# Bee Honey Dose-dependently Ameliorates Lead Acetate- mediated Hepatorenal Toxicity in Rats

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**Abstract:** Lead poisoning is a serious condition caused by increased levels of the heavy metal lead in the body. Lead interferes with a variety of body processes and is toxic to many organs and tissues including the heart, bones, intestines, kidneys, liver as well as reproductive and nervous systems. The present study aimed to evaluate the amleiorative effect of bee honey against lead acetate toxicity. Honey was administered orally at doses of 0.2, 0.4 and 0.4 g/kg for one month. Lead treatment (200mg/kg, p.o) started 10 days before the honey treatment and continoued with honey for one month. Positive control group were treated with lead acetate for 40 days. Lead intoxication caused a significant increase in serum malondialdehyde (MDA), decreased glutathione (GSH), increased uric acid, urea and creatinine. Moreover, lead administration increased bilirubin and the enzymatic activiry of GOT, GPT and alkaline phosphatase. In addition, lead intoxication increased total cholesterol, HDL, LDL and triglycerides. Bee honey dose- dependently reversed the adverse effect of lead on liver and kidney function and decreased MDA level and noormalized GSH level. In conclusion, induction of oxidative stress and disturbing lipid metabolism may be responsible for the adverse cellular effects of lead and that these cellular events may mediate the hepatotoxic and nephrotoxic manifestations in lead intoxication. Also, the beneficial effect of honey is likely- partially- due to its antioxidant property and its modulatory effect on the metabolic processes.
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**Key word:** Lead acetate- honey- liver- kidney

## 1. Introduction

Heavy metals including lead are naturally occurring elements that have a high atomic weight and a density at least five times greater than that of water and most of the heavy metal salts are soluble in water and form aqueous solutions and consequently cannot be separated by ordinary physical means of separation (El-Zahrani and El-Saied, 2012). Their multiple industrial, domestic, agricultural, medical, and technological applications have led to their wide distribution in the environment, raising concerns over their potential effects on human health and the environment (Tchounwou *et al*., 2012). Their toxicity depends on several factors including the dose, route of exposure, and chemical species, as well as the age, gender, genetics, and nutritional status of exposed individuals (Vassallo *et al*., 2011). During evolution, different organisms have developed diverse strategies to maintain an equilibrated relation with heavy metal ions present and available in the surrounding medium. Cells face two tasks, the first is to select those heavy metals essential for growth and exclude those that are not, and the second to keep essential ions at optimal intracellular concentrations (Cobbett and Goldsbrough, 2002; Perales-Vela *et al.,* 2006).

Lead is a persistent metal and commonly present in our living environment. Even at low doses of developmental stages, lead exposure resulted in embryonic toxicity, behavioral alteration, and adult learning/memory deficit (Chen *et al*., 2012). Consistently, Gargouri *et al*. (2012) studied the effect of lead acetate exposure in drinking water to mothers during gestation, from the 5th day of gestation to day 14 postpartum, revealing tha caused lead deposition was found in the brain and cerebellum of newborns as well as cerebellum tissue damages and significant decrease in weight and protein content of these tissues. Oxidative stress and changes in antioxidant enzyme activities in brain tissues were also recorded (Gargouri *et al*., 2012). Moreover, lead induced deficits in learning and memory through overactivation of hippocampal serine/threonine protein phosphatases PP1 and PP2A (Rahman *et al*., 2012).

In addition, lead acetate disturbed biochemical and hematological indices and induced nephrotoxicity and hepatoxicity through profound elevation of reactive oxygen species (ROS) production and oxidative stress, leading to increased lipid peroxidation level and depletion of intracellular reduced glutathione (GSH) level in kidney and liver (Ibrahim *et al*., 2011, Wang *et al*., 2011, Liu *et al*., 2012). Consistently, lead acetate has been found to induce biochemical and histological abnormalities in blood, kidney, liver and brain tissues (Ozsoy *et al*., 2011). Ademuyiwa *et al*. (2005) reported a significant positive correlation between blood lead and total cholesterol and LDL levels. Among studies of

inflammation, *in vitro* studies and a study of occupationally exposed workers found a relationship between lead and tumor necrosis factor-α (TNF-α), but not between lead and interleukin-6 (IL-6) (Valentino *et al*. 2007).

The health benefits of honey have long been realized by humans to treat a variety of ailments. Besides its sugar composition, honey consists of a number of bioactive compounds such as phenolic compounds, flavonoids, carotenoid-like derivatives, organic acids, Maillard reaction products, catalase, ascorbic acid, and other compounds which function as antioxidants (Bogdanov *et al*., 2008). Several therapeutic and medicinal effects such as antibacterial, antimutagenic, antiproliferative, hepatoprotective, hypoglycemic, and antioxidant effects have been ascribed to honey through last years (Erejuwa *et al*., 2010 a; Erejuwa *et al*., 2010 b; Ghashm *et al*., 2010).

The aim of the present study is to evaluate the protective effect of bee honey on lead-induced kidney and liver toxicity.

## 2. Materials and Methods:

Experimental animals: male adult Sprague Dawley rats (150-200 g) were kindly provided from our breeding center at NODCAR and kept for a week for acclimatization under normal conditions and constant temperature (25±1C°) with *ad. libitum* water and food until starting the experiment.

Chemicals: Lead acetate ($C_4H_6O_4Pb.H_2O$) was purchased form Sigma-Aldrich.

Bee honey was purchased from the Egyptian market.

A total number of 40 rats was divided into five equal groups, the first is the negative control group, the second group is the positive control group, which is treated with lead acetate (200mg/kg/day,p.o) for 40 days, the other three groups represent the combined treatment where the animals were treated with lead acetate (200mg/kg) for 40 days and bee honey was administered of three doses (0.2; 0.4 and 0.8g/kg, p.o) concurrently with lead at the last 30 days. At the end of treatments, animals were sacrificed. Blood samples were collected in clean and dry tubes and centrifuged at 3000 r.p.m for 10 min. for serum separation.

Determination of urea, creatinine, uric acid, bilirubin, total cholesterol, triglycerides, and high density lipoprotein (HDL) were analyzed colorimetrically using commercial available kits (STANBIO Lab. TX, USA). Low density lipoprotein was calculated mathematically by Friedwald's formula (1972). The activities of GOT, GPT and ALP were determined according to Reitman and Frankel (1957). Determination of reduced glutathione and malondialdehyde levels were determined by HPLC methods according to the Jayatilleke and Shaw (1993) and Karatepe (2004) respectively.

Statistical Analysis:

Data presented as means ± SE. One-way ANOVA followed by LSD test were used to evaluate significant differences from the control and lead-treated group groups. Statistical processor system support (SPSS) for Windows software, release 10.0 (SPSS, Inc, Chicago, IL) was used.

## 3. Results:

Result in Table 1 depicted that lead treatment increased levels of uric acid, creatinine, urea and bilirubin compared to control group. Moreover, lead-treated animals exhibited high levels of total cholesterol, HDL, LDL and TAG (Table 2). Table 3 showed that lead acetate treatment significantly increased the enzymatic activities of GOT, GPT and ALP enzymes. Bee honey dose-dependently attenuated the elevating effect of lead on, kidney and liver function parameters and GOT, GPT and ALP enzyme activities and normalized kidney and liver function and lipid profile (Tables 1,2 and 3). Figures 1,2 and 3 represent the correlations between doses of honey and the tested parameters. Figure 4 showed that lead acetate significantly increased level of MDA and decreased the level of reduced glutathione in serum. Honey treatment antagonized the effect of lead acetate on both GSH and MDA levels in a dose-dependent manner. Figures 5 represent the correlations between doses of honey and the levels of glutathione (GSH) and malondialdehyde.

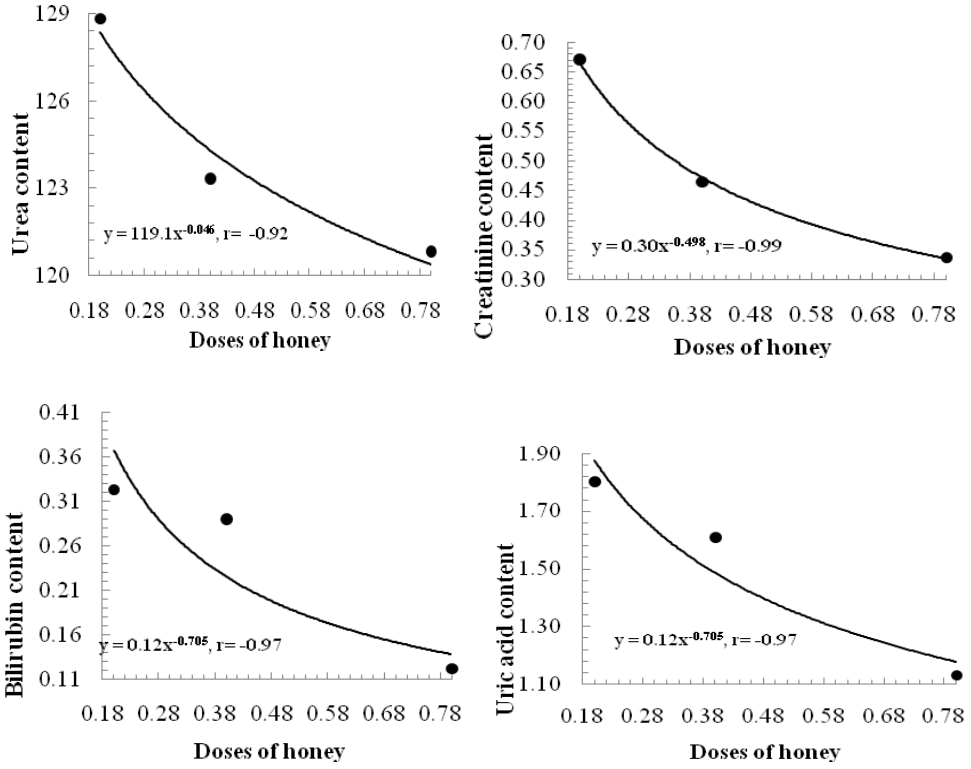


Figure 1: Relationship between the administered doses (0.2, 0.4, 0.8 g/kg b. wt.) of honey bee and the concentration of urea (mg/dl), creatinine (mg/dl), bilirubin (mg/dl) and uric acid (mg/dL) in serum of male albino rats. Each value is a mean of six rats.

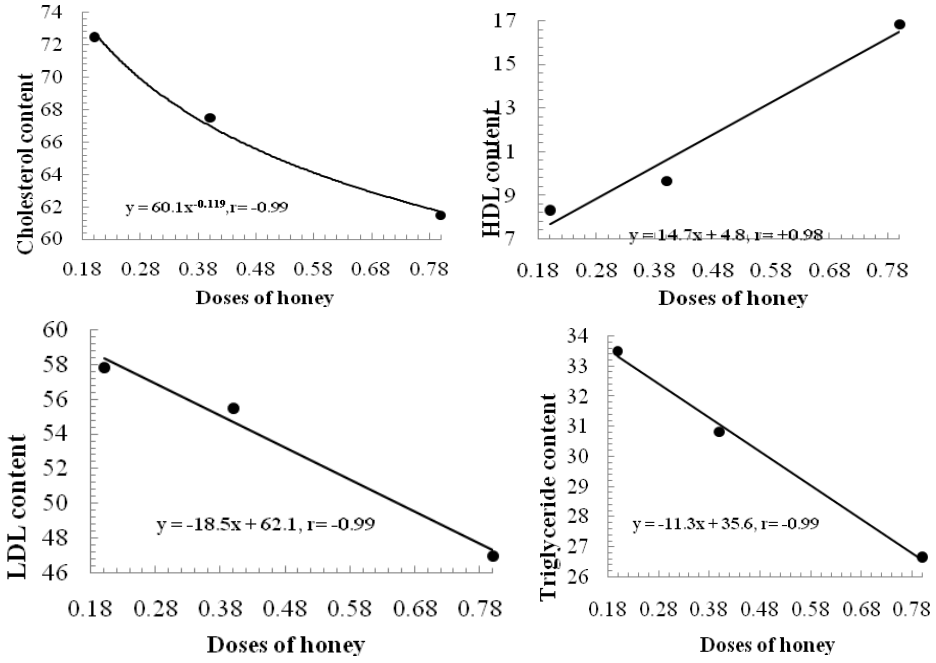


Figure 2: Relationship between the administered doses (0.2, 0.4, 0.8 g/kg b. wt.) of honey bee and the concentration of cholesterol (mg/dl), HDL (mg/dl), LDL (mg/dl) and triglyceride (mg/dl) in serum of male albino rats. Each value is a mean of six rats.

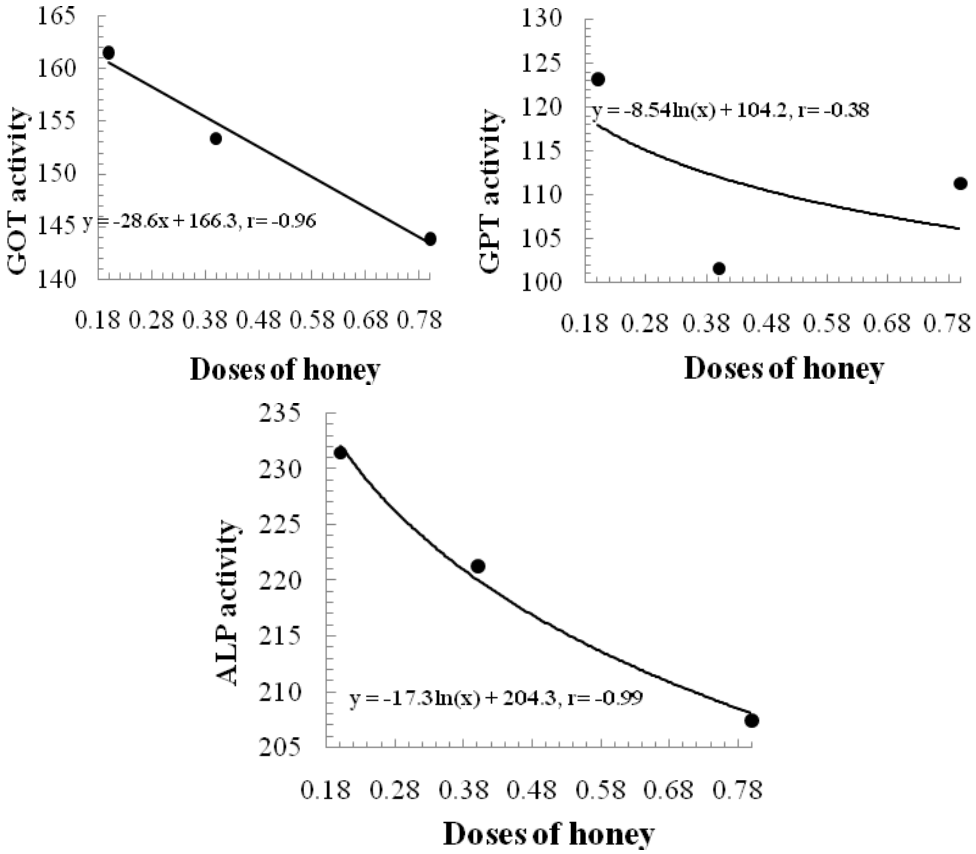


Figure 3: Relationship between the administered doses (0.2, 0.4, 0.8 g/kg b. wt.) of honey bee and the activities of GOT (U/l), GPT (U/l)and alkaline phosphatase ALP (U/l) in serum of male albino rats. Each value is a mean of six rats.

Table 1: Effect of Lead Acetate, Bee Honey (0.2, 0.4 and 0.8 g honey /kg b. Wt) alone or in combination on Levels of Urea, Uric Acid, Creatinine and Bilirubin in Serum of Male Albino Rats.

| Experimental groups | Urea (mg/dl) | Uric acid (mg/dl) | Creatinine (mg/dl) | Bilirubin (mg/dl) |
|---|---|---|---|---|
| Control | 130.3 ± 0.687 | 1.98 ±0.049 | 0.710 ±0.052 | 0.423 ±0.017 |
| Pb – administered rats | 152.3 ±1.14* | 2.73 ±0.049* | 1.850 ±0.043* | 0.783 ±0.047* |
| Pb+H1 administered rats | 128.8±0.70 | 1.80 ±0.051 | 0.672 ±0.031 | 0.323 ±0.004 |
| Pb+H2 administered rats | 123.3±0.49* | 1.61 ±0.029* | 0.465 ±0.021* | 0.290 ±0.012* |
| Pb+H3 administered rats | 120.8±0.60* | 1.13 ±0.204* | 0.337 ±0.022* | 0.122 ±0.005* |
| ANOVA (Doses of honey effect) | $F_{3,20}$= 342, $P$<0.000 | $F_{3,20}$= 38, $P$ <0.000 | $F_{3,20}$= 514, $P$ <0.000 | $F_{3,20}$= 131, $P$ <0.000 |
| ►Correlation coefficient | -0.82 | -0.93 | -0.81 | -0.87 |

Values are presented as mean ± standard error. (N=6)
$P$ <0.000: significant effect of administered doses of honey at d.f. 3, 20 and α= 0.0001
► correlation coefficient between the administered doses of honey and the studied parameters.
* significant difference in comparison with the corresponding controls at α= 0.05

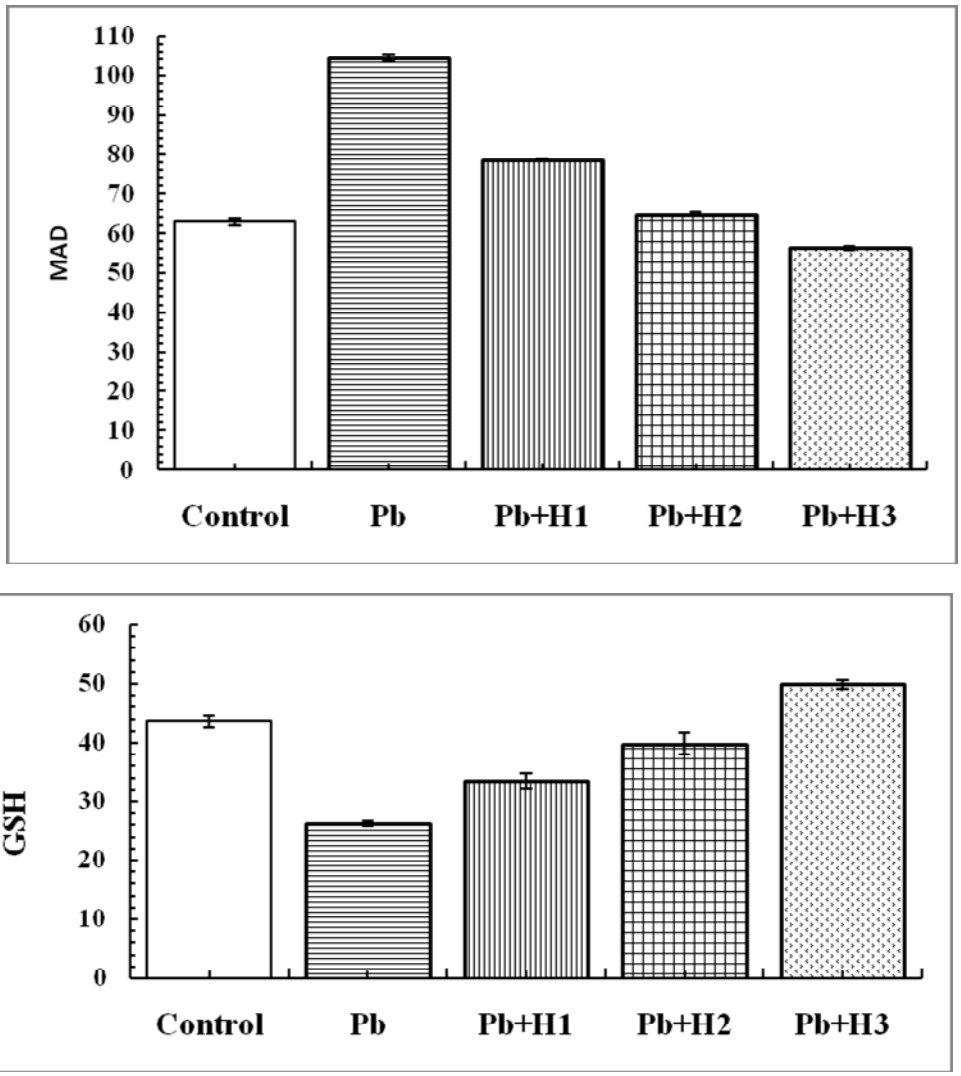


Figure 4. Effect of Lead Acetate, Bee Honey ( 0.2, 0.4 and 0.8 g/kg b.wt.) on the Levels of MAD and GSH in Serum of Male Albino Rats

Table 2: Table 1: Effect of Lead Acetate, Bee Honey (0.2, 0.4 and 0.8 g honey /kg b. Wt) alone/ or in Combination on Levels of Cholesterol, Triglycerides, HDL and LDL in Serum of Male Albino Rats.

| Experimental groups | Cholesterol (mg/dl) | Triglyceride (mg/dl) | HDL (mg/dl) | LDL (mg/dl) |
|---|---|---|---|---|
| Control | 73.33±0.882 | 34.50±0.764 | 10.50± 0.764 | 60.2±1.30 |
| Pb – administered rats | 78.50± 0.428* | 40.83±0.910* | 5.83± 0.307* | 65.2±0.70* |
| Pb+H1 administered rats | 72.50± 0.428 | 33.50±0.563 | 8.33± 0.422* | 57.8±0.60 |
| Pb+H2 administered rats | 67.50± 0.764* | 30.83±0.307* | 9.67± 0.422 | 55.5±0.43* |
| Pb+H3 administered rats | 61.50± 0.563* | 26.67±0.882* | 16.83± 0.477* | 47.0±0.73* |
| ANOVA (Honey Effect) | $F_{3,20}$= 165, $P$ <0.000 | $F_{3,20}$= 70, P $P$ <0.000 | $F_{3,20}$= 132, $P$ <0.000 | $F_{3,20}$= 143, $P$ <0.000 |
| Correlation coefficient | -0.98 | -0.95 | +0.98 | -0.98 |

Values are presented as mean ± standard error. (N=6)

$P$ <0.000: significant effect of administered doses of honey at d.f. 3, 20 and α= 0.0001

► correlation coefficient between the administered doses of honey and the studied parameters.

* significant difference in comparison with the corresponding controls at α= 0.05

Table 3: Effect of Lead Acetate, Bee Honey (0.2, 0.4 and 0.8 g honey /kg b. Wt) alone. or in Combination on Levels The enzymatic Activity of GOT, GPT and ALP in Serum Male Albino Rats

| Experimental groups | GOT (U/l) | GPT (U/l) | ALP (U/l) |
|---|---|---|---|
| Control | 160.5±0.76 | 123.50± 0.764 | 229.50± 0.764 |
| Pb – administered rats | 191.2±2.21* | 136.83± 1.014* | 261.50± 2.320* |
| Pb+H1 administered rats | 161.5±0.96 | 123.17± 0.477 | 231.50± 0.847 |
| Pb+H2 administered rats | 153.3±0.67* | 101.67± 1.346* | 221.33± 0.558* |
| Pb+H3 administered rats | 143.8±2.50* | 111.33± 1.520* | 207.50± 3.344* |
| ANOVA (Honey Effect) | $F_{3,20}$= 135, $P$ <0.000 | $F_{3,20}$= 111, $P$ <0.000 | $F_{3,20}$= 119, $P$ <0.000 |
| Correlation coefficient | -0.89 | -0.71 | +0.93 |

Values are presented as mean ± standard error. (N=6)

$P$ <0.000: significant effect of administered doses of honey at d.f. 3, 20 and α= 0.0001

► correlation coefficient between the administered doses of honey and the studied parameters.

