Research Article

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Ameliorative Synergistic Effect of Honey and *Moringa Oleifera* on lead-induced alteration of Biochemical and Haematological Indices in *Clarias gariepinus*

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ABSTRACT: Lead confers deleterious damage to the cells and it is necessary to explore further and develop a more effective way to ameliorate lead toxicity. This study aims to investigate how honey and *Moringa oleifera* (MO) can synergistically provide a more effective way to ameliorate lead toxicity. Groups of ten fish (*Clarias gariepinus*) were given Pb (0.30 g) and supplemented feed (T1) containing both honey (5 g) and MO (5 g). Others received pb (0.30 g) and supplemented feed (T2) containing either honey (10 g) or (T3) containing MO (10 g). Finally, hematological and biochemical analysis were conducted and a decline in the hematological parameters was observed. Also, the level of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), urea, creatinine, and malondialdehyde (MDA) significantly increased while glutathione (GSH) and superoxide dismutase (SOD) reduced in the group given lead only. However, these biochemical indices and hematological parameters are greatly restored in the group that received both honey and MO compared to the group that received either honey or MO. The findings of this study reveal that co-administration of both honey and MO synergistically damages caused by lead-induced toxicity better.

Keywords: synergistic, hematological, honey, *Moringa oleifera*, *Clarias gariepinus*

INTRODUCTION

A major global problem is heavy metal contamination of the aquatic environment because metals are indestructible, and most have harmful effects on species (Adekola and Eletta, 2007). In addition to the runoff of treated and untreated liquid waste into water bodies. There are many ways that heavy metals enter rivers and lakes, including through soils and rocks that are exposed to surface water. Such hazardous waste and other toxic manufacturing by-products are significant sources of pollutants for the environment (Okareh and Adeolu, 2015). Metal accumulation may lead to a high rate of mortality or cause several hematological and biochemical changes (Mahmoud, Ebied, and Mohamed, 2013). Fish can concentrate and transfer significant quantities of certain metals from the water in the web chain into the water (Abdel-Mohsien and Mahmoud, 2015).

The biological effects of lead, a harmful metal with the most extensive research, depend on the time and intensity of exposure. Since lead is frequently present in soil, water, and food, it is one of the most dangerous metals (Fihri *et al.*, 2016). Lead, commonly spread in nature, is regarded as one of the main environmental toxins. The consequence of toxicity caused by lead results in serious health ailments. Neural, neurological, hepatic, and renal damage are the most important (Bandyopadhyay e*t al.*, 2014).

Honey administered to rats exposed to lead poisoning ameliorated lead-induced anemia and prevented damage to hepato-renal lead (Fihri et al., 2016). Results indicated that daily use of honey may assist in alleviating some of the negative effects of Pb (Tandon and Singh, 1994). Furthermore, honey also protects the heart through improving lipid metabolism, antioxidant activity, regulation of blood pressure, restoration of heartbeat, reduction of myocardial infarction region, antiaging properties, and attenuation of cell apoptosis (Idrus et al., 2020). The hepatoprotective effect of (MO) leaves on lead-induced liver damage in adult Wistar rats was revealed by several researchers (Jo et al., 2012; Sharayu and Asmita, 2017). Several publications have shown that MO is capable of hepatoprotection. A study conducted on moringa (Fakurazi et al., 2008) showed that MO improves the efficacy of enzyme antioxidation and

counteracts hepatotoxicity caused by paracetamol. Previous research on Moringa's effect on the liver has shown that the plant is safe for use and has an ameliorative and therapeutic effect.

Accordingly, there is a need to develop a much more potent and effective treatment that will ameliorate lead toxicity and reverse the cellular damage caused by lead exposure. This present study attempt to explore the ameliorative synergistic effect of honey and MO against lead toxicity. Hence, biochemical analysis and haematological analysis were methods employed in this study to examine the biochemical indices.

The specific objectives were established for the present study on the basis of the above-discussed issues to analyze this synergistic effect of honey and MO on the biochemical indices and haematological parameters in fish (*Clarias gariepinus*) after exposure to lead.

MATERIALS AND METHODS

This study initially conducted a systematic literature review to explore the ameliorative effect of honey and moringa against lead toxicity. *Clarias gariepinus* (100-200 g in body weight and 12-15 cm in total length) was chosen for this study and all the necessary precautions for maintaining the fish were followed. The healthy fishes were sorted into five groups and were exposed to lead acetate for 28 days. The fish were fed the formulated diet containing various levels of *M. oliefera* leaves and honey at various concentrations as experimentally designed. The blood samples were then prepared for hematological and biochemical analysis. Later, a statistical analysis was carried out with the data.

Experimental design

The healthy fish were sorted into five groups and each group consisted of 10 in each duplicate groups, this was done to increase the statistical power and reliability of the result. Table 1 shows the experiment conducted for different groups. The fish were exposed to lead acetate for 28 days. They were fed the formulated diet containing various levels of M. oliefera leaves and honey at various concentrations. All fish were weighed at the start and at the end of the experiment. Fish were fed at 4% of their body mass, and each tank's diets were offered daily. Contaminant was directly exposed to fish using $Pb(NO_3)_2$ diluted as a freshly prepared mixture with 200ml of water. Table 2 shows the composition of the various groups considered in this study.

Animal management and administration of Honey and MO

Clarias gariepinus (100-200 g in body weight and 12-15 cm in total length) was chosen for its high economic value.

Table 1. Various groups and composition

Groups	Number of fishes	Labeling
Control	10	Control
Group A	10	Lead acetate only
Group B	10	Lead + honey
Group C	10	Lead + moringa leaf
Group D	10	Lead, honey + moringa leaf

Table 2. Administration for different groups

Control	10 g of supplemented feeds only
Group A	0.30 g of lead acetate and fed with 10g of supplemented feeds
Group B	0.30 g of lead acetate and fed with 10g of supplemented feeds containing 10.0g of honey.
Group C	0.30 g of lead acetate and fed with 10g of supplemented feeds containing 10.0g of moringa leaf extract.
Group D	0.30 g of lead acetate and fed with 10g of supplemented feeds containing honey and moringa leaf extract (5.0g each).

Note: The daily doses were done and preceded for 28 days (4 weeks)

It is one of the most cultivated and consumed fish in Africa and Nigeria (Adewunmi and Olaleye, 2001). Juvenile *C. gariepinus* was adapted under laboratory conditions for 30 days and supplied with de-chlorinated tap water prior to experimentation. All the necessary precautions for maintaining the fish followed the Nigeria

Table 3. Gross Composition of the experimental diet

Ingredient	T1	T2	T3	T4
Maize	2.5	19.0	19.0	18.0
Fish Meal	36.0	34.0	35.0	36.0
Soybeans Meal	22.0	20.0	20.0	21.0
Moringa Leaf Meal	0.0	10.0	5.0	0.0
Honey	0.0	0.0	5.0	10.0
Rice Bran	12.0	12.0	11.0	10.0
Bone Meal	1.0	1.0	1.0	1.0
Limestone	0.5	0.5	0.5	0.5
Premix	1.5	1.5	1.5	1.5
Salt	0.5	0.5	0.5	0.5
Oil	1.5	1.5	1.5	1.5

Water Works Corporation recommendations. Water quality conditions (pH, temperature, and salinity) were measured daily. The amount of food fed was approximately 4% of their body mass and was given once daily. Abnormality and mortality in each test group were recorded once every 24 hours. The fish was watched carefully for signs of disease, stress, physical damage, and mortality. A 16-hour light and 8-hour dark photoperiod was maintained.

Material Collection

Fresh leaves of *Moringa oleifera* were bought from Ogbomoso farmer's market in Nigeria. Then was properly authenticated by a Plant Taxonomist at the Biology Department, Ladoke Akintola University of Technology, from Oluwadamilare agro farms located in Ijebu Ode. The processed honey was purchased from the Department of Animal Nutrition and Bio-Technology, Ladoke Akintola University of Technology.

