Bee Venom - Lead Acetate Toxicity Interaction

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Abstract: Venom immunotherapy is a highly effective treatment, capable of improving health-related quality of life. Bee venom (BV) is considered an effective rheumatoid arthritis modulator that removes reactive oxygen species (ROS). The hypothesis behind the present work was that, the ability of BV to remove reactive oxygen species (ROS) may help in protecting against lead acetate induced hepatic injury. Accordingly, the present study was designed to investigate the possible interaction between BV and lead acetate, with an emphasis on the involvement of IL-6 and oxidative stress in the BV-lead acetate hepatic effects in mice. Adult male mice were divided into 2 main groups namely, BV group and BV/Lead acetate group. The BV group was equally divided into 5 subgroups, given BV in the dose levels 0, 1, 2.5, 5 or 10 μg/kg, sc. The BV/Lead acetate treated animals were parallely divided into 5 subgroups, according to the given dose of BV. They were orally administered 100 mg/kg lead acetate, 1 hr after BV administration. Each of BV and lead acetate was given every other day for one month. Twenty-four hours after the last dosage, serum biochemical markers (ALT and AST), liver oxidative stress markers (superoxide dismutase, SOD, glutathione GSH, malondialdehyde MDA, and catalase, CAT), liver total nitrate/nitrite content, and interleukin-6 (IL-6) were assessed. The obtained results revealed that, while administration of BV induced decrease in liver IL-6 content, it induced increase in the hepatic total nitrate/nitrite content. The liver GSH levels and SOD activity were inversely correlated with the lower used doses of BV (1, 2.5 and 5 μg/kg). The high-utilized dose 10 μg/kg induced a significant increase in liver MDA content. Lead acetate decreased the survival rate and the relative liver weight and increased ALT activity. Pre-administration of BV restored each of the liver weight, ALT activity and the liver nitrite content to their normal ranges. In conclusion, exposure to lead acetate resulted in mild to moderate liver toxicity, whose certain manifestations were alleviated on co-exposure to Low dose level of BV.

Key words: Bee venom, lead acetate, hepatotoxicity, oxidative stress, interleukin 6.

INTRODUCTION

Lead is a toxic metal that induces a broad range of physiological, biochemical and neurological dysfunctions in humans (Skerfving and Bergdahl 2007). Although atmospheric lead pollution due to tetraethyl lead, from gasoline, has been improved over the last decades, humans are still exposed to lead via contaminated foods and water through industrial activities (Barbosa et al., 2005). Lead-induced oxidative stress in blood and other soft tissues has been postulated to be one of the possible mechanisms of lead-induced toxic effects (Pande et al., 2001). Disruption of pro-oxidant/antioxidant balance might lead to tissue injury. It was reported that lead increases the level of lipid peroxidation (Upasani et al., 2001) and brain thiobarbituric acid-reactive substances and that it alters the antioxidant defense system (Adanaylo and Oteiza, 1999). As liver is a common target organ for injury from exposure to a broad range of chemicals, drugs and naturally occurring agents, similar effects were also reported in the hepatic tissues (Sandhir and Gill, 1995). Recent studies confirmed the possible involvement of reactive oxygen species (ROS) in lead-induced toxicity (Gurer and Ercal, 2000, Wang et al., 2007). Recently, it was demonstrated that interleukin-6 (IL-6) is induced by various metal compounds such as lead, and that it plays an important role in the induction of metallothionein in the liver (Kobayashi et al., 2007). Metallothionein is a low-molecular-weight metal-binding protein, known to play an important role in the protection against heavy metal toxicity, and is involved in the scavenging of free radicals (Suzuki et al., 1993).
Unconfirmed circumstantial evidence for the favorable effects of bee venom (BV) on a wide variety of ailments goes back for centuries (Lee et al. 2004). With the increasing popularity of alternative medicine and the adoption of Eastern medicine in the Western hemisphere, BV therapy has increasingly gained acceptance among physicians in Europe (Lee et al., 2005a&b), U.S. and -recently- in Egypt. Bee venom has been reported to be a possible remedy for various ailments ranging from multiple sclerosis, asthma, polyneuritis and neuralgia, to malaria, wound healing and epilepsy (Stuhlmeier, 2007). The list of reported ailments that can be ameliorated by BV include its usefulness for the treatment of some immune-related diseases, especially rheumatoid arthritis (Lee et al., 2004&2005a&b, Kwon et al., 2002).

Several studies have reported radio-protective, anti-mutagenic, anti-inflammatory, anti-nociceptive and anticancer effects of BV, both in vitro and in vivo. Bee venom is demonstrated to have a radio-protective effect against basal and oxidative DNA damage in Wistar rat lymphocytes. It is not genotoxic and do not produce oxidative damage at low concentrations (Gajski and Garaj-Vrhovac, 2009). In addition, it was found that BV acupuncture decreases the level of reactive oxygen free radical species (ROS) induced oxidative damage to synovial fluid proteins (Suh et al., 2006). It also suppresses the generation of nitric oxide in mouse macrophage cell line (Raw 264.7 and synovioocytes obtained from rheumatoid arthiritis patients (Park et al, 2007) and suppresses LPS-induced iNOS expression and inhibits the expression of pro-inflammatory molecules including cyclo-oxygenase-2 (Cox-2) and interleukin-1 beta (IL-1β) in rat C6 glioma cells (Lee et al., 2009). On investigating the anti-arthritic effect of BV in adjuvant induced arthritis in rats, it was found that serum concentrations of tumor necrosis factor alpha (TNF-α) and IL-1 β were significantly lower in rats of the BV treatment (Luo et al., 2006). In addition, it was found that a time-and dose-dependent response as well as the type of treated cell line, determine the immunosuppressive and/or immunostimulant property of BV that could be effective in future therapeutic strategies (Hamedani et al., 2005).

On the other hand, some reports argued that it seems unlikely that BV treatment will be of great benefit, as it was found that in fibroblast-like synoviocytes, dermal fibroblasts and mononuclear cells exposed to BV, mRNA levels of several pro-inflammatory genes and Cox-2 protein levels are increased (Stuhlmeier, 2007). Furthermore, large quantities of oxygen radicals are produced in a dose-dependent manner in leukocytes exposed to BV (Stuhlmeier, 2007).

Based on the observation that free radicals were generated during the pathogenesis processes induced by lead exposure, it was presumed that supplementation of antioxidants could be an alternative method for chelation therapy (Flora et al., 2003). However, some authors have raised questions pertaining to the potential risk for side effects of intake of large doses of vitamins (Aiguo, 2001), as it was presumed that these vitamins might act as pro-oxidant for the introduction of apoptotic cells in the presence of oxygen and the generation of H2O2. So, it may be beneficial to investigate the effects of agents other than the ordarinary antioxidants.

Based on the current conflicting properties for BV, and the need for testing agents other than ordarinary anti-oxidant, the present study was therefore conducted to investigate whether BV is a protector or a potentiator of hepatotoxicity-induced by lead. To gain insight regarding the liver antioxidant profile of the BV, changes in some oxidative stress markers and IL-6 content in the liver were estimated following exposure to different dose levels of BV, either alone or with concomitant exposure to a dose of lead acetate induced liver injury.

**MATERIALS AND METHODS**

**Animals:**
Adult male mice of the Swiss albino strain aged 12-14 weeks, weighing 30-35 g., obtained from the animal house of the National Organization for Drug Control and Research (NODCAR) were used. Mice were housed six per cage and were maintained under standard laboratory animal housing conditions fed standard diet and water *ad libitum*.

**Doses Preparation and Routes of Administration:**
Lyophilized Apis Mellifera purified bee venom (VACSERA, Egypt, 1mg/ vial) was used. It was subcutaneously administered in the dose levels of 0, 1, 2.5, 5 and 10 μg/kg in a dose volume of 5ml/kg.

Lead acetate (LA) was prepared as solution in distilled water. It was orally administered in a dose of 100 mg/kg in a dose volume of 25 ml/kg.

Both BV and lead acetate were administered every other day for 30 days.
Experimental Design:

After an acclimation period of 7 days, 142 mice were enrolled in the experiment and were divided into 2 main groups namely, BV group and BV-LA group. The first group was served to study the dose-response relationship of BV and the second group to study the BV-LA interaction. Animals of these groups were further subdivided into four subgroups, according to the given dose levels of BV. Animals of each BV and BV-LA subgroup were subcutaneously injected, either of 0, 1, 2.5, 5 or 10 μg/kg dose level of BV. To BV-LA interaction group LA was administered orally, in a dose of 100 mg/kg, 1 hr after BV injection. Each subgroup of the dose-response BV group was consisted of 10 mice and of 12 mice in the BV-LA interaction group. In addition, parallel control group (10 mice) received the utilized vehicle (dist. H2O) and another group of 24 mice that received oral administration of lead acetate only were utilized. Both BV and lead acetate were administered every other day for 30 days.

After 24 hr of last dosing, blood was withdrawn from the orbital sinus of each animal in separated heparinized tubes for plasma separation. Animals were then decapitated; livers were rapidly excised for the estimation of alteration in lipid peroxidation indices, malondialdehyde (MDA), antioxidant enzymes such as superoxide dismutase (SOD) and catalase (CAT) as well ad nonenzymatic antioxidants such as glutathione (GSH). Furthermore, plasma alanine transaminase (ALT) activity was assessed. Livers were weighted for assessment of relative liver weight and fixed small parts of the livers were fixed in 10% formalin for histopathological examination. The plasma aliquots and liver samples were stored at -80 °C before the assays. Fixed weights of livers were homogenized in KCl for determination of tissue superoxide dismutase (SOD), catalase (CAT). Other fixed liver weights were homogenized in ice-cold protease inhibitor phenyl-methyl-suphanyl florid (PMSF) for determination of liver IL-6 content.

Antioxidant Enzymatic activities:

Tissue superoxide dismutase (SOD) activity:

Superoxide dismutase (SOD) activity was determined by the method of Marklund and Marklund (1974). Briefly, the reaction mixture consists of homogenate, buffer and pyrogallol. The buffer consist of 50 ml of 0.1 M Tris to 49 ml distilled water and 1 ml of 0.1 M diethylenetriamine pentaacetic acid, pH 8.2 and was aerated by stirring vigorously at room temperature for 20 min before use. To 5 μL of liver homogenate adequate volume 50m M tris-HCl buffer (pH 8.2) containing 1mM diethylenetriamine pentaacetic acid to become 2 ml final volume was added. The reaction was initiated by adding 25 μL of 24 mM mmol/L of pyrogallol (Sigma-Aldrech, USA) dissolved in 10 mM/L HCl, prepared immediately before use. The change in the optical density at 420 nm was recorded for 2-3 min. The blank was run using the Tris alone plus pyrogallol, and the exact increase in the optical density for this system should be 0.02 absorbance units/min.

Tissue Catalase (CAT) Activity:

Tissue catalase was determined according to the method of Evans and Diplock (1991). Liver homogenates of mice were diluted with buffer, to obtain an adequate dilution of the enzyme. Then, 2 ml of the enzyme dilution were added to a cuvette and mixed with 1 ml of 30 mmol/L H2O2 and the absorbance at 240 nm was measured for 100 seconds. Initial absorbance of the reaction mixture should be round 0.5. The enzyme activity (U/g tissue) is expressed as the first order constant that describes the decomposition of H2O2 at room temperature. Liver protein content was estimated by Lowry et al., (1951) method.

Non-enzymatic antioxidant

Liver glutathione (GSH) content:

Liver samples were homogenized in ice-cold 150 mol/L KCl for determination of GSH levels. The chilled liver was homogenized in KCl (10% (v/v) final homogenate). A spectrophotometric method using Ellman's reagent (Beutler, 1975) was used for determination of GSH content. The absorbance was measured within 5 min at 412 nm using a double beam spectrophotometer (Helios a thermospectonic). Results were expressed as μmol/g tissue.

Lipid Peroxidation:

The formation of reactive oxygen species (ROS) was quantified by measuring the product of the reaction of ROS with membrane lipid. Hepatic lipid peroxidation was assessed by determining the malondialdehyde (MDA) content of tissue homogenates by using a colorimetric assay, as previously described (Uchiyama an Mihara, 1978). Briefly, to 0.5 ml liver homogenate, 1 ml of 20% trichloroacetic (TCA) was added. After precipitating the protein with TCA, 3ml of 1% orthophosphoric acid (1% H3PO4) and 1 ml of 0.6%
thiobarbituric acid (0.6 TBA) was added and then incubated in a boiling water bath for 40 min. After cooling, the samples were extracted with n-butanol and centrifuged at 4000 rpm. The absorbance of samples was determined at 520 and 535 nm. 1,1,3,3-tetraethoxypropane was used as a standard. Concentrations of MDA were expressed as nmol/g tissue.

**Determination of Liver Nitrite and Total Nitrite/nitrate Contents:**
Total nitrate/nitrite accumulation in liver was performed according to Miranda et al., (2001) as an indication of NO production. The procedure for NO determination was based on the reduction of nitrate by vanadium (III) chloride (VCl3) combined with detection by Griess reaction. Before NO estimation, liver homogenate were deproteinized. Two hundred μl of absolute ethanol was added to 100 μl sample followed by centrifugation at 3000 rpm for 5 min. Saturated solution of 200 mg VCl3 (Sigma-Aldrich) was prepared in 1 M HCl (25 ml), after filtration, the blue solution was stored in the dark at 4°C. Griess reagent was composed of 1:1 mixture of 0.1% N-(1-naphthyl) ethylenediamine in deionized H2O and 2% sulfanilamide (Sigma-Aldrich) in 5% HCl and premixed immediately prior application. Experiments were performed by adding 100 μl VCl3 to 100 μl sample followed by rapid addition of 100 μl Griess reagents (1:1:1). The absorbance at 540 was measured using a spectrophotometer (Heilos & thermospectonic) following 30 min incubation. Nitrate standard solution was serially diluted and the absorbance was referred to the nitrate standard curve.

**Determination of liver interleukin-6 (IL-6) content:**
IL-6 levels were determined in liver homogenate by enzyme-linked immunosorbent assay (ELISA) kit. The fixed liver weight samples were homogenized in 1 ml of ice–cold protease inhibitor, phenyl-methyl-sulphanyl florid (PMSF, Pharmacia LKB Biotechnology, Uppsala, Sweden). Single-use aliquots of the homogenates were stored at -80°C before measurement of liver IL-6 content. The enzyme-linked immunosorbant assays was performed according to the manufacturer's instructions (Invitrogen), and read at 450 nm using ELISA reader (BioTEk Instruments Inc., ELx 808, USA). The concentration of interleukin was normalized by the wet weight of the tissue before homogenization to yield the amount of IL-6 in pg/gram of tissue.

**Histopathological Examination:**
The fixed liver samples were trimmed, dehydrated, embedded in paraffin, sectioned, mounted on glass slide, stained with hematoxylin and eosein and examined by light microscopy. Other liver sections were stained with special stain (Sirius red) for detection of fibrosis (Gurr, 1971) and were examined by light microscopy.

**Statistical Analysis:**
Statistical analysis was performed using SPSS 10.0 statistical software (SPSS Inc, Chicago, IL). Means were used and data were analyzed by one-way analysis of variance (ANOVA). If the test showed a significant difference, the least significant difference test was used as a Post hoc Tukey's test for multiple comparisons. The differences were considered significant if the probability was associated with p<0.05.

**RESULTS AND DISCUSSION**

**Effect of Lead Acetate (LA) and Bee Venom (BV) on the Relative Liver Weight:**
While LA significantly decreased relative liver weight by 7% (p<0.05), pre-administration of either doses of BV could restore the relative liver weight to its normal value (Fig. 1).

**Fig. 1:** Effect of Bee venom and lead acetate on relative liver weight
**Effect of Lead Acetate and Bee Venom on Liver Glutathione (GSH) levels:**

The liver glutathione content of mice injected subcutaneously with either of the utilized BV dose levels (1, 2.5 and 5 μg/kg) exhibited significant increases by 147% (p<0.001), 86% (p<0.001) and 59% (p<0.001), respectively as compared to the control group (fig. 2).

Although LA induced insignificant reduction in the liver GSH content, pre-administration of the either dose levels of BV (1, 2.5, 5 and 10 μg/kg) could restore liver GSH content to its normal value (fig. 2).

**Fig. 2:** Effect of BV and lead acetate on hepatic glutathion content

**Effect of Lead Acetate and Bee Venom on Liver MDA levels:**

The present results revealed that neither LA nor BV at all different utilized doses had influence on liver MDA content (fig 3). However, the higher utilized dose of BV (10 μg/kg) induced significant increase in liver MDA by 34% as compared to the control group (fig. 3).

**Fig. 3:** Effect of Bee venom and lead acetate on hepatic MDA content

N.B. a: significant from control group

**Effect of Lead Acetate and Bee Venom on Liver Superoxide Dismutase (SOD) activity:**

The results represented in figure (4) revealed that immunization with BV resulted in a negative correlation between the SOD activity and the dose level, where SOD activity exhibited significant increase by 114% (p<0.01), 81% (p<0.001) and 52% (p<0.001) at dose levels 1, 2.5 and 5 μg/kg, respectively as compared to the control value (fig. 4).

**Fig. 4:** Effect of Bee venom and lead acetate on liver SOD activity
Effect of Lead Acetate and Bee Venom on Liver Catalase (CAT) activity:
The obtained results revealed that none of the treatment BV or LA or their companied treatment had influence on catalase activity (fig 5).

![Liver Catalase Activity](image1)

**Fig. 5:** Effect of Bee venom and lead acetate on liver catalase activity

Effect of Lead Acetate and Bee Venom on Plasma alanine transferase (ALT) activity:
Lead acetate induced significant increase in plasma ALT activity by 21% (p<0.05) as compared to control group (fig. 7). Pre-administration of the lowest dose level of BV (1 μg/kg) could restore the ALT enzymatic activity to its normal value (fig. 6).

![Plasma ALT Activity](image2)

**Fig. 6:** Effect of Bee venom and lead acetate on serum ALT (GPT) activity

B. a: significant from control group

Effect of Lead Acetate and Bee Venom on liver total nitrite/nitrate and liver nitrite contents:
The utilized doses of BV (1, 2.5, 5 and 10 μg/kg) induced significant increase in total nitrite/nitrate content to reach 491% (p<0.001), 584% (p<0.001) 498% (p<0.001), and 506% (p<0.001), respectively, as compared to the control value (fig.7). Although administration of 100 mg/kg of LA every other day for one month induced insignificant increase in liver total nitrite/nitrate content (fig. 7 ), it induced significant rise in liver nitrite content by more than four folds to reach 459% (p<0.001) as compared to the control group (fig. 8). Pre-administration of either of the utilized doses of BV prior LA could restore both liver total nitrite/nitrate and liver nitrite contents to their normal value (figs.7&8).

![Liver Total Nitrite/Nitrate Content](image3)

**Fig. 7:** Effect of Bee venom and lead acetate on liver total nitrate/nitrite content
Effect of Bee Venom and Lead Acetate on Hepatic Nitrite Content:

The results in figure (9), revealed that the utilized dose levels of BV (2.5, 5 and 10 μg/kg) induced significant decrease in liver IL-6 content by 28% (p<0.05), 34% (p<0.01) and 29% (p<0.05) respectively as compared to the control group (fig. 9). Pre-administration of the lowest utilized dose level of BV (1μg/kg) induced significant reduction in liver IL-6 content by 27% as compared to LA non-treated group (fig 9).

Histopathological and Fibrotic Changes:

As shown in figures (10&16), the liver tissues of control group showed normal architecture. Liver tissues of mice administered low dose level of BV (1μg/kg) showed almost normal liver tissues with less fibrotic reaction within the hepatic lobules (fig. 17) and few vessels showed dilatation and congestion (fig.11) with less fibrotic reaction (fig.17). However, administration of the high utilized dose levels of BV induced toxic effects on liver tissues manifested mainly in the vasculature, hepatic tissues, and increase in extracellular matrix with the presence of fibrocytes (fig. 18). These pathological alterations was strongly evident in liver tissue of mice treated with 10 μg/kg BV for one month (fig.12), where many necrotic areas with fibrocytes appearance (fig. 18) accompanied by vacuolated hepatocytes. In addition, dilated congested blood vessels and expanded portal areas accompanied by inflammatory reactions were also seen (fig. 12). By decreasing BV dosage the areas of necrosis were gradually decreased, few vessels showed dilatation and congestion, less fibrotic reaction within the hepatic lobules. The incidence and severity of these histopathological changes reduced by decreasing the dose, where the liver of group treated with 1μg/kg BV, showed normal liver tissues  (figs. 11&17 ).

The examined liver tissues of mice administered LA revealed mild to moderate toxicity. Many dilated central veins, expanded portal areas with periportal inflammatory responses could be observed (fig.13). Scattered wide areas of vacuolated hepatocytes together with scattered inflammatory cells aggregates focally seen around focal necrotic areas. In addition, foci of extravasated red blood cells were seen (fig.13).

Animals groups pre-treated with BV prior lead, showed sign of improvement in liver profile compared to those exposed to lead only. Such improvement was symptomized in the reduction of necrotic areas, occurrence of wide areas of intact hepatocytes. In liver of mice pre-treated with the lowest doses of BV less toxicity was seen, where mild necro-inflammatory areas were widely scattered, but reduced in size (fig. 14). More reduction in hepatocytic vacuolation with mild necrosis and inflammatory reaction (fig. 14).
However, the liver of mice pre-treated with BV at dose level of 2.5μg/kg showed evidence of inflammatory reaction mainly in perivascular zone and sinusoids. Liver tissues of mice pre-administered 5 μg/kg exhibited focal necrotic changes with inflammatory cells was observed confined to few perivenular areas, also hepatocytic vacuolation is only seen in 50% of the population.

Pre-administration of BV prior LA produced less toxic impact on liver tissues, where in 10 μg/kg treated group, hepatocytic vacuolