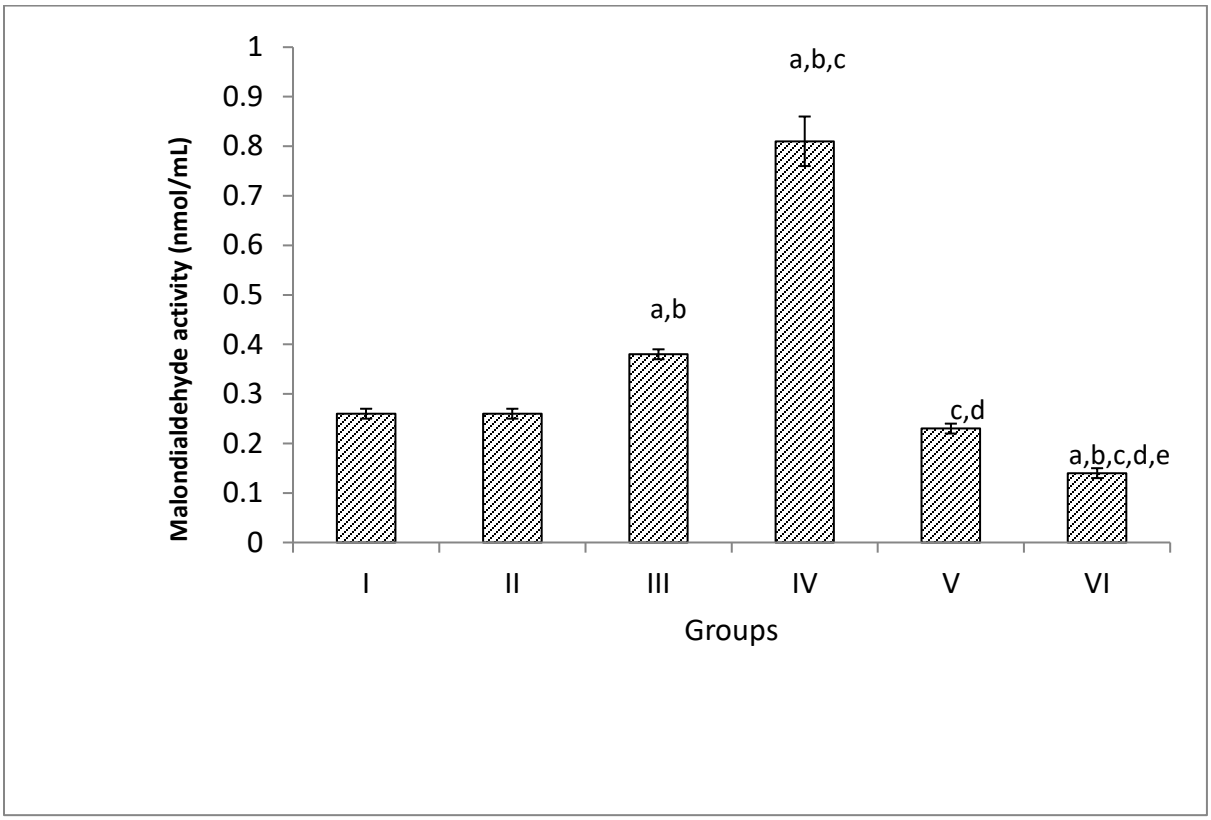

Figure 3 The malondialdehyde (MDA) activity in the six groups presented as mean ± SEM. a = versus I, b = versus II, c = versus III, d = versus IV, e = versus V at $p<0.05$

4. DISCUSSION

The immuno-modulation and anti-oxidative roles of Bee honey, olive oil on Wistar rats administered crude oil was investigated. It was observed that the oral administration of NBLCO to male Wistar rats interfered significantly with immunoglobulin's status as IgA, IgG and IgM were significantly increased. The significant elevation of these antibodies as recorded in the present study is in agreement with similar study in literature where crude oil was reported to cause significant elevation in immune response (McLoone et al., 2021). Normally, these antibodies are involved in the body's defense mechanism when exposed to bacteria, viruses, toxins, allergies, parasites and chemical substances. The NBLCO may have presented this effect because of the toxicants in it; these toxicants may have probably instigated generation of free radicals which are capable of causing lipid peroxidation. These activities in turn initiated oxidative stress and severe inflammatory response by the immune system.

The presence of these free radicals causes hypersensitivity reaction which stimulates the release of cytokines leading to an increased production of IgA, IgG and IgM by B cells to the site of injury (Angel et al., 2023). This alteration can have direct impact on immune cells as continuous/prolong exposure to crude oil which causes an increase in immunoglobulin can further deplete these antibodies hence weakening the body's defense and exposing the body to various infections causing an immune deficiency (Matveeva et al., 2022). In contrast, intervention with olive oil and bee honey significantly reduced IgA, IgG and IgM. Olive oil and bee honey have been explicitly studied in various literatures to exert antioxidant and anti-inflammatory properties due to the presence of polyphenols and flavonoids (Samarghandian et al., 2017). The co-administration of olive oil, bee honey with crude oil drastically altered the oxidative impact caused by reactive oxygen species generated by crude oil due to the presence of antioxidant deposits in olive oil and bee honey.