Plant Preparation

The Moringa leaves were cleansed to eliminate any impurities prior to air drying at room temperature. This procedure aimed to preserve the leaves green color and decrease the presence of antinutritional components. Subsequently, the dried leaves were pulverized into a fine powder using a hammer mill, following the methodology described by Irabor *et al.* (2021).

Supplemented food preparation

Different groups of animals were fed various experimental feeds. The different groups of feed are described below;

T1: Normal commercial fish feed (Blue Crowns feed) was given to both the Control and Lead Only groups.

T2: Feed Supplemented with Moringa only

T3: Feed Supplemented with Moringa and Honey in equal proportion

T4: Feed Supplemented with Honey only

The commercial fish food (Blue Crown Feeds) was supplemented with *Moringa oleifera* leaves powder and honey per gram of fish food. All ingredients were mixed with grounded commercial fish food and distilled water. Then, the prepared diets were extruded through a minced-meat machine, allowed to dry at 70 °C for 48 hours, and stored at room temperature until use. These doses of 10% *M. oleifera*-supplemented feed are selected based on the previous report (Irabor *et al*., 2021) that they do not show toxicity to the fish. Also, 10% of Honey was also used as a supplement. Table 3 shows the gross composition of the experimental diet, according to the methodology of Irabor et al. (2021).

Chemicals and reagents

Chemicals and reagents used, such as Thiobarbituric acid (TBA)**,** epinephrine, reduced glutathione, hydrogen peroxide (H_2O_2), and methanol, were procured from Sigma-Aldrich, kits such as ALT, AST, and ALP were gotten from Randox UK.

Samples collection and preparation

After euthanasia with an overdose of MS-222, the liver was removed and preserved in 10% neutrally buffered formalin for future analysis. The blood sample was taken at the midline of the anal fin. The needle was inserted via the musculature into the ventral surface of the fish spine. Samples for hematological analysis were collected in heparin tubes. The remaining blood was clotted at room temperature and centrifuged for 10 minutes. Plasma was withdrawn and stored at -20 °C until assayed.

Hematological analysis

Anticoagulant (EDTA) preserved blood was used for the estimation of various hematological parameters like hemoglobin content, red blood cell count, white blood cell count, and packed cell volume performed in accordance with (Torts *et al.*, 1998). These values were then used in calculating mean corpuscular hemoglobin (MCH). Mean corpuscular volume MCV and mean corpuscular hemoglobin concentration (MCHC) according to Dacie and Lewis (1977). According to routine clinical methods, hematological analysis was carried out at the Medical Laboratory University Health Centre, LAUTECH Ogbomoso (Torts *et al*., 1988).

Table 4. Calculated Nutrient Composition

Ingredient	T1 (Mean ± S.E.M)	T2 (Mean ± S.E.M)	T3 (Mean ± S.E.M)	T4 (Mean ± S.E.M)
Crude Protein	38.27 ± 0.7852	38.04 ± 1.207	37.67 ± 1.022	37.63 ± 0.5138
Crude Fibre	7.240 ± 0.6697	7.000 ± 1.160	6.570 ± 0.9064	6.170 ± 0.5312
Ether Extract	5.820 ± 0.9007	7.560 ± 0.5831	6.700 ± 0.3291	6.600 ± 1.109

Values were expressed as Mean ± SEM (n=3)
Note: (T1) normal commercial fish feed (blue crowns feed), which was given to both the control group and the lead only group, (T2) feed supplemented with moringa only, (T3) feed supplemented with moringa and honey in equal proportion, (T4) feed supplemented with honey only

Hematological analysis and Biochemical analysis

Anticoagulant (EDTA) preserved blood was used for the estimation of various hematological parameters like hemoglobin content, packed cell volume, red blood cell count, and white blood cell count was done according to (Torts *et al.*, 1998), Mean corpuscular hemoglobin concentration (MCHC). Mean corpuscular volume MCV and Mean corpuscular hemoglobin (MCH) were calculated according to Dacie and Lewis (1977). According to the procedures of Reitman and Frankel outlined by Ochei and Kolhatkar, the activities of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) in the serum and tissues were determined. According to Kind and Kind, plasma alkaline phosphatase (ALP) activity was measured. Tietz et al. 1983 protocol of colorimetric Biuret technique was used to quantify the concentration of serum total proteins. The Misra and Fridovich (1972) approach was used to assess the superoxide dismutase (SOD) activity. The principle of Varshney and Kale (1990) was used to calculate the concentration of lipid peroxidation. Malondialdehyde (MDA) and thiobarbituric acid (TBA) combine to generate an MDA-TBARS adjunct that absorbs significantly at 532 nm, which is used to estimate lipid peroxidation. The liver's reduced glutathione (GSH) level was measured using an Ellman-38 approach that Hissin and Hilf (1973) adapted. The Aebi (1983) technique was used to measure the catalase activity.

Statistical analysis

Prism 6 was used to analyze the data. The mean and standard error of the mean (Mean±SEM) were used to express the results. One-way analysis of variance (ANOVA) was used to compare values, and then a student t-test was performed. A $p>0.05$ was considered statistically significant, while a $p>0.05$ was considered insignificant.

STUDY FINDINGS AND DISCUSSION

The nutritional composition of the supplemented feed was calculated and determined seen in Table 4, it was discovered that moringa leaves had been previously documented to have a crude protein content of 22.60 ± 0.17%, ash content of 11.24 ± 0.17%, crude fat content of 13.40±0.25%, crude fiber content of 8.07 ± 0.17%, and carbohydrates content of 44.69 ± 0.41% (Lesten & Emmanuel, 2018). Similarly, Honey derived from *Apis mellifera* was found to contain a moisture content of 23.17 mg/100 g, ash content of 2.38 mg/100 g, protein content of 29.06 mg/100 g, free fatty acids content of 38.70 mg/100 g, and carbohydrates content of 17.96 mg/100 g (Baba *et al.*, 2020). After supplementation, moringa and honey slightly increased the feed ether extract composition thereby providing energy, essential fatty acids and fat-soluble nutrients for normal growth and development of fish as reported by Kaushik and Cowey (1991). There was no significant difference in the Crude fiber and Crude protein.

Table 5. Determined Nutrient Composition

Ingredient	T1	T2	T3	T4
Crude Protein	39.06	38.73	38.01	38.02
Crude fibre	6.91	7.10	6.66	5.89
Ether Extract	6.22	7.99	8.01	7.87

Note: (T1) normal commercial fish feed (blue crowns feed), which was given to both the control group and the lead-only group, (T2) feed supplemented with moringa only, (T3) feed supplemented with moringa and honey in equal proportion, (T4) feed supplemented with honey only.

The findings (Figure 1A) showed a significant increase in AST, ALT, and ALP activities in lead-induced group (Group A) when compared with the control group. However, a decrease was noticed in treatment with Honey Supplemented (Group B), Moringa Supplemented (Group C), and Group D (Honey and Moringa Supplemented) when compared with lead-induced group.

The result presented in (Figure 1B) showed a significant decrease in T.P level in lead-induced group (Group A) and a group treated with Group C (Moringa Supplemented) when compared with the control group, while an increase was observed in Group B and D when compared to Group A. Similarly, when compared with the control group, there was a decrease in albumin and globulin level in a lead-induced group (Group A).

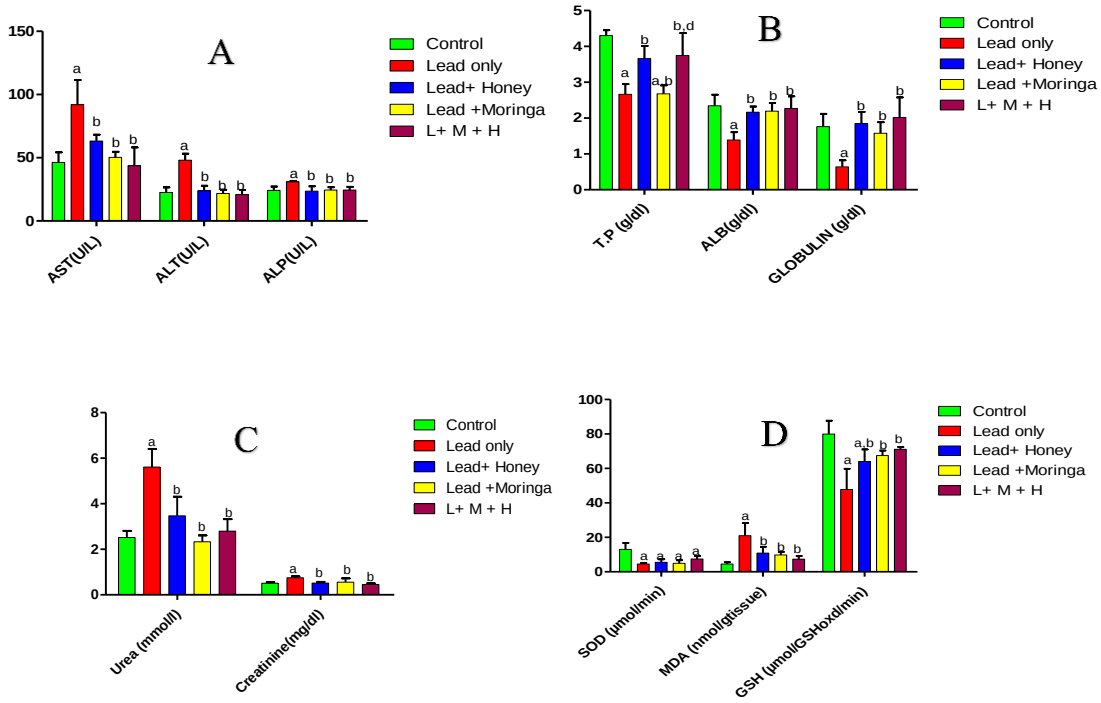

Figure 1. Effects of *M. oleifera* and Honey-supplemented on AST, ALT, and ALP (A), T.P, ALB, and GLB (B), Urea and Creatinine (C), Superoxide dismutase (SOD), methylenedioxy-amphetamine (MDA), glutathione (GSH) and in *C. gariepinus.*

Where L+ M + H represents Lead + Moringa + Honey. Values are expressed as Mean ± S.E.M
(a) Statistical significance when compared with the control group at $p < 0.05$.
(b) Statistical significance when compared with Group A (12.4 mg/l of lead only) at $p < 0.05$
(c) Statistical significance when compared with Group B (Lead + 10% Honey) at $p < 0.05$.
(d) Statistical significance when compared with Group C (Lead + 10% Moringa) at $p < 0.05$.

However, treatment with Honey (Group B), Moringa (Group C), and Honey and Moringa (Group D) significantly increased ALB and GLB activities when compared with lead-induced group. From Figure 1C, the level of urea and creatinine increased in the lead-induced group (Group A) compared to the control group. However, treatment Group B, C, and D significantly decreased urea concentration and creatinine levels when compared with lead-induced group. In contrast, the result in (Figure 1D) showed a significant decrease in SOD activity and GSH level in a lead-induced group (Group A) compared to the control group. However, treatment Group B, C, and D increased SOD activity and GSH levels when compared with lead-induced group (Group A), although not significantly. Also, Group D has slightly higher SOD activity than the other treated groups, although not significantly.

The result in showed (Figure 1D) a significant increase in MDA concentration in lead-induced group (Group A) when compared with the control group. However, treatment Group B, C, and D significantly decreased MDA concentration when compared with lead-induced group. The decrease observed in MDA concentration in Group C and D was not statistically significant compared to Group B.

The significant increase in MDA concentration observed in the lead-induced group (Group A) compared to the control group indicates the presence of oxidative stress and lipid peroxidation induced by lead exposure. MDA is byproduct of lipid peroxidation, and its elevated levels indicate cellular damage caused by oxidative stress. However, the single or co-administration of honey and MO in Group B, C, and D resulted in a significant decrease in MDA concentration when compared to the lead-induced group (Group A), following that both honey and MO have been reported to possess potent antioxidant properties as they contain various bioactive compounds such as polyphenols, flavonoids, and other antioxidants, which can scavenge free radicals and reduce oxidative stress in cells. By neutralizing reactive oxygen species (ROS), honey and MO may protect cellular structures and lipids from peroxidation, thereby decreasing MDA levels.