* significant difference in comparison with the corresponding controls at α= 0.05

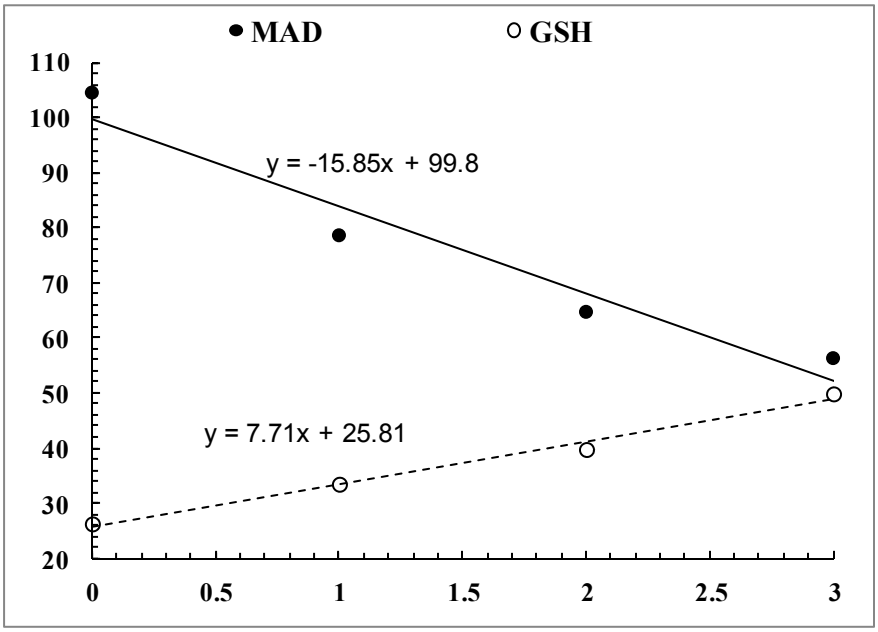


Figure 5. Relationship between the administered doses of honey and the concentrations of MAD and GSH in male albino rats, after 30 days of administration.

**4. Discussion:**

The present study showed that lead increased urea, uric acid, creatinine and bilirubin levels, caused an elevation in the enzymatic activity of both GOT and GPT and disturbed lipid profile. This might indicate that lead exposure induced adverse effect upon liver and kidney function. Elevated liver enzymes may indicate inflammation or damage to liver cells. Inflamed or injured liver cells leak higher than normal amounts of certain chemicals, including liver enzymes, into the blood-stream, which can result in elevated liver enzymes on blood. Consequently, it is plausible that inflamed or injured liver cell resulted in disturbed lipid profile. In accordance, a recent study indicated that oral administration of lead acetate increased the activity of blood enzymes: alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, lactate dehydrogenase and a decrease of creatinine level in rats (Ibrahim *et al*., 2012). In addition, the present study indicated that lead exposure induced a significant increase in both total cholesterol, triglycerides, HDL and LDL levels. Similar findings have been reported for lead-exposure by Peters *et al*. (2012). whereas, Cocco *et al*. (1991) indicated that lead induced remarkable increase HDL but decreases in total cholesterol and LDL levels. However, another study of occupational lead exposure found a positive correlation between blood

lead and total cholesterol and LDL, but not between blood lead and HDL (Ademuyiwa *et al*. 2005). However, low doses of lead were associated with a decrease in total cholesterol and HDL and an increase in triglyceride levels (Skoczynska *et al*.,1993).

The elevated MDA and decreased GSH levels might indicate increased lipid peroxidation and oxidative stress. This effect might be interpreted that lead may induce metabolic dysfunction through the enzymatic inhibition and/or disturbing the oxidant/antioxidant status. In good keeping to this interpretation, a recent study showed that lead decreased blood glutathione (GSH), GSH peroxidase, adenosine triphosphatase, and catalase but increased oxidized GSH, thiobarbituric acid reactive substance, and intracellular calcium in rat (Wang *et al*., 2011 and Flora *et al*., 2012).

Lead exposure induced metabolic disorders and biochemical changes in the liver (Lazarenko and Mel'nykova, 2012). Consistently, a previous study indicated that lead exposure caused changes in lipid metabolism which indicated that lead intoxication resulted in induction of phospholipidosis in the kidney and brain. (Ademuyiwa *et al*. , 2005). In accordance, several studies demonstrated normalizing effect of honey on lipid parameters and its potential benefits on risks of coronary heart disease (Münstedt *et al*., 2009, Alagwu *et al*., 2011 and Nemoseck *et al*., 2011).

It is worthy to note that the kidney is the first target organ of heavy metal toxicity because of its ability to reabsorb and accumulate divalent metals. The extent of renal damage by heavy metals depends on the nature, the dose, route and duration of exposure. In accordance, it has been reported that both acute and chronic intoxication cause nephropathies (Barbier *et al*., 2005; Shelley *et al*., 2012), In addition, a previous study revealed that lead inhibits the spontaneous refolding of chemically denatured proteins by forming high-affinity multidentate complexes with thiol and other functional groups and inhibited the chaperone-assisted refolding of chemically denatured and heat-denatured proteins (Sharma *et al*., 2008).

From the obtained results, it is concluded that oral administration of honey bee dose-dependently showed significant suppression of lead-induced harmful effects on liver and kidney function and restored the normal lipid profile. Moreover, honey exhibited antioxidant potential against lead induced-oxidative stress. This effect might be due to the antioxidant and the lipid metabolism-enhancing effect of honey. In accordance to the present findings, a previous study indicated that honey exhibited a protective potential by improving the disrupted liver biochemical markers and alleviating the increase of lipid peroxidation induced by Aluminum chloride (Shati and Alamri, 2010). In addition, a previous study indicated that honey has a remarkable total antioxidant capacity and radical scavenging activity (Oddo *et al*., 2008, Cavuşoğlu *et al*., 2009 and Küpeli *et al*., 2010).

In conclusion, oxidative stress and to lesser extent abnormal lipid metabolism have been implicated in the hepatotoxicity of lead. Whereas, oxidative stress is the main culprit in the nephrotoxicity of lead intoxication. The beneficial effect of honey is due to is ability to counteract the oxidative damage and protect liver and kidney tissues and restore the normal metabolic processes.

**References**


1. Ademuyiwa O, Ugbaja RN, Idumebor F. and Adebawo O. (2005): Plasma lipid profiles and risk of cardiovascular disease in occupational lead exposure in Abeokuta, Nigeria. Lipids Health Dis. 4:19.
2. Alagwu EA, Okwara JE, Nneli RO. and Osim EE. (2011): Effect of honey intake on serum cholesterol, triglycerides and lipoprotein levels in albino rats and potential benefits on risks of coronary heart disease. Niger J Physiol Sci., 26(2):161-165.
3. Barbier O, Jacquillet G, Tauc M, Cougnon M,. and Poujeol P.(2005): Effect of heavy metals on, and handling by, the kidney. Nephron Physiol. 99(4):p105-10.
4. Bogdanov, S. Jurendic,T. Sieber, R.and Gallmann,P.(2008): Honey for nutrition and health: a review. *Journal of the American College of Nutrition*, 27(6): 677–689.
5. Cavuşoğlu K, Yapar K. and Yalçin E. (2009): Royal jelly (honey bee) is a potential antioxidant against cadmium-induced genotoxicity and oxidative stress in albino mice. J Med Food. 12(6):1286-92.
6. Chen J, Chen Y, Liu W, Bai C, Liu X, Liu K, Li R, Zhu JH. and Huang C. (2012): Developmental lead acetate exposure induces embryonic toxicity and memory deficit in adult zebrafish. Neurotoxicol Teratol. pii: S0892-0362(12)00149-3. (ahead of print).
7. Cocco PL, Cocco E, Anni MS, Flore C, Melis A. and Salis S. (1991): Occupational exposure to lead and blood cholesterol in glucose-6-phosphate dehydrogenase deficient and normal subjects. Res Commun Chem Pathol Pharmacol. 72(1):81–95.
8. Cobbett, C. and Goldsbrough, P. (2002): Phytochelatin and metallothioneins: Roles in heavy metal detoxification and homeostasis. Annu. Rev. Plant Biol. 53, 159–182.

