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Research Article

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In-vitro Study of Hepatoprotectant and Anti-Atherosclerotic Effect of Raw and Boiled Leaf Extract of *Moringa Oleifera*

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ABSTRACT

This in vitro study aims to investigate the antioxidative property and the potential of raw and boiled aqueous extracts of Moringa oleifera (MO) against lead acetate (LA) induced toxicity in goat liver homogenate and LDL (low density lipoprotein) oxidation in human blood plasma. Thereby to prove its efficiency as a hepatoprotectant and antiatherosclerotic agent. The effect on biochemical indices such as lipid peroxidation (LPO), protein level (PL), and enzymatic activities (EA) of vital liver enzymes (alkaline phosphatase, acid phosphatase and succinate dehydrogenase) was examined. Results showed a significant decline in the PL and the EA and increased LPO in LA exposed groups compared to the unexposed control groups. CO administration of the raw and boiled extracts separately to the liver homogenate along with LA increased the PL and EA. It also significantly reduced the formation of TBARS due to LPO caused by LA exposure. Thus, the aqueous extracts of MO might be hepatoprotective. Also the LDL oxidation in human blood plasma induced by LA exposure was reduced in the presence of raw and boiled extracts separately. Thereby confirming that the aqueous extracts of the plant can prevent the formation of atherosclerotic plaques, which is the major risk in cardiovascular diseases. The investigation proves that both raw and boiled aqueous extracts of MO due the presence of potent antioxidants, exerted an ameliorative effect and maintained the studied parameters close to the control groups. This confers its protective role against LA induced hepatotoxicity and prevents the formation of atherosclerotic plaque.

Keywords: *Moringa oleifera*; Lead acetate; Lipid peroxidation; Protein levels; Enzymatic activities

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INTRODUCTION

Medicinal plants have been used for centuries before the advent of orthodox medicine and around 40% of modern medicine is derived from medicinal plants [1]. A wide variety of nutritional and medicinal virtues has been attributed to the roots, bark and leaves flowers, fruits, and seeds of *Moringa oleifera* [2-4]. *Moringa oleifera* is one of the 14 species of family Moringaceae and is commonly known as 'Drumstick tree' or the 'horse radish tree' [5]. *Moringa oleifera* is a type of local medicinal Indian herb which has turn out to be familiar in the tropical and subtropical countries [6]. The leaves are the most nutritious part of the plant, being a significant source of B vitamins, vitamin C, provitamin A as beta-carotene, vitamin K, manganese and protein [7]. Traditionally, it is used to treat many diseases throughout the world and many of them are scientifically proved, which mainly include; antitumor, antipyretic, antiepileptic, antiinflammatory, antiulcer, antispasmodic, diuretic, antihypertensive, cholesterol lowering, antiatherosclerotic, antidiabetic, hepatoprotective, antibacterial and antifungal activities and are being employed for the treatment of different ailments in the indigenous system of medicine, particularly in South Asia [3]. *Moringa oleifera*, which is considered to be full of medicinal properties, was chosen for the study of its Hepatoprotectant and Anti-atherosclerotic properties against lead induced Hepatotoxicity and LDL oxidation.

EXPERIMENTAL SECTION

Chemicals

The analytical reagent (AR) grade lead acetate trihydrate was obtained from HIMEDIA Laboratory Pvt. Ltd., India. All other reagents and chemicals used were of analytical grade procured from local sources. Deionized distilled water was used in the entire study. The plant for the study was collected at Telecom Layout, HBR 5th Block, Bangalore, which was a one year old plant. The leaves were randomly selected and collected freshly from the plant for each and every trial carried out during the course of the project work.

Determination of Hepatoprotectant Property of *Moringa Oleifera*

Experimental design:

The hepatoprotectant property of the leaves of *Moringa oleifera* was studied using 1% and 5% of both raw and boiled aqueous extracts for different assays. In the present study, experimental protocol was designed into two separate phases. During the first phase, goat liver homogenate cultures were exposed for the specific period of time to different concentrations of lead acetate selected on the basis of LD50 value and reported literature [8] in order to evaluate the lead induced alterations in selected biochemical parameters like lipid peroxidation, protein content and enzymatic activities of alkaline phosphatase, acid phosphatase as well as succinate dehydrogenase *in vitro*. The second phase involves co-administration of plant aqueous extract of *Moringa oleifera* to lead acetate exposed homogenate for specific time duration to investigate a Protective effect of the antidote against lead toxicity [9].

Preparation of plant (*Moringa oleifera*) extract:

5 g of freshly collected *Moringa oleifera* leaves was homogenised with 100 ml distilled water (raw extract). 5 g of freshly collected *Moringa oleifera* leaves was boiled for 10 mins (boiled extract). The plant extract content thus obtained was first filtered through ordinary filter paper and then centrifuged at 4000 rpm for 10 min. The supernatant was collected and either 1% or 5% extracts of both raw and boiled were used for the present investigation.

Preparation of lead acetate solutions:

0.010 gm of lead acetate was dissolved in 100 ml of double distilled water to prepare the stock solution of 100 ppm. A definite volume of this stock solution was used in a final volume of the reaction mixture, so as to get the required concentrations of lead acetate. 1 ppm and 3 ppm concentrations of lead acetate were used in the present study.

Liver Tissue Source

The goat liver was used as a vital organ for the study. Liver samples weighing approximately 250-350 gm of healthy adult goat (Capra Hircus) were obtained from the approved Dhodi slaughter house, Frazer Town. After sacrificing the animal, fresh liver tissue was brought to the laboratory under frozen condition and used immediately. The appearance of fresh tissue was dark reddish-brown colour. Liver tissue was washed in normal saline, blotted dry by pressing between 2-3 folds of filter paper and divided into different experimental groups.

Experimental Groups

The experimental protocol includes different experimental groups: (I) Control Group, (II) Herbal Antioxidant (Raw/Boiled *Moringa oleifera* extract) Exposed Group, (III)Lead Acetate (1 ppm/3 ppm) Exposed Group, (IV) Lead Acetate (ppm) and Herbal Antioxidant (Raw/Boiled *Moringa oleifera* extract)(co-administration) Exposed Group.

In-vitro Study

The tissues of different experimental groups were subjected to the process of homogenization with constant pace and speed under suitable condition of 4°C in chilled glass mortar pestles in order to maintain the viability. The fresh liver homogenates obtained were whitish red in appearance due to haemolysis occurred during the homogenization process. The liver homogenate samples in all the tubes were exposed to aqueous solutions of various concentrations of lead acetate (1 ppm and 3 ppm) and plant extract of *Moringa oleifera* mg/mL for 30 minutes time duration. The unexposed control and exposed liver homogenates were maintained at same conditions in shaker incubator at 37°C and subjected to study of various biochemical indices for investigating Protective effect of the antidote against lead

induced hepatotoxicity [9].

Biochemical Analysis

Lipid peroxidation assay:
To analyze free radical induced cell injury by lead acetate, the levels of lipid peroxides were determined in liver homogenates. A 10% of tissue homogenate of liver was prepared in ice cold 0.1 M phosphate buffer solution (pH=7.4) for the estimation of lipid peroxidation levels. The measurement of lipid peroxidation (LPO) in the liver homogenates of control and exposed groups were done by the method of Ohkawa et al. [10]. 1% of the plant extract was used for this assay. The extent of Lipid Peroxidation was determined by the formation of thiobarbituric acid reacting substances (TBARS). The lipid peroxidation inhibition percentage was calculated by using the formula below:

Lipid peroxide Inhibition% = (OD of control – OD of test) × 100/OD of control

Protein assay:
For investigation of toxic effect of lead acetate on the protein metabolism, levels of soluble proteins were estimated in goat liver homogenates by the method of Lowery et al. [11]. 2% liver homogenate was prepared using double distilled water and 5% of the MO extract of both raw and boiled aqueous extract was used for this assay. The percentages of protein content in the experimental groups were calculated by using the formula below:

% of Protein content = OD of sample × 100/OD of control

Alkaline phosphatase (ALP) and Acid phosphatase (ACP) assay foam test:
The alkaline phosphatase and acid phosphatase activities were analyzed by the method of King and Armstrong. 2% liver homogenate was prepared using double distilled water and 5% of the MO extract of both raw and boiled aqueous extract was used for this assay. The percentage of the enzyme activity was calculated by using the formula below:

% of enzyme activity = OD of Sample × 100/OD of control

Succinate dehydrogenase (SDH) assay:
Tetrazolium method is an improved spectrophotometric method for measuring succinate dehydrogenase activity. The toxic effect of lead acetate on SDH activity was estimated using this method. 2% liver homogenate was prepared using double distilled water and 5% of the MO extract of both raw and boiled aqueous extract was used for this assay. The percentage of the enzyme activity was calculated by using the formula below:

% of enzyme activity = OD of Sample × 100/OD of control

In-vitro* Studies of Anti-Atherosclerotic Property of *Moringa oleifera

Experimental design:
The anti- atherosclerotic property of the leaves of *Moringa oleifera* was studied using 1% of both raw and boiled extracts for its anti-oxidant property. In the control group, the serum was exposed 3 ppm lead acetate for the specific period of time in order to evaluate the lead induced oxidation of lipoproteins which poses the major cause for atherosclerosis. In the test group, serum was co-administered with plant aqueous extract of *Moringa oleifera* and lead acetate for specific time duration to investigate a Protective effect of the antidote against lead toxicity.

Extraction of plasma:
Freshly drawn human blood was used for the study. Plasma was obtained from EDTA-treated blood and separated by low-speed centrifugation at 2330 g at 4°C for 10 min.

Inhibitory effect on lead-induced LDL oxidation:
The blood serum was extracted from freshly drawn blood by centrifugation. The serum appeared straw yellow colour after the removal of blood cells. The serum samples were exposed to aqueous solutions of lead acetate (3

ppm) and plant extract of *Moringa oleifera* (1% extract) for 120 minutes time duration. The control and exposed serum were maintained at same conditions in shaker incubator at 37°C and was subjected to LPO assay for investigating Protective effect of the antidote against lead induced oxidation [12].

Biochemical Analysis

To measure the effect of the leaf extract on lead acetate induced oxidation of serum lipoproteins, 0.4 mL serum was first exposed to 1% *Moringa oleifera* raw and boiled leaf extract separately. Ascorbic acid (25 mg/ml) was used as the standard antioxidant. Oxidation reaction of lipoproteins was then initiated by adding freshly prepared 3 ppm lead acetate solution and incubated for 120 minutes time duration. The control and exposed serum were maintained at same conditions in shaker incubator at 37°C. Serum along with 3 ppm lead acetate was used as control. The measurement of lipid peroxidation (LPO) in the serum of control and exposed groups were done by the method of Ohkawa et al. 1% of the plant extract was used for this assay. The extent of Lipid Peroxidation was determined by the formation of TBARS.

RESULTS AND DISCUSSION

Scavenging Activity of MO Aqueous Extracts on DPPH Radicals

The free radical scavenging activity of *Moringa oleifera* is shown in the Figure 1. The antioxidant activity of both raw and boiled extract was compared with ascorbic acid (40 µg/mL) and its percentage of anti-oxidant property was found to be 86.6% and the percentage of anti-oxidant property of raw and boiled extract were found to be 64.07% and 63% respectively. Free radical scavenger is a significant mechanism for the inhibitory activity towards lipid peroxidation and in addition excellent marker for antioxidant activity [13]. It was observed that both raw and boiled extract of *Moringa oleifera* showed antioxidant effect, this infers that boiling didn't remove much of the antioxidants.