It was observed that oral administration of NBLCO significantly reduced the activities of SOD and CAT. The co-administration of olive oil and bee honey significantly reversed the value of the aforementioned parameters. The observed results are in agreement with documented studies that continuous ingestion of crude oil reduces SOD activity Adebola et al., (2018) and CAT (Adedara et al., 2012). Physiologically, SOD is a major antioxidant enzyme that is important in defense mechanism of all living cell against reactive oxygen

species, hence perform essential roles in defense against oxidative stress in the body (Fujii et al., 1999; Amadi et al., 2023). Crude oil which contains complex hydrocarbons generates several reactive oxygen species, therefore, when ingested causes depletion of the antioxidant system as continuous ingestion of crude oil causes excessive utilization of SOD hence the reduction observed in this study. Free radicals generated by crude oil when orally ingested initiates severe oxidative stress leading to the depletion of the anti-oxidant system of the body.

But co-administration of olive oil, bee honey with crude oil significantly increased SOD and CAT activities. This agrees with previous studies that olive oil and bee honey have antioxidant properties and helps to protect against oxygen radicals (Adebola et al., 2018; Adeyemi and Adeyemi, 2021). The observed lower activities of the antioxidant enzymes following NBLCO administration is suggestive of its overwhelming oxidative activity, which may probably arise from the suppression of the enzymatic antioxidant mechanisms. The co-administration of olive oil and bee honey shows their ability to reduce free radicals, hence positively improve the immunity in the Wistar rats. Studies have proposed that crude oil is capable of causing severe damages to cells and tissues by inducing oxidative stress and lipid peroxidation, as prolonged ingestion of crude oil has been reported to cause oxidative stress through generation of free radicals (Achuba and Osakwe, 2003).

The marked elevation in CAT activity perhaps may be due to the antioxidant component of olive oil and bee honey which exacts a boost upon the depleting antioxidant system caused by free radicals generated by crude oil. This agrees with previous studies that olive oil and bee honey are active antioxidant compounds (Odo et al., 2012). The findings of this study where administration of NBLCO significantly elevated MDA further agrees with earlier report by Ayman et al., (2019), where a similar elevated MDA level was reported. These workers postulated that the elevated MDA level was probably due to free radicals produced from lipid peroxidation and oxidative stress induced by crude oil; particularly as MDA is one of the final products of unsaturated fatty acids peroxidation in cells (Gawel et al., 2004). The ability of crude oil to increase MDA levels is suggestive of an overloaded antioxidant system which is caused by oxidative activities.

There are reports in literature of cases where consumption of feed contaminated with crude oil induced lipid peroxidation in rats which was accompanied by increased generation of reactive oxygen species that may inactivate the antioxidant system (Anozie and Onwurah et al., 2001). The human body is well furnished with innate antioxidant system that protects against oxidative injuries and repairs damaged tissues (Valko et al., 2007). Continuous exposure of the body to polycyclic hydrocarbons produced by crude oil can instigate oxidative stress such that natural antioxidants known for their overwhelming antioxidant reserve are depleted (Kabaran, 2018; Sarfraz et al., 2018). As seen in this study, olive oil and Bee honey were able to reverse the toxic effects of crude oil on oxidative stress due to their overwhelming antioxidant reservoir.

5. CONCLUSION

The results of this study indicate that ingestion of crude oil significantly increased antibodies (IgA, IgG and IgM), induced oxidative stress (decreased SOD and CAT) as well as increased MDA, which olive oil and bee honey intervention ameliorated. Intervention with olive oil and Bee honey significantly reduced antibodies concentrations and increased antioxidant enzymes. This may probably be due to the antioxidant properties of olive oil and Bee honey, thus neutralizing the impact of crude oil on these markers. In this present study olive oil and bee honey have been established to possess antioxidant properties which are capable of salvaging the oxidative stress and perturbation of the immunoglobulin status following administration of crude oil.

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Authors' Contributions

Ita SO: Study conception and planned detail of laboratory protocol; Nwikoleh NL: Actual laboratory work and development of manuscript for publication; Abia ER: Design of experimental protocol and analysis of raw statistical data.

Consent for publication

Not applicable

Informed consent

Not applicable

Ethical approval

The study was approved by the Medical Ethics Committee of The Faculty of Basic Medical Sciences Ethical Committee of University of Uyo. (Ethical approval code UU_FBMSREC_2023_004). The Animal ethical guidelines are followed in the study for experimentation.

Conflicts of interests

The authors declare that there are no conflicts of interests.

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Data and materials availability

All data associated with this study are present in the paper.

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