9. El-Zahrani HA. and El-Saied AI. (2012): Bioremediation of heavy metal toxicity from factory effluents by transconjugants bacteria. J Egypt Soc Parasitol. 41(3):641-50.
10. Erejuwa, O. O. Gurtu, S. Sulaiman, S. A. Wahab, M. S. A. Sirajudeen, K. N. S. and. Salleh, M. S. M. (2010 a ): Hypoglycemic and antioxidant effects of honey supplementation in streptozotocininduced diabetic rats," *International Journal for Vitamin and Nutrition Research*, 80(1): 74–82.
11. Erejuwa, O.O. Sulaiman, S. A. Wahab, M. S. Sirajudeen, K. N. S. Salleh, M. S. M. D. and Gurtu, S. (2010 b): Antioxidant protection of Malaysian tualang honey in pancreas of normal and streptozotocin-induced diabetic rats," *Annales d'Endocrinologie*, 71 (4): 291–296.
12. Flora SJ, Gautam P. and Dwivedi N.(2012): Dose-dependent effects of ethanol on lead-induced oxidative stress in rats. J Environ Pathol Toxicol Oncol.;31(1):61-73
13. Friedewald WT, Levy RI. and Fredrickson DS.(1972): Estimation of the concentration of low density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clinical Chemistry 18: 499-502.
14. Gargouri M, Ghorbel-Koubaa F, Bonenfant-Magné M, Magné C, Dauvergne X, Ksouri R, Krichen Y, Abdelly C. and El Feki A.(2012): Spirulina or dandelion-enriched diet of mothers alleviates lead-induced damages in brain and cerebellum of newborn rats. Food Chem Toxicol. 50 (7):2303-10.
15. Ghashm A. A. Othman, N. H Khattak,. M. N. Ismail, N. M and Saini, R (2010): Antiproliferative effect of Tualang honey on oral squamous cell carcinoma and osteosarcoma cell lines," *BMC Complementary and Alternative Medicine*, 10 (49).
16. Ibrahim NM, Eweis EA, el-Beltagi HS,. And Abdel-Mobdy YE.(2011): The effect of lead acetate toxicity on experimental male albino rat. Biol Trace Elem Res. 144 (1-3):1120-32.
17. Ibrahim, N.M., Eweis, E. A., El-Beltagi, H.S. and Abdel-Mobdy, Y.E.(2012): Effect of lead acetate toxicity on experimental male albino rat. Asian Pacific Journal of Tropical Biomedicine 2 (1) 41–46.
18. Jayatilleke E. and Shaw S.(1993): A high-performance liquid chromatographic assay for reduced and oxidized glutathione in biological samples. Analytical Biochemistry, 214(2), 452-57
19. Karatepe M.(2004): Simultaneous determination of ascorbic acid and free malondialdehyde in Human Serum by HPLC-UV. Liquid Chromatography & Gas Chromatography North America, 22: 362-65
20. Küpeli Akkol E, Orhan DD, Gürbüz I. and Yesilada E.(2010): In vivo activity assessment of a "honey-bee pollen mix" formulation. Pharm Biol. 48(3):253-9.
21. Lazarenko IA. and Mel'nykova NM (2012): Comparison of blood biochemical indices in rats exposed to lead in macrodispersed form and nanoform. Ukr Biokhim Zh. 84 (1):85-9.
22. Liu CM, Ma JQ. and Sun YZ. (2012): Puerarin protects rat kidney from lead-induced apoptosis by modulating the PI3K/Akt/eNOS pathway. Toxicol Appl Pharmacol. 258(3):330-42.
23. Münstedt K, Hoffmann S, Hauenschild A, Bülte M, von Georgi R. and Hackethal A.(2009): Effect of honey on serum cholesterol and lipid values. J Med Food. 12(3):624-628.
24. Nemoseck TM, Carmody EG, Furchner-Evanson A, Gleason M, Li A, Potter H, Rezende LM, Lane KJ. and Kern M. (2011): Honey promotes lower weight gain, adiposity, and triglycerides than sucrose in rats. Nutr Res. 31(1):55-60.
25. Oddo LP, Heard TA, Rodríguez-Malaver A, Pérez RA, Fernández-Muiño M, Sancho MT, Sesta G, Lusco L, and Vit P.(2008): Composition and antioxidant activity of Trigona carbonaria honey from Australia. J Med Food 11(4):789-94
26. Ozsoy SY, Ozsoy B, Ozyildiz Z. and Aytekin I. (2011): Protective effect of L- carnitine on experimental lead toxicity in rats: a clinical, histopathological and immunohistochemical study. Biotech. Histochem. 86 (6): 436-43.
27. Perales-Vela, H.V., Julia´n Mario Pen˜a-Castro, J.M. and Rosa Olivia Can˜izares-Villanueva, R.O. (2006): Heavy metal detoxification in eukaryotic microalgae Chemosphere 64: 1–10
28. Peters JL, Kubzansky LD, Ikeda A, Fang SC, Sparrow D, Weisskopf MG, Wright RO, Vokonas P, Hu H, Schwartz J. (2011): Lead concentrations in relation to multiple biomarkers of cardiovascular disease: the Normative Aging Study. Environ Health Perspect.120(3):361-6.
29. Rahman A, Khan KM, Al-Khaledi G, Khan I. and Al-Shemary T. (2012): Over activation of hippocampal serine/threonine protein phosphatases PP1 and PP2A is involved in lead-induced deficits in learning and memory in young rats. Neurotoxicology. 33(3):370-83.
30. Rietman, S. and Frankel, S. (1957): A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases *Amer. J. Clin. Pathol.* 28: 56-63.

31. Sharma SK, Goloubinoff P. and Christen P.(2008): Heavy metal ions are potent inhibitors of protein folding. Biochem Biophys Res Commun; 372(2):341-345.
32. Shati AA. and Alamri SA.(2010): Role of saffron (Crocus sativus L.) and honey syrup on aluminum-induced hepatotoxicity. Saudi Med J. 31(10):1106-13.
33. Shelley R, Kim NS, Parsons P, Lee BK, Jaar B, Fadrowski J, Agnew J, Matanoski GM, Schwartz BS, Steuerwald A, Todd A, Simon D. and Weaver VM.(2012): Associations of multiple metals with kidney outcomes in lead workers. Occup Environ Med. [ahead of print]
34. Skoczynska A, Smolik R. and Jelen M.(1993): Lipid abnormalities in rats given small doses of lead. Arch Toxicol 67(3):200–204.
35. Tchounwou PB, Yedjou CG, Patlolla AK. and Sutton DJ (2012): Heavy metal toxicity and the environment. EXS. 101:133-64.
36. Valentino M, Rapisarda V, Santarelli L, Bracci M, Scorcelletti M, Di Lorenzo L, Cassano F. and Soleo L. (2007): Effect of lead on the levels of some immunoregulatory cytokines in occupationally exposed workers. Hum Exp Toxicol. 26(7):551–556.
37. Vassallo DV, Simões MR, Furieri LB, Fioresi M, Fiorim J, Almeida EA, Angeli JK, Wiggers GA, Peçanha FM. and Salaices M. (2011): Toxic effects of mercury, lead and gadolinium on vascular reactivity. Braz J Med Biol Res. 44 (9):939-46.
38. Wang L, Wang H, Li J, Chen D. and Liu Z. (2011): Simultaneous effects of lead and cadmium on primary cultures of rat proximal tubular cells: interaction of apoptosis and oxidative stress. Arch Environ Contam Toxicol. 61(3):500-511.

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