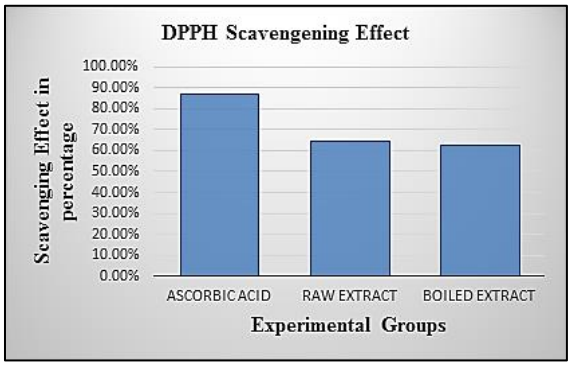

Figure 1: Showing DPPH scavenging activity

Determination of Hepatoprotectant Property of *Moringa Oleifera*

Lipid peroxidation:

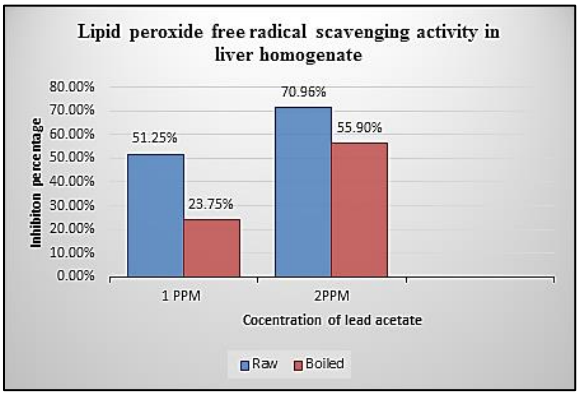

Figure 2: Showing the inhibitory effect of raw and boiled extracts on lipid peroxidation

The percentage of inhibition of TBARS formation as results of LPO in the goat liver homogenate exposed to different concentrations of lead acetate, in the presence of aqueous extracts of Moringa oliefera is shown in Figure 2. The percentage of inhibition due to the exposure to raw and boiled extract separately with respect to their control, in the presence of 1 ppm, was found to be 51.25% and 23.75% respectively. While the percentage of inhibition in the presence of 2 ppm was found to be 90.96% in raw extract exposed group and 55.90% in boiled extract exposed group. The lead acetate exposure alone was found to increase production of TBARS significantly, which is marked by increased LPO levels in goat liver homogenates. The increase in lipid peroxidation was dose dependent. Addition of aqueous extract of Moringa oliefera (1% extract) to homogenate did not cause any significant effect on LPO level. However, simultaneous addition of lead acetate (1 ppm/2 ppm) and aqueous raw and boiled extracts separately, in goat liver homogenate significantly reduced lead induced lipid peroxidation as compared to lead exposed groups.

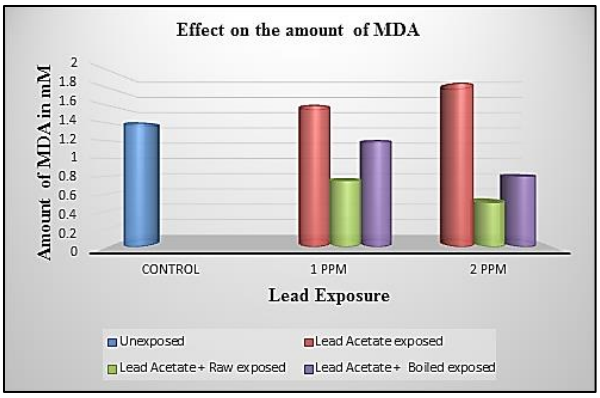

Figure 3: Showing the reduction of MDA

Lead acetate exposure in liver homogenate significantly increased the production of MDA (malondialdehyde). On simultaneous addition of raw and boiled aqueous extract separately in the presence of 1 ppm and 2 ppm lead acetate, marked the reduction in the amount of MDA. The lead acetate exposure alone was found to increase the production of MDA to 1.6 mm and 1.85 mm against 1ppm and 2 ppm concentration respectively. While unexposed group showed the presence of 1.4 mm MDA which may be due stress experienced during homogenising of the liver. On simultaneous co-administration of lead acetate (1 ppm) and aqueous raw and boiled extracts separately, in goat liver homogenate significantly reduced the MDA to 0.75 mm and 1.2 mm respectively. While in the case of exposure to lead acetate (1 ppm) and aqueous raw and boiled extracts separately, reduced the MDA to 0.5 mm and 0.8 mm respectively. Supplementation of aqueous extract of *Moringa oleifera* as an ameliorative agent resulted in significant reduction in elevated MDA levels in lead acetate exposed group (Figure 3). The significant increased levels of MDA in lead acetate exposed groups compared to control group, might be due to formation of highly reactive species having unpaired electrons known as free radicals [9]. When the balance between antioxidant system and reactive oxygen species (ROS) is lost, it results in oxidative stress [9]. Several studies shows similar increase in the amount of ROS in lead-exposed animals [14,15]. Depletion of the intracellular free radical scavenger glutathione might enhance the production of reactive oxygen species such as superoxide ions, hydroxyl radicals and hydrogen peroxide [9]. Most important consequence includes the peroxidation of membrane lipids, with an increase in the permeability of cell membrane rendering the tissue susceptible to free radical injury [16]. The results emphasized that co-administration of herbal extract of *M. oleifera* to lead exposed liver homogenates cultures significantly reduced the increased levels of lipid peroxides. The various antioxidants present in the herbal extract were found to exert ameliorating effect by lowering free radical levels. The plant extract of *M. oleifera* inhibits the chain reaction and exerts an antioxidant effect.

Protein levels:

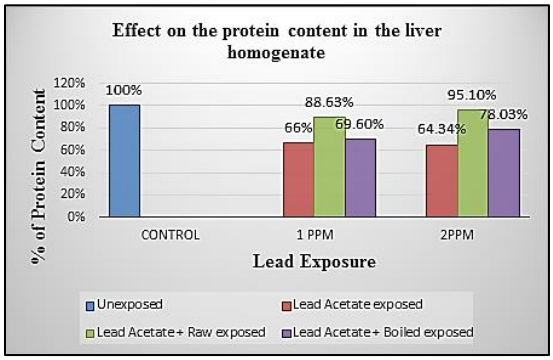

Figure 4: Showing the effect on protein levels in liver homogenate

Lead Acetate exposure caused a significant decline in the protein levels in goat liver homogenate (Figure 4). Considering control to show 100% protein content, at 1 ppm and 2 ppm lead acetate exposure groups, there was significant reduction in the protein contents to 66% and 64.34% respectively. Administration of aqueous raw and boiled extract of *M. oleifera* (5% extract) did not show any significant effect. However, simultaneous supplementation of lead acetate and *M. oleifera* aqueous raw and boiled separately in liver homogenates significantly increased the protein content to 88.63% in raw extract exposed group and 69.20% in the boiled extract group. Supplementation of aqueous extract of *M. oleifera* as a therapeutic agent resulted in significant maintenance of protein levels against lead intoxication. Reduction in protein content in the lead acetate exposed homogenates might be due to binding of lead with the sulfhydryl (–SH) group containing proteins and interference with number of enzyme systems essential to cellular metabolism [9]. Further, MDA formed during lipid peroxidation could react with –SH groups of proteins to damage them, thus inhibiting enzymes requiring –SH groups for their activities [17]. The other factors responsible for alteration in protein metabolism might be inhibition of protein biosynthesis due to the impairment of peptide chain initiation [9]. The reduction in proteins might be due to increased proteolysis and reduced incorporation of amino acids into proteins [18] or increased deamination of amino acids in the liver [9].