Hematological parameters such as WBC, RBC, HGB, HCT, MCV, Platelet, MCH, MCHC, and LYMPH content in the lead-induced group (Group A) decreased significantly compared to the control group. However, treatment Group B, C, and D significantly increased WBC, RBC, and HGB content when compared with the lead-induced group. (Figure 2A), (Figure 2B), (Figure

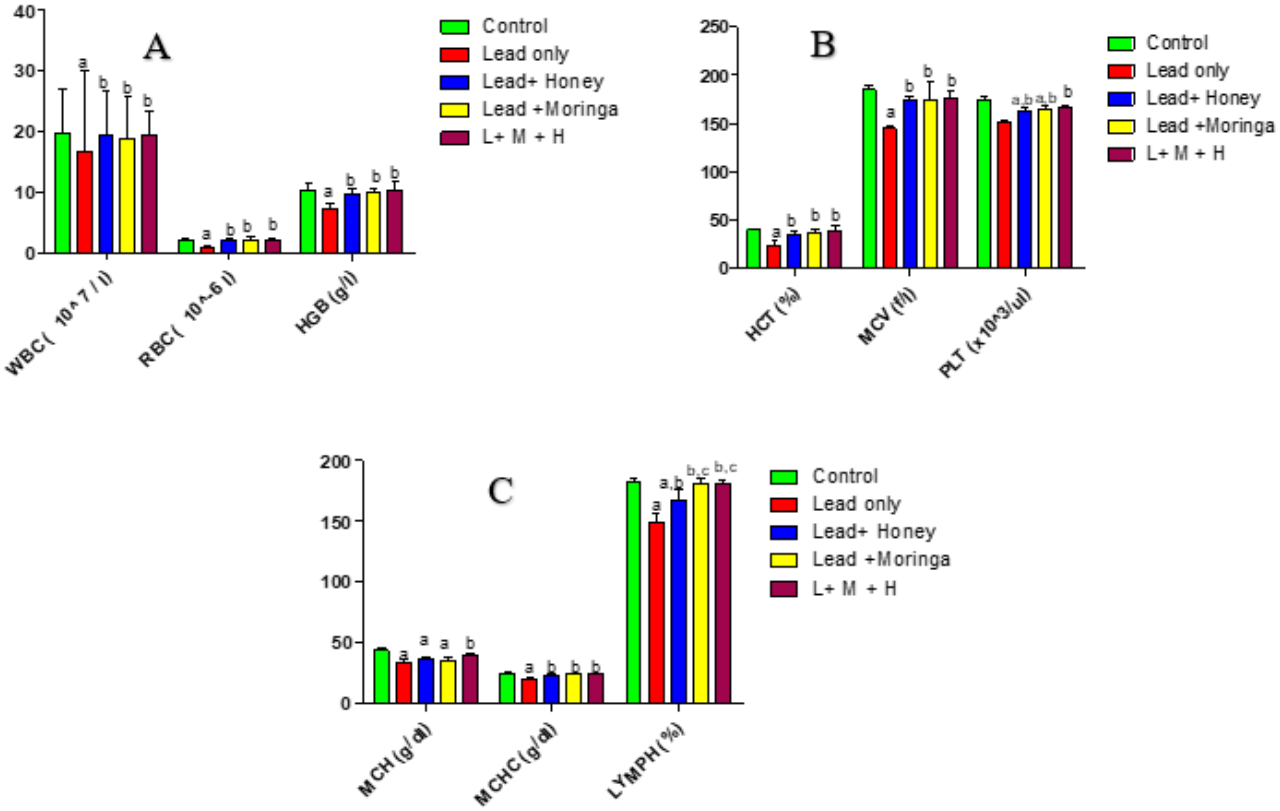

Figure 2. Presents a bar chart showing the results obtained for the effects of *M. oleifera* and Honey-supplemented on AST, ALT and ALP (A), T.P, ALB and GLB (B), Urea and Creatinine (C), Superoxide dismutase (SOD), methylenedioxy amphetamine (MDA), glutathione (GSH) and in *C. gariepinus*.

Where L+ M + H represent Lead + Moringa + Honey. Values are expressed as Mean ± S.E.M
(a) Statistical significance when compared with Control group at $p < 0.05$.
(b) Statistical significance when compared with Group A (12.4 mg/l of lead only) at $p < 0.05$
(c) Statistical significance when compared with Group B (Lead + 10% Honey) at $p < 0.05$.
(d) Statistical significance when compared with Group C (Lead + 10% Moringa) at $p < 0.05$.

2C). Noticeably, the ALT, AST, ALP, urea, creatinine, and MDA significantly increased while GSH and SOD reduced in the group given lead only. But these biochemical indices and hematological parameters increased more significantly in the group that received both honey and MO compared to the group that received either honey or MO. The findings of this study were utilized to establish that combining both honey and *MO* provides a more effective way to treat lead-induced toxicity.

The result revealed that single and co-administration of Honey and MO improve the biochemical indices as well as hematological parameters, and ameliorate the toxic effect of lead significantly when compared to the groups administered lead only. However, co-administration of Honey and MO showed a better ameliorative effect, although not statistically significant when compared to sole administration of MO, as seen in the Hematological parameters and also in the higher SOD and GSH activity, lower MDA concentration.

(Fihri *et al.*, 2016) The study supports the above result by indicating that Honey has a defensive effect against hepato-renal toxicity and lead-induced anemia. Then it was concluded that honey protects against the toxic effects of lead-induced blood, hepatic and renal damage. It was also indicated by (JO, 2012) and (Jo et al., 2012) that moringa oleifera has a healing and prophylactic effect in adult Wistar Rats on Lead Mediated damage to Haematological and Bone Marrow Components.

(Sharayu and Asmita, 2017) pointed out in their analysis that Lead acetate is a strong mutagen that induces oxidative stress in various test systems that have demonstrated cytotoxic effects in human erythrocytes in onion root tips and hemolysis. MO displays potent anti-cytotoxic and anti-hemolytic properties against cytotoxicity and hemolysis caused by lead acetate. “Supplementation of honey with the administration of Pb for 7 weeks reduced the Pb-induced increase in the levels of urinary 8-aminolevulinic acid, blood zinc protoporphyrin and Pb in blood, liver, and kidney, and decrease in blood hemoglobin. Therefore, the results suggest that the regular consumption of honey may

reduce some adverse effects of Pb" (Tandon and Singh, 1994).

CONCLUSION

This study aimed at the synergistic and ameliorative and potency of the combination of both honey and moringa-supplemented feed diets against lead toxicity on the fish which is more pronounced. Initially, the fishes were exposed to lead and were fed with the formulated diet containing various levels of *M. oliefera* leaves and honey at various concentrations as experimentally designed. Later, the samples were prepared for hematological and biochemical analysis. Further, the study conducted a statistical analysis with the data. The study employs evaluation of the biochemical indices and haemalogical parameter to see how the use of honey and *Moringa oleifera* can synergistically ameliorate lead induced toxicity and the results revealed that the combination of honey and *Moringa oleifera* provides an effective and efficient way to treat lead induced toxicity, although it is important to notice that the sole administration of honey or *Moringa oleifera* have similar ameliorative effect as the combination Honey and *Moringa oleifera* at equal percentage against lead toxicity, although treatment with combination showed better non-significant antioxidant properties (as seen in SOD, GSH activities and MDA concentration). Therefore, different proportion of MO and Honey Combination should be investigated to provide more insight on the therapeutic effect of the co administration of Honey and MO. Accordingly, it is suggested that the administration of honey and *Moringa oleifera* both individually and synergistically will have positive impacts on the health of the fish as well as the consumers nationwide.

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