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EFFECT OF OLIVE OIL EXTRACT ON LEAD INDUCED IN MICE, THE STUDY OF GLYCEMIA AND LIPIDEMIA IN NORMAL AND DIABETIC TREATED MICE

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AUTHORS' CONTRIBUTIONS

This work was carried out in collaboration among all authors. Author AS designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors VRA and KD managed the analyses of the study. Author KS managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Lead is one of the environmental contaminant, which can threaten living organism in many ways. Mice treated with lead acetate found to exhibit significant production in hemoglobin content between normal and experimentally treated mice. The hematological values indicated a significant production in total white blood cells (WBC), red blood cells (RBC) count between normal and experimental. The level of high-density lipoprotein (HDL) in lead induced mice was found to be low with18.0 mgs/dl where as the control mice revealed an elevated high-density lipoprotein (HDL) level of 32.0 mgs/dl. Similarly the level of low-density lipoprotein (LDL), cholesterol was found be elevated than the control. Another biochemical parameter is the estimation of serum cholesterol which was found to be 90.0 mgs/dl with lead acetate induced mice and a remarkable decrease in serum cholesterol was established which exhibited 65.0mgs/dl. The results for serum triglycerides with lead induced mice showed 49.5 mgs/dl than compared to normal mice. Analysis of serum biomarkers such as lactate dehydrogenase (LDH) revealed an increased lactate dehydrogenase (LDH) level than the normal control mice. To study the kidney dysfunction the level of serum creatinine revealed an elevated level of 0.90 mgs/dl which is the remarkable effect leading to renal failure. The level of total bilirubin was found be eye in lead induced mice than the control treated mice. Studies pertaining to body weight absorbed during the periods of 15 days it was found be reduced in lead induced toxicity mice. To study of diabetic condition induced with streptozotocin (STZ) over a period of 15 days which exhibited significance rise in blood glucose concentration than the normal control mice. The study further extended the supplementation of olive oil in order to toxicity of lead acetate by olive oil revealed serum biomarker such as level of serum glutamate-pyruvate transminase (SGPT) in control showed 46.0 U/ML. Whereas in olive oil treated mice along with STZ induced mice revealed a moderate enzyme activity. Antipyretic study of olive oil by assessing the rectal temperature showed 40°C for control where as olive oil administered mice showed a rectal temperature of 40.5°C.

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1. INTRODUCTION

Lead exposures in construction industry are intense, and although exposure to lead in most industrial sectors has been effectively controlled by standards promulgated by the US Occupational Safety and Health Administration (OSHA), construction work had until 1993 been exempted from those protections. The commonly used animal models of diabetes include spontaneous model, genetic model and chemical- induced model [1]. Lead accounts for most of the cases of pediatric heavy metal poisoning [2].

The diagnosis of lead toxicity has traditionally been based on significantly elevated blood lead levels. However, data now indicates that low- level exposures resulting in cognitive dysfunction, neurobehavioral disorder, neurological damage, hypertension, and renal impairment. Olive oil (olea europaea) contains oleic acid as potential fat source with moderate degrees of other fatty acids. Olive oil has been commercially as a food supplement which can be in the form of syrup and capsules. The potential therapeutic effect of olive oil includes antioxidant properties, hypotensive, hypoglycemia, cardiovascular, hepato protective and anti- asthmatic effects [3]. Olive oil has positive effects on regulation of cholesterol and oxidation of had cholesterol (i.e, LDL) Olive oil is rich in monounsaturated fatty acids (MUFA) in the form of oleic acids which accounts for 70% - 80% of total fatty acids.

The aim of the present investigation is to explore whether role of olive oil and it impact potential protective effect against lead-induced oxidative stress. Heavy metals may come into the human [4] body during food, water, air, or assimilation through the skin when they come in contact with humans in agriculture and in manufacturing, pharmaceutical, industrial, or housing. Industrial exposure accounts for a widespread route of exposure for adults.

The majority of this lead is required for batteries [5]. The rest is used for cable wrapping, plumbing, bullets, and fuel additives. Lead is a toxicant for virtually all organs of the body and has significant debilitating effects on the nervous, renal, hepatic and hematopoietic systems. Lead is found to increase oxidative stress by the production of free radicals and decreasing antioxidant capacity resulting in cell apoptosis. Drinking water is also a major source of lead exposure, predictable to be accountable for around 20 percent of the total daily exposure experienced by the majority of the U.S. population [6].

Hence the present investigation to collect olive oil commercially and to prepare extracts, standardize toxicity bioassay of lead, administer lead acetate solution orally to animal model (mice) and maintain control mice. To feed mice by oral supplementation of olive oil on potential therapeutics and lead acetate, induce the diabetic condition with streptozotocin in mice model and to study the Glycemia and Lipidemia supplemented with olive oil, further to analyze catalase, SOD activity between control and experimental treated mice, assess the serum biomarkers of SGPT (Serum Glutamate Pyruvate Transaminase) and SGOT (Serum Glutamate Oxalo Transaminase) ACP (Serum Acid Phosphatase) ALP (Serum Alkaline Phosphatase) between normal and experimental mice, study were further analyses haematological parameters such as HB (Hemoglobin), WBC (White blood cells), RBC (Red blood cells), and platelets between normal and experimental treated mice, similarly study on biochemical parameters such as Triglycerides, HDL(High density lipoprotein), LDH(Lactate dehydrogenase), and cholesterol, and to assess the lead toxicity and to estimate the antioxidant enzymes between control and experimental treated mice. Simultaneously, to study the antipyretic effect of olive oil, beyond which where study the lead toxicity and its influence on major enzymes between control and experimental treated mice.

2. MATERIALS AND METHODS

2.1 Maintenance of Animals

The study was conducted on male and female white albino mice, *mus muscules*, aged one month. The animals were kept in standard compartmented rectangular and well-ventilated cages. They were maintained on standard healthy laboratory conditions at temperature of 18-20°C and twelve hours light and darkness. Animals were adapted to the new environment for seven days prior to study start. All mice had free access of drinking water and food, *adlibitum*, during the experimental period.

2.2 Administration of Lead to Mice for Toxicity Study

2.2.1 Experimental animals

The animals were divided into four equal groups. Each group comprised of five animals and was marked as group I, II, III, IV. The first group

represented the healthy control animals, the second group animals were given 0.03 g/kg body weight of sub lethal doses of lead acetate respectively in their daily supply of drinking water for twelve weeks. The third group animals were given lead acetate (0.03 and 0.05 g/kg) and olive oil (1 ml orally). Each mouse was weighed every week and its daily water intake was determined.

2.3 Establishment of Lead- Poisoned Mice Model

Lead acetate was soaked in normal saline, which was given to the mice via intragastric administration at a dose of 0.03 g/kg per day for 20 consecutive days. After 20 days, successful generation of the lead poisoning model was confirmed by observing abnormal behaviors and measuring blood levels. Mice exhibiting the abnormal behaviors of arability and hyper activity, with a blood lead level ≥2 mg/dl, were considered to have undergone successful modeling.

2.4 Intragastric Administration of Olive Leaf Extract

Two hours after establishment of lead received 0.5 ml of olive oil orally for a period of 20 days. The controls were given deionized water. All animals were allowed diet and water ad libitum during the period of study after oral gavage of oil.

2.5 Blood Collection and Analysis

In order to study the haematological and biochemical investigation, blood was collected from each mouse individually. The animals were fasted for twelve hours prior to blood collection. All animals were anesthetized by chloroform and blood samples were collected immediately from their heart using heart puncture technique with the aid of disposable sterile syringe and needle. Blood sample of each mouse was then transferred to a sterile capped tube containing anticoagulant. EDTA for haematological indices including total erythrocyte count (TEC), total leukocyte count (TLC), packed cell volume(PCV), haemoglobin (HB) concentration, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MHC) and the amount of platelets.

Serum biochemical parameters were analyzed using auto serum analyzer, in accordance of manufactures instructions. These parameters included aspartate aminotransferase (AST) or glutamic oxaloacetic transaminase (GOT), alanine aminotransferase (ALT) or glutamic-pyruvic transaminase (GPT), bilirubin concentration, lactate dehydrogenase (LDH), alkaline phosphatase (AKP), and serum creatinine concentration.

II. Induction of Diabetes by Administration of STZ

Preparation of Streptozotocin: STZ was purchased from sigma chemicals containing 100 mg each vial and before used, it must be stored in -20°c streptozotocin was freshly dissolved in saline 1% tween 80 mixture in order to get concentration stoke of 10 mg/ml.

Induction of Diabetes: Diabetes was induced by a single intraperitoneal injection with STZ (65 mg/kg body weight) [7]. Mice were fast overnight before injection with STZ.

Experimental Design: The animals were divided into 3 groups (each of 4 mice). All international and local rules and regulation for handling animals in experiments were followed. They experimental groups were illustrated as follows.

Group 1: Healthy mice fed on pellet diet served as normal controls.
Group 2: Diabetic mice fed on pellet diet.
Group 3: Diabetic mice fed on pellet diet plus olive oil extract (0.05 ml/day)

Symptoms accompanying diabetes mellitus such as polydipsia, polyuria, and loss of body weight were observed every day STZ induced mice are considered as diabetes mellitus when fasting body glucose level above 11.1 mol/l. Diabetes severity grade is classified into 3 types, serve, moderate and mild diabetes, based on fasting blood glucose level. At the end of the experiment, the animals were anesthetized with chloroform after 12 hours fasting and whole blood samples were taken from hepatic portal vein. The blood samples left for 15 minutes at 37°C for serum separation, then centrifuged at 3000 rpm for 10 minutes, then sera were separated and kept in plastic vials at -20°c until analyses [8].

Biochemicals Assay: Serum glucose was determined by enzymatic colorimetric method according to the method described by Thulesen, et al., 1997.Total cholesterol (TC) was determined in serum according to the enzymatic colorimetric method and Triglycerols were determined in serum according to the enzymatic colorimetric method [9].

Estimation of Serum Cholesterol: 0.1 ml of serum were added to 4.9 ml of Fecl3 precipitating reagents and mixed well. It was centrifuged at 4000 rpm, 2.5 ml for supernatant 5 minutes was taken and it this reagent. In addition 2.5 ml Fecl3 diluting reagent and

4 ml of concentration H_2SO_4 were added and mixed thoroughly. Preparation of standard graph by taking 0.5, 1.0, 1.5, and 2.0 ml working standard are diluted to 5 ml with Fecl3 diluting reagent and treated as for the test. The color developed was read at 560 nm in spectrophotometer, the method [10].

Estimation of Triglycerides: 0.1 ml of lipid extract was taken in a centrifuged tube. To this add 4 ml of isopropanol and mixed well and 0.4gm of alumina was added and the tube and placed in mechanical rotator for 15 minutes and were tubes centrifuged for 3000 rpm 10 minutes. 2.5 ml of supernatant was taken in a try test tube and to this 0.6 ml of the saponification reagent were added and the tube was placed in a water bath at 65°c for 1 minutes. After cooling the tube containing 1 ml of sodium metaperoidate reagent was added and 0.5 ml of acetyl acetone was added. The tube is then incubated at 65° c for 30 minutes. The color developed was measured at 465 nm. A blank was made with 0.1 ml of distilled water instead of lipid extract.

Estimation of HDL Cholesterol: 0.5 ml of serum is taken in a centrifuge tube and to this 0.05ml of precipitating reagent is added and mixed well and mixed well and kept at room temperature the contents are then precipitated LDL, VLDL, settle down at the bottom and HD2 was left along in the supernatant. This supernatant is removed and analyzed by ZAX'S method.

Estimation of LDL Cholesterol: LDLS from a complex with be parin which, in the presence of $CaCl_2$, form residues. Add into test tubes 2ml of 0.025M.$CaCl_2$ and 0.2ml of blood serum in each. Into one of them, pour 0.04M of 1% heparin solution. Mix and after 4 minutes perform a colorimetric measurement, using a red light filter.

LDL concentration can be calculated using the formula

Here EK light absorption of the control solution.

E X light absorption of the test solution OD.

The value of LDL cholesterol can be calculated it the value of triglycerides and HDL cholesterol are known by friede wald's equation

Determination of catalase (CAT) activity in serum: Catalase activity in the serum was determined according to the method [11]. Erythrocyte sediment was prepared from the heparinized serum and washed 3times with isotonic saline. A stock haemolysate containing approximately 5 g Hb/ dl was prepared. By the addition of 4 parts by volume of distilled water a1:500 dilution of this concentrated haemolysate with soium-potassium phosphate buffer (0.05 M, pH=7) was prepared immediately before the assay. Reference cuvette contained 1 ml of buffer and 2 ml of haemolysate and test cuvette contained 2 ml distilled haemolysate. The reaction was started by addition of 1 ml of H_2O_2 (30 mM in the buffer) to the test cuvette, mix well andthe decrease in extinction was measured at 240 nm for 1minute by 15 sec interval.

Determination of superoxide dismutase (SOD) activity in serum: Blood SOD activity was determined according to the method [12] after removing the haemoglobin. 0.1 ml of the heparinised serum was haemolysed by 0.9 ml of cold water (4°C).The haemolysate was treated with 0.25 ml of CHCl3 and 0.5 ml of ethanol with vigorous mixing to remove the haemoglobin. The mixture was centrifuged at15000 rpm for 60 min. 0.025 ml of the clear supernatant was used for the SOD assay as described. The activity was expressed as U/g Hb.

Antipyretic Activity: Treatment was carried out as:

Group I: Control group animals (Normal saline 5 ml /kg)
Group II: Paracetamol (10 mg /kg)
Group III: 100 mg /kg methanol extract
Group IV: 200 mg /kg methanol extract

Yeast induced pyretic method: A suspension of Brewer's yeast (15 %) in saline (0.9%) was prepared. Four groups each containing 3 mice of either three were taken. The *thermocouple* was inserted 2cm deep into the rectum and the rectal temperatures were recorded. The animals were fevered by injection brewer's yeast suspension (10 mg /kg) subcutaneously in the back the nape of the neck. The injection site was massaged in order to spread the suspension beneath the skin. The room temperature was kept at 22to24°C. Immediately after yeast administration, food was withdrawn and the rise in rectal temperature was recorded. The measurement was repeated after 30 minutes. The dose of the test compound and standard drug was given orally, the rectal temperature was recorded again after 1, 2, and 4 hours. Paracetamol (10 mg/ kg) was selected as standard drug.

2.6 Measurement of Bio-Markers of Hepatic Injury

Estimation of Serum Glutamate Pyruvate Transaminase *(SGPT) activity*-Non-hemolyzed serum (0.05 ml) was mixed with 0.25 ml of glutamatepyruvate transaminase substrate and incubated for 3Omin at 37°C. Then 0.25 ml of 2, 4-

dinitrophenylhydrazine (DNPH) solution was added, mixed and kept for 20 min at room temperature. Then 2.5 ml of 0.4(N) NaOH was added, mixed and kept at room temperature for 10 min. The intensity of the developed colour was noted at 540 nm after setting the UV/VIS spectrophotometer (Biorad; Model Smartspec Plus) to zero with water. The decrease in absorbance represents the decrease in the concentration of - keto-glutarate from whichthe activity was calculated. The level of the enzymeactivity was expressed as IU/L.

Estimation of Serum Alkaline Phosphatase *(ALP) activity*-Serum alkaline phosphatase (ALP), at analkaline pH, hydrolyses di-sodium phenyl phosphateto phenol. The phenol formed, reacts with4-aminoantipyrine in the presence of potassiumferricyanide, as an oxidising agent, to form a redcoloured complex. The intensity of the colour formedis directly proportional to the activity of ALP present inthe sample.

ALP activity was quantitatively determined using ALP-kit, based on the method as described byKind & King. The valuesof the enzyme activity were expressed as KA (King-Armstrong) Units. *Estimation of serum bilirubin level*-The principle of formation of a pink coloured azo-bilirubin by the reaction between bilirubin and the diazo reagent was utilized. The total bilirubin level was measured using a previously reported method.

Estimation of blood glucose level-Blood glucose level was estimated by the method of Nelson and Somogyi. In separate 10 ml marked test tubes, 0.5 ml of blood filtrate and 0.5 ml of working standard glucose solution was taken. To each of these tubes, 1 ml of alkaline copper reagent was added. The contents of the tubes were mixed thoroughly and heated in a boiling water bath in an upright position for 20 min. After cooling to room temperature, 1 ml of arsenomolybdate colour reagent was added to each of the tubes and the contents of each tube were diluted up to 10 ml with distilled water. The intensity of the developed colour was noted at 540 nm using a UV/VIS spectrophotometer after setting the instrument to zero density with blank.

Measurement of the glycogen content of liver-Liver tissue (200 mg) was weighed on a torsion balance and finely homogenized with 20 ml. of 5% TCA in an all glass homogenizer [13]. The precipitate of the proteins was filtered off and the clear filtrate was subjected to analysis. Iodine reagent was prepared (16.5 ml of Lugol's solution, prepared by dissolving 1 g of iodine and 2 g of KI in 20 ml. of water, was added to 983.5 ml. of an aqueous solution, containing 25% (w/v) of KCI.

Catalase-Catalase was assayed by measuring the breakdown of hydrogen peroxide (H_2O_2). The weighed amounts of the hepatic tissue were homogenized in 5% ice-cold 50 Mm phosphate buffer pH 7.2. The homogenates were then centrifuged at 12000 × *g* for 12 min. The supernatant thus obtained was then carefully collected and incubated with 0.01 ml of absolute ethanol at 4°C for 30 min. Thereafter, 10% Triton X-100 was added to have a final concentration of 1%. The sample, thus obtained, was used to determine the catalase activity by measuring the breakdown of H_2O_2 spectrophotometrically at 240 nm. The enzyme activity was expressed as micromoles of H_2O_2 consumed per min per mg protein.

Measurement of the lead content in the hepatic tissue by atomic absorption by spectrophotometry. The tissue samples were incubated overnight at 37°C. The dry weight of the tissues was subsequently taken. The dried tissues were then placed with nitric acid and double distilled water in the conical flask. The conical flasks with their contents were then placed on the hot plate and heated at 65 to 70°C for digestion of the hepatic tissue. Then, per chloric acid was added for the precipitation of the protein and heated until white fumes came out. The contents of the conical flasks were then transferred quantitatively into 25 ml marked volumetric flasks and the volume was finally made up to 25 ml. The samples thus prepared were used for the measurement of lead content using an atomic absorption spectrometer.

3. RESULTS AND DISCUSSION

Lead is one of the main environmental contaminants, which can threaten living organisms in many ways. Lead toxicity may affect multiple organs of human body and it is associated with number of physiological, biochemical and morphological alteration. The present investigation, which was aimed to study the risk of exposure to different dosage of lead acetate which may affect the function of kidney and liver.

Table 1 and Plate 1 depicted lead toxicity on the body. An organ weight of experimental animals. The lead acetate was induced to three different groups. Group I serve as control, which exhibit the body weight of 78 g and liver and kidney exhibit 3.18 g and 1.60 g.

Group: 2 the lead induced mice after 25 days of exposed they were reduced to the body weight of 70.5g liver and kidney 2.71g ,1.53g reduced respectively. Group: 3 revealed administration of lead indicated the slightly elevated body weight of 75.3g liver and kidney 2.95g, 1.55g respectively

The result indicated that mice treated with lead acetate showed significant reduction in haemoglobin in lead acetate administered mice. The level of haemoglobin exhibited 8.5ml/dl whereas the normal control treated mice revealed the haemoglobin content of 9.5 ml/dl. Similarly, the result depicted in Table 2: indicated significant reduction in total WBC count with 2700 Cells /cumm, whereas the RBC Count of normal control mice showed 2900 cumm. In comparison between control and experimental treated mice it was observed a remarkable decrease in total RBC count exhibiting 4.65 million cells/ cumm in lead acetate mice over the control, which the RBC count revealed 5.05 million cells/ cumm.

Further which, the haemotological values observed for platelet in lead acetate treated mice showed 3.26 lakhs cells/ cumm, while the control treated mice exhibited platelet count of 3.08 lakhs cells/ cumm. The reduction of haemotological parameters and decreased level of other indices were found to be the concordant haemotological values altered due to toxic effect of lead. The other haematological parameters WBC for control mice 4.75 million cells/ cumm. Whereas the experimental treated mice (lead induced) revealed the WBC count of 2.95 million cells/cumm. This reduction may be due to the effect of lead. The results were noted in Fig. 1.

Effect of Biochemical Parameters on Lead Toxicity: On investigating the serum analysis, the level of HDL was found be 18.0 mgs/dl whereas on control treated groups revealed 32.0 mgs/dl. Simultaneously, the results for LDL, Cholesterol, exhibiting changes in the serum level of 21.0 mgs/dl. Whereas when compared to the control, the level of HDL cholesterol was found be elevated to 30.0 mgs/dl. The results on serum total cholesterol showed 90.0 mgs/dl with lead acetate administered mice where as in control treated groups, the remarkable decrease in serum total cholesterol exhibiting 65.0 mgs/dl. The results were exhibited in Fig. 2.

The results for serum triglycerides under the effect of lead acetate was also increased exhibited 49.5 mgs/dl. When compared to the control treated mice which showed 45.0 mgs/dl. To assess the effect of lead acetate on the activities of enzymes indicated elevation of lactate dehydrogenase (LDH) was found gradually increased with the increasing lead acetate dose of all treated animals rather compared to control. On analysis of LDH for lead acetate experimental group which revealed 190.2 U/L whereas the control treated group revealed 146.0U/L. To assess the effect of lead on kidney function, the activity of the serum of serum creatinine showed an elevated level of 0.90 mgs/dl with lead acetate group of mice whereas the control treated mice showed a decreased serum creatinine of 0.65 mgs/dl. The results were exhibited in Fig. 3.

The present study also investigated the changes in the serum level of total bilirubin which showed an increasing serum of total bilirubin activity in lead acetate induced mice exhibiting 0.75 mgs/dl. Whereas the control treated mice exhibited 0.60 mgs/dl of total bilirubin. The values of haematological parameters which include total RBC count and the creatinine level in the blood were depicted in the Fig. 4. In control group, the weight of the body of mice was average between 210-220 g, while the weight of olive oil supplemented along with lead acetate treated mice239 g revealed before the start of the experiment.

After a period of 25 days, the corresponding weight averaged to be around 232 g in mice. It has been observed that the reduction in body weight in lead induced toxicity was observed. The RBC count depicted in the Fig. 5 revealed 5.40million cells/cumm, whereas the control exhibited 7.31 million cells/cumm.

Besides the level of serum creatinine in blood treated with lead acetate with olive oil revealed 0.65 mgs/dl. Whereas, the control treated group revealed a creatinine level of 0.93 mgs/dl. To assess the effect of diabetogenic activity of STZ induced mice model were shown in Fig. 6. The intravenous injection at the dose of 100 g/kg caused moderate diabetic condition exposed for 15 days. All the animals developed diabetics within 15 days after administration of STZ. The significant rise in blood glucose concentration was observed till second week in comparison with control. While the metabolic parameters such as serum triglycerides, were observed between olive oil and STZ induced mice model.

The serum triglycerides in the control treated mice revealed 51.0 mgs/dl. Whereas the serum triglycerides level of olive oil and STZ induced mice model revealed 40.0 mgs/dl. The activity of antioxidant enzymes such as catalase, were also assessed. Simultaneously administration of olive oil was carried out for 20 days. Analysis of enzymatic activity in male and female of animal model compared to healthy control revealed SGOT 51.0 U/L, whereas the experimental treated mice revealed an enzymatic activity of SGOT of 55.2 U/L and 52.5 U/L. Similarly the estimation of SGPT observed in control nice

revealed 42.0 U/L. whereas the level of SGPT treated with olive oil and the STZ induced mice revealed a moderate level of enzyme activity with 40.5 U/L and streptozotocin induced mice revealed 39.5 U/L respectively.

Antipyretic activity of olive oil was evaluated by determining its effect on yeast induced pyrexia in albino mice. Fig.7 and Plate. 3. Fever was induced in mice by subcutaneous injection of Brewer's yeast suspension at a dose of 10mg/kg body weight of mice, below the nape of the neck. The injected neck side was then massaged to spread the suspension into the tissues. The rectal temperature was recorded for all groups of mice were after 10 hrs of yeast administration. The rectal temperature was markedly elevated in 40°C, for control, where as in paracetamol treated group as standard group revealed 41.7°C. After a specific time olive oil was administered. The result revealed that olive oil administered mice showed a significant reduction in rectal temperature with 100 mg/kg showed 40.5°C rectal temperature which is followed by when compared the untreated groups.

Simultaneously the rectal temperature was also monitored were paracetamol (standard) treated groups which exhibited a temperature of 39.4°C after 4 hours of administration. The result confirmed that the olive oil extracts possess remarkable antipyretic activity as compared with paracetamol.

To determine the effect of lead toxicity an antioxidant enzymes in the serum of mice with different treated groups was studied is shown in Fig. 8 treatment of mice induced with lead acetate revealed the presence of antioxidant enzymes catalase was found to be 102.3±3.01 which is a drastic production due to lead toxicity compared to control treated mice. Which exhibited 11.43±0.51. To elevate antioxidant capacity in the lead poisoned mice catalase, Superoxide dismutase activity supplemented with olive oil treatment group were significantly increased than the control group. The lead acetate plus olive oil treated group revealed 110.21±2.30 was the presence of catalase expressed as µg/g of protein.

Superoxide dismutase another antioxidant enzyme are presented in the Fig. 8 where in lead acetate induced mice revealed 84.2±1.36 quantum of Superoxide dismutase mg/g than the normal control treated mice which exhibited 89.0±0.30 mg/g f protein. Similarly, lead acetate supplemented with olive oil revealed 87±2.10 of Superoxide dismutase expressed in mg/g of dl. Lead toxicity may affect multiple organ of human body and it is associated the number of physiological, biochemical and morphological alteration exposure of lead has been shown to increase production of reactive oxygen species and consequently alter antioxidant defense system in mice resulting in oxidative damage the lead toxicity on the activity of major enzymes of the experimental treated mice after 25 days of treatment. The results revealed control treated mice revealed 22.8 U/ML of Serum Alkaline Phosphatase were as the experimental treated lead induced mice indicated a significant production in Serum Alkaline Phosphatase with 55.0 U/ML. Continuous exposure to lead might adversely affect mitochondrial enzyme synthesis were in Serum Acid Phosphatase analysis were exposure a control treated mice expressed 41.1 U/ML whereas the experimental treated mice revealed 4.2 U/ML of Serum Acid Phosphatase.

Besides the other important parameter which affects the kidney of experimental treated mice induced with lead toxicity are serum creatinine, serum urea, and serum Aspartate Transaminase (AST). Control normal mice expressed a serum creatinine level 0.65mgs/dl where as in lead induced mice observed in kidney showed the serum creatinine of 1.0mgs/dl. Simultaneously serum urea was also found be elevated in lead induced toxicity of the body and organs optimize revealed 28.0mgs/dl a comparatively and increased serum urea. Similarly a progressive effect of lead on liver function was monitored with serum Aspartate Transaminase (AST). Aspartate Transaminase (AST) is used to elevate liver function. The present finding of the study indicated significant increase in enzyme activity of Aspartate transaminase (AST) of the intoxicated mice (Lead induced) compared to control mice.

The results of this investigation of the body weight of the experimental animals were significantly decreased after 25 days of exposure with lead acetate (Plate.4). The above observation was in total accordance with the results were coincides with [14]. The weight loss might be due to interruption of lead acetate in absorption and metabolism in nutrition essential for health. Mechanism for dealing with oxidative stress may be particular relevance in the brain, given the high rate of oxygen metabolism. In the present study, stress due to carcinogenicity of lead led to brain tumors and oxidative stress, Lead exposure induced protection of reactive oxygen species (ROS). Lead has been shown to deplete antioxidant protein. Lead exposure cause brain tumor hence the present study by examining the lead content in mice model and is mechanism dealing with oxidative stress was studied. Lead has been shown to decreased variant enzymes like LDH, catalase, and SOD. The results were depicted in Plate 5 and 6. In the present study, the enzyme activity was carried out in treatment groups of normal control, lead acetate treated and lead acetate supplemented with olive oil was carried out to detect

specific enzyme activity in mice where in, over expression of SOD and reduced oxidative damage. Dietary phenolic compounds present in the olive leaf extract showed strong lead scavenging activities where in, olive leaf can prevent damage from lead poisoning. Besides in order to assessthe role of lead poisoning, the mice model which affect the kidney function was studied by analyzing serum creatinine between the normal control and lead induced mice.

The result suggested that serum creatinine exhibiting an elevating level of 0.90 mgs/dl in lead induced mice rather than control mice which exhibited 0.65 mgs/dl of serum creatinine. A similar observation was also carried out to detect the serum level of total bilirubin. The total bilirubin content in lead induced mice showed 0.75 mgs/dl over the control treated mice which exhibiting 0.60 mgs/dl.

Haematological investigation revealed a decreasedlevel of haemoglobin, WBC, RBC, and platelet count between the normal and lead acetate treated groups. These haematological changes might be attributed to the toxic effect of lead on cell metabolism intraction with some reactions leading to inhibition of enzymatic activity.Such as amino-levulic acid dehydrodase which play key role in haemobiosynthesis. Platelets count revealed considerable increase in lead induced mice compared to normal mice. This may be due to the reduction of haematological values might be attributed to binding of lead to red blood cells which increase membrane fragility and RBCs destruction [15]. It has been reported that lead induced inflammation which lead to increase in white blood cells [16] which was observed in this study. This may be due to thrombocytopenia after lead intoxication [17] followed by thrombocytosis. Olive leaf was used for the treatment of malaria and associated fever [18]. The physiological substances of olive leaf are hydroxytrysol, trysol, caffeic acid, P-Goumaric acid, vanillic acid, vanillin, oleuropein, luteolin, diosmetin, rutin,verbascoside, lutelin-7-glucoside, apigenin-7-glucoside,and diosmetin-7-glucoside [19].

Olive leaf offered a capacity to lower blood pressure and increase blood flow in the coronary arteries [20]. The phenolic compounds extracted from olive leaf possessed antioxidative activity [21, 22], as well as antimicrobial activity against *Helicobacter pyroli*, *Campylobacter Jejuni*, *Staphylococcus aureus* [23]. The optimum extraction condition for the supercritical fluid extraction of olive leaf polyphenols were examined [24].

Table 1. Lead toxicity on the body and organs weight of the experimental animals after 25 days of exposure

Lead acetate Dose	**Sex**	**Body weight (g)**	**Liver weight (g)**	**Kidney weight (g)**
Group I (control)	Male	78	3.18	1.60
Group II (lead acetate)	Male	70.5	2.71	1.53
Group III (lead acetate + olive oil	Male	75.3	2.95	1.55

Table 2. Heamatological changes in blood serum of the experimental animals after 25 days of treatment with lead acetate and olive oil

Haematological Analysis	**Control**	**Experimental**
RBC	7.31 million cells/cumm	5.40 million cells/cumm
Creatinine	0.93mgs/dl	0.65 mgs/dl

Table 3. Lead toxicity and its influence on major enzymes of serum alkaline phosphatase and serum acid phosphatase in mice model

Enzyme analysis	**Group I (control)**	**Group II (lead acetate induced)**
Serum Alkaline Phosphatase (AKP)	67.8 U/L	55.0 U/L
Serum Acid Phosphatase (ACP)	41.2 U/L	4.2 U/L

Fig. 1. Haematological changes in blood serum of the experimental animals after 25 days

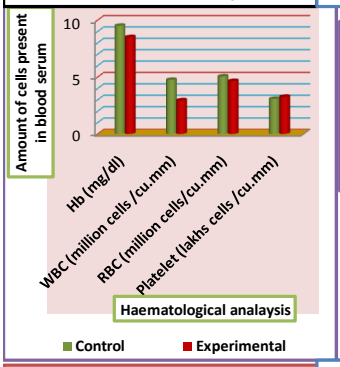


Fig. 2. Changes in the biochemical parameters of experimental animals after 25 days of lead exposure

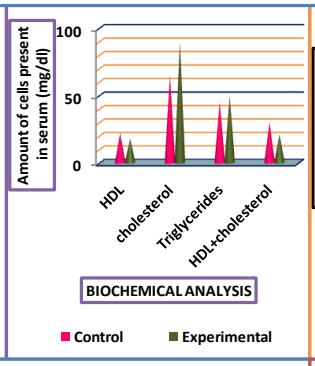


Fig. 3. Lead toxicity of the liver and kidney of the experimental mice in 12 weeks

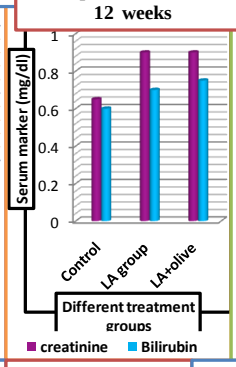


Fig. 4. Haematological changes in mice after 25 days of treatment with lead acetate and olive oil

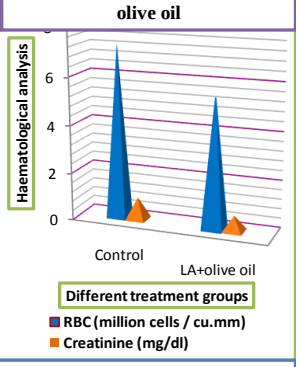


Fig. 5. Changes in the Triglycerides level of olive oil with STZinduced mice model after 15 days

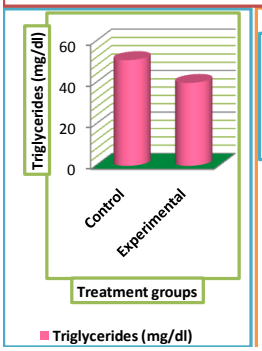


Fig. 6. Changes in the enzymatic activities in blood serum of olive oil with Streptozotocin after 15 days of treatment in mice model

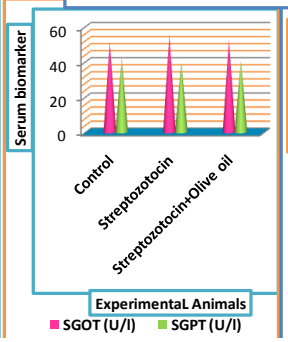


Fig. 7. Antipyretic effect of olive oil in mice model

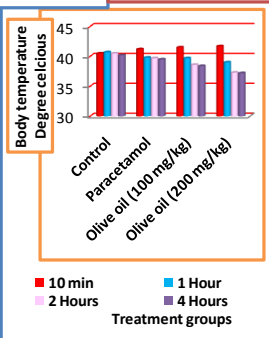


Fig. 8. Effects of lead toxicity of antioxidant enzymes on the serum of mice with different treatment groups

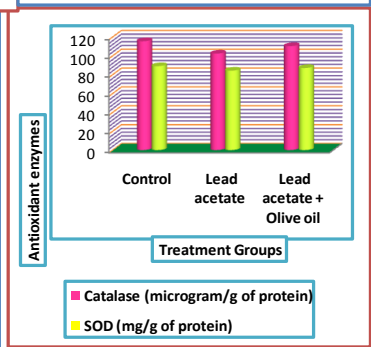


Plate:1. Dissection of organs to determine weight on lead induced mice

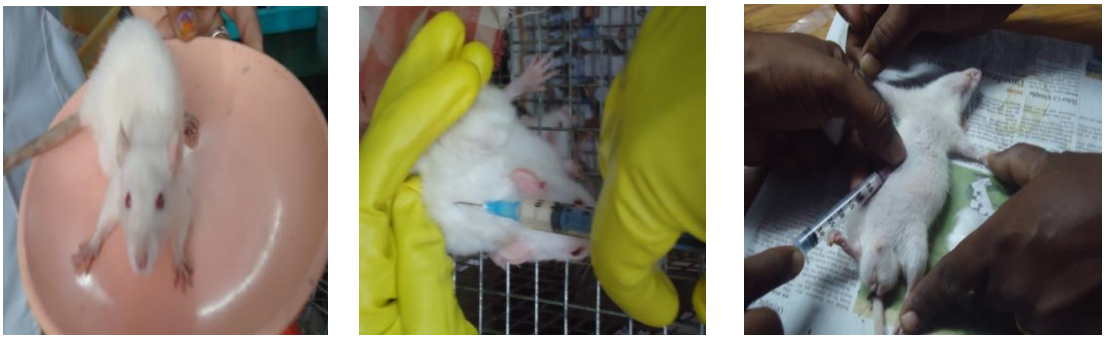
Plate:2. Oral Administration of lead, Streptozotocin and olive oil in experimental mice

Plate:3. To determine the antipyretic activity with a supplementation of olive oil

Plate:4. Mice were weighed before and after Treatment

Plate:5. To determine yeast induced Pyrexia method

Plate:6. Collection of blood by heart puncture method in mice



3.1 Lead Scavenging Method

The present study was performed to evaluate the potential chemo preventive effects of the individual and combined phenolic in olive leaf extract using the nitrite-scavenging method. The nitrite scavenging abilities of oleuropein, rutin, vanillin, caffieic acid, and their mixture at 500µm were 72.7%, 47.8%, 6.3%, 92.2%, and 66.6% respectively. At 1000 µm, these values were increased for 86.6%, 88.1%, 11.3, 97.8%, and 84.8%. All the phenolic compounds (except Vanilln) in the olive leaf extract showed strong nitrite scavenging abilities, as both individual and combined phenolic. These result suggested that the protection against damage from nitrite and nitro samine-related cancer. Oxidative stress was involved in the pathology of oxidation- linked diseases such as cancer, heart disease, atherosclerosis, and rheumatoid arthritis, and may play a role in neuro degenerative disease and aging processes [25]. Dietary phenolic compounds have generally been considered as a non-nutrient and their possible benefits to human health through their phenolic linked antioxidant effects which have just recently been considered. The body weight loss might be resulting from the interruption of lead acetate in absorption and metabolism of feed nutrients essential for health [26]. In the present study, lead acetate is known induce hepatotoxicity and it is easily available as hepatotoxicity agent. When liver plasma membrane is damaged, a variety of enzymes were released into the circulatory system which results in elevated level of SGOT enzymes.

Pyrexia fever is caused as a secondary impact due to infection, inflammation, malignancy and other diseased condition. The body's natural or innate resistance to create an environmental where infections are damaged tissue cannot survive. This infected tissue or damaged tissue enhanced pro-inflammatory mediators.(ex) like cytokine, interleukin, and tumor necrosis factor. This increased the synthesis of prostaglandin (PGE2) and direct to hypothalamus there by triggering the hypothalamus to elevate the body temperature. The body temperature increase in dilate the blood vessels and increased sweating to reduce the temperature. Normally high fever is the indirection of progressive and faster disease leading to tissue catabolism and degradation.

In the present study, antipyretic potential of methanolic extract of olive oil was elevated by determining is effect on yeast induces pyrexia in mice model. This result showed an increased temperature for control treated mice induced with yeast after 10 hours of infection. The methanol extract of olive oil provided an enhanced antipyretic activity which was also a dose dependent marked as 100 mg and 200 mg respectively. It is astonished to note that olive oil with different dose of 100 mg and 200 mg caused low body temperature up to4 hours following as administration. The effect of olive oil extract an yeast induced pyrexia show the rectal temperature was marked by elevated to 41.4°C after the oral supplementation of methanolic extract of olive oil the temperature was found to be reduced. Then 4 hours showed is sizeable decreased and was comparable to paracetamol a standard drug. At 100 mg and 200mg a remarkable antipyretic activity was recorded than the control. The result revealed olive oil have remarkable antipyretic activity an compared with standard paracetamol.

Catalase is an antioxidant enzymes which helps in converting hydrogen peroxide to water and oxygen and thus posses this ability to control the concentration of reactive oxygen species within the cells. An enhancement in the activity of catalase indicates oxidative stress which revealed that mice treated with lead acetate for 15 days decreased the catalase activity. In comparison to the activity measured in the control mice our results were in total agreement with Rabinowitz et al., [27].

The study of Superoxide dismutase has been demonstrated in lead induced mice showed a remarkable decreased the enzyme activity. The study was further extensively to elevated lead induced mice along with olive oil revealed a site increased in Superoxide dismutase activity. Antioxidant enzymes as a cellular protective role against oxidative stress resulting in liver tissue damage due to lead toxicity. The decreased activity of Superoxide dismutase and an increased activity of catalase to indicate possible accumulation of reactive oxygen intermediate. The results indicate olive oil supplemented has the potential to ameliorate lead induced hepatic injury due to oxidative stress in mice model. This protective effect may be due to antioxidant properties of olive oil may have feature therapeutic relevance.

To assess the enzymatic activity of Serum Glutamic Oxaloacetate Transaminase and Serum Glutamic Pyruvic Transaminase as serum biomarkers in mice model the level of Serum Glutamic Oxaloacetate Transaminase in control treated ice revealed 54.0 U/L. Whereas Serum Glutamic Pyruvic Transaminase was found to exhibit 46.0 U/L. The level of Serum Glutamic Pyruvic Transaminase activity significantly increased in comparison to the activity level measured in the control mice. Treatment of mice with lead acetate for 15days caused a significant increased the levels of Serum Glutamic Pyruvic Transaminase indicating serious hepatic tissue damaged. Serum Glutamic Pyruvic Transaminase is an important biomarker of hepatic function our findings are in total conformitywith the work of [28]. To assess the effect of lead on brain function the activities of Serum Alkaline Phosphatase, and Serum Acid Phosphatase were investigated. The activities of these enzymes

were elevated with increased in lead acetate. This elevation might be due to increasing of cell membrane permeability or cell membrane damage and the influence of lead. The above results were in total accordance with other findings by Shalan, et al., [29, 30] in which emphasized stimulation of Serum Alkaline Phosphatase had been noted in mice. Under the effect of lead in case of the kidney function, the concentration of urea, and creatinine were examined to check the functioning of kidney in intoxicated mice compared to normal healthy mice. The level of creatinine elevation might be due to kidney dysfunctions and considered as functional evidence of lead induced nephrotoxicity.

4. CONCLUSION

In the present findings include lead toxicity even at low doses has harmful effect on experimental animals. Lead is one of the main persistent environmental contaminants which can threaten living organisms. Lead toxicity may affect multiple organs of human body and it is associated with number of physiological, biochemical and morphological alteration. Herbal medicines derived from plant extracts such as olive leaf oil extract and increasingly utilized to treat wide variety of diseases and toxicity which affect health.

ETHICAL APPROVAL

Animal Ethic committee approval has been taken to carry out this study, Madurai Kamaraj University was considered and cleared by the IRB&EC for implementation.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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