The plant extract of *Moringa oleifera* is able to prevent cell injury by maintaining sulfhydryl groups of membrane binding proteins. Lead acetate exposure to goat liver homogenate for 30 minutes brought about a significant reduction in the alkaline phosphatase activity. Result revealed that alkaline phosphatase activity markedly decreased as the dose of lead acetate was increased in goat liver homogenate, and it remained less than control always (Figure 5). The decline in the enzyme activity at 1 ppm and 2 ppm exposure was represented as 63.1% and 50% respectively. Addition of aqueous raw and boiled extracts of *M. oleifera* separately to liver homogenate did not cause any significant effect. However, simultaneous addition of lead and aqueous extracts (5% extract) in goat liver homogenate significantly increased the activity of enzyme. The enzyme activity in raw extract exposed groups was increased to 97.5% and 88.7% in the presence of 1ppm and 2ppm lead acetate respectively. This increase in the enzyme activity was very close to control. Similar result was obtained in the case of boiled extract exposed groups, where the increase in the enzyme activity was found to be 95.6% and 80.6% in the presence of 1ppm and 2 ppm lead acetate respectively. Supplementation of aqueous extract of *M. oleifera* significantly maintained alkaline phosphatase activity closest to the control group and exerted positive effects against lead toxicity.

Alkaline phosphatase:

The products of LPO such as hydro peroxides can inhibit protein synthesis and alter enzyme activity [19]. The results obtained shows that lead acetate exposure caused significant depletion in a–SH activity of alkaline phosphatase in liver. Alkaline phosphatases are a group of enzymes, which hydrolyse phosphate esters at alkaline pH [9]. Reduced alkaline phosphatase activity might be attributed to the alteration in cell membrane permeability in addition to lead induced imbalance between synthesis and degradation of enzyme [9].

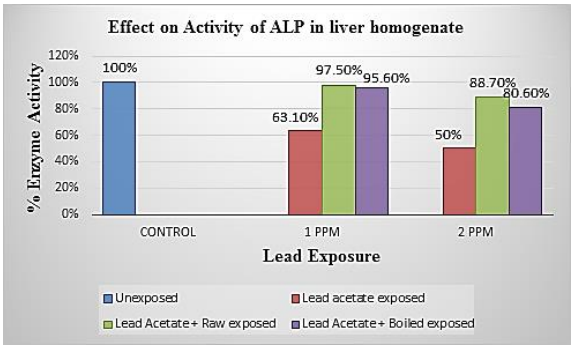

Figure 5: Showing effect on ALP activity

Acid phosphatase:

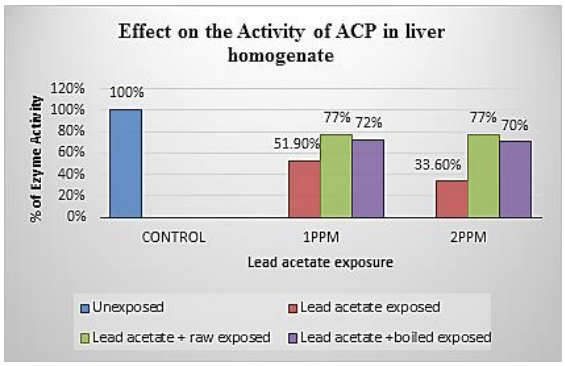

Figure 6: Showing the effect on ACP activity

Lead acetate exposure to goat liver homogenate for 30 minutes brought about a significant reduction in the acid phosphatase activity. Result revealed that acid phosphatase activity markedly decreased as the dose of lead acetate was increased in goat liver homogenate and it remained less than control always (Figure 6).The decline in the enzyme activity at 1ppm and 2ppm exposure was represented as 51.9% and 39.6% respectively. Addition of aqueous raw and boiled extracts of *M. oleifera* separately to lead acetate exposed liver homogenate did not cause any significant effect. However, simultaneous addition of lead and aqueous extracts (5% extract) in goat liver homogenate significantly increased the activity of the enzyme. The enzyme activity in raw extract exposed groups was increased to 77% and 77% in the presence of 1 ppm and 2 ppm lead acetate respectively. This increase in the enzyme activity was very close to control. Similar result was obtained in the case of boiled extract exposed groups, where the increase in the enzyme activity was found to be 72% and 70% in the presence of 1ppm and 2 ppm lead acetate respectively. Supplementation of aqueous extract of *M. oleifera* significantly maintained acid phosphatase activity closest to the control group and exerted positive effects against lead toxicity. This inhibition in enzyme activities by heavy metals may be due to the direct binding of the metal with enzyme protein [20] or the toxic effects produced by them on tissues leading to decreased synthesis of enzymes.

The observed decrease in acid phosphatase activity in the liver homogenate is explained as due to the inhibition of the enzyme by the influx of metal ions, the destabilization of the plasma membrane and the resultant depletion of the enzyme away from the tissues [9].

Succinate dehydrogenase:

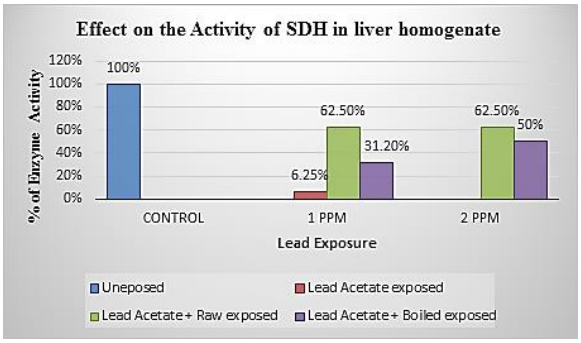

Figure 7: Showing the effect on SDH activity

Lead acetate exposure to goat liver homogenate for 30 minutes brought about a significant reduction in the succinate dehydrogenase activity. Result revealed that succinate dehydrogenase activity markedly decreased as the dose of lead acetate was increased in goat liver homogenate, and it remained less than control always (Figure 7).There was significant decline in the enzyme activity at 1 ppm and 2 ppm exposure was represented as 6.25% and no activity was observed respectively. Addition of aqueous raw and boiled extracts of *M. oleifera* separately to lead acetate exposed liver homogenate did not cause any significant effect. However, simultaneous addition of lead and aqueous extracts (5% extract) in goat liver homogenate significantly increased the activity of the enzyme. The enzyme activity in raw extract exposed groups was increased to 62.5% in the presence of both 1 ppm and 2 ppm lead acetate respectively. This increase in the enzyme activity was very close to control. Similar result was obtained in the case of boiled extract exposed groups, where the increase in the enzyme activity was found to be 31.2% and 50% in the presence of 1 ppm and 2 ppm lead acetate respectively. Supplementation of aqueous extract of *M. oleifera* significantly maintained acid phosphatase activity closest to the control group and exerted positive effects against lead toxicity. The result obtained shows a considerable loss of SDH activity in the liver homogenate exposed to lead acetate. Any change in the SDH activity of liver reveals alterations in its oxidative energy metabolism indicating lesions in TCA cycle, which may affect conversion of succinate to fumarate leading to blockage in the Krebs's cycle which would result into reduction in ATP synthesis. Reduction in SDH activity would also indicate a possible alteration in mitochondrial structure and functions of mitochondrial enzymes due to accumulation of lead in mitochondria. Lead may uncouple oxidative phosphorylation, which may reflect on the slow rate of TCA cycle. Thus, depression of SDH activity reflects upon the altered state of oxidation and energy metabolism of a damaged liver [9]. The *Moringa oleifera* extract maintained the alkaline phosphatase, acid phosphatase and succinate dehydrogenase enzyme activities in goat liver homogenate nearest to control. The mechanism of action of the herbal extract seemed to be mainly due to the presence of potent antioxidants that participates in oxidation – reduction reactions. *Moringa oleifera* aqueous extract thus acts an effective hepatoprotective agent due to its antioxidative property. The plant extract reduces oxidative stress, subsequently providing protection against lead induced alterations in protein content and enzyme activities in goat liver homogenate.

In-vitro* Studies of Anti-Atherosclerotic Property of *Moringa oleifera

Inhibitory effect on lead acetate-induced LDL oxidation in blood plasma:

The effect of raw and boiled aqueous extracts of the leaf on the percentage inhibition of the formation of TBARS resulting from the oxidation of human LDL is compared with Ascorbic acid as standard (Figure 8).The percentage inhibition of Ascorbic acid was found to be 53.3%, while the inhibitory effect of raw extract was 33.33% and that of boiled extract was 23.33%. The results show that ascorbic acid and aqueous extracts significantly inhibited the TBARS formation. In addition, from the results obtained, this study demonstrates that the raw and boiled aqueous extract of *Moringa oleifera* leaves both equally and significantly reduce the formation of atherosclerotic plaque. These results agree well with earlier findings where the water extract of the fruits of this plant significantly lowers the levels of serum cholesterol, very low-density lipoprotein and LDL in hypercholesterolemia rabbits [21].

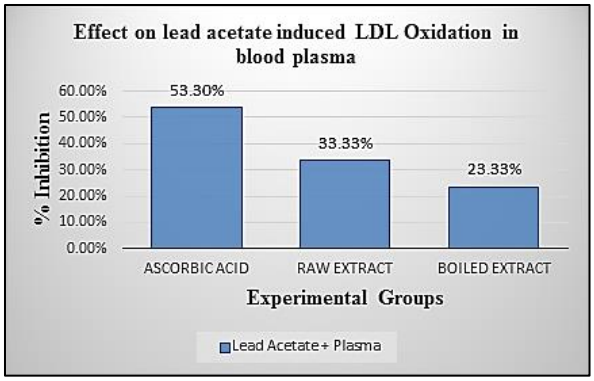

Figure 8: The inhibitory effect on lead acetate-induced LDL oxidation

We have shown that the *Moringa oleifera* leaf extract possessed strong radical scavenging activity and antioxidant activity. Polyphenols other than vitamin E have been known to exert powerful antioxidant effect *in vitro*. They inhibit lipid peroxidation by acting as chain-breaking peroxyl-radical scavengers, and can protect LDL from oxidation [22]. It has been found that the *Moringa oleifera* leaf extract contains 2% (w/w) of polyphenols, therefore, the antioxidant effects of the leaf extract may depend on its phenolic components [12]. Polyphenolic compounds also possess a variety of other biological activities, such as reduction of plasma lipids, which might be due to the up-regulation of LDL receptor expression [23], inhibition of hepatic lipid synthesis [24] and lipoprotein secretion [25], and increase in cholesterol elimination via bile acids [26]. It is possible that the activity in lowering lipid levels and aortic plaque formation of the *Moringa oleifera* leaf extract may result from the phenolic compounds present in the extract. However, the precise mechanisms underlying these effects need to be elucidated in future studies. In the present study, the effects of *Moringa oleifera* leaf extract on oxidative modification of LDL by determining levels of TBARS formed were demonstrated. It has been found in this study that after the incubation of human plasma with lead acetate, the production of TBARS was increased. In contrast, in the presence of *Moringa oleifera* leaf extract, the oxidative modifications of LDL were significantly reduced the levels of TBARS formation. These findings indicate that *Moringa oleifera* leaf extract suppresses the initiation and propagation of lipid peroxidation, and owing to its phenolic content, it may help suppress atherosclerosis by scavenging hydrogen oxide radicals [12].

CONCLUSION

From our result it is clear that both raw and boiled extracts of *Moringa oleifera* leaves are efficient and effective in exhibiting hepatoprotectant and anti-atherosclerotic activities. Based on the results of our studies and other previous studies it can be suggested that *Moringa oleifera* may serve as a safe and cheap source for the prevention of hepatotaxicity and cardiovascular diseases since this plant has long been used as food and vegetable in Asian countries and moreover without any reports of toxic effects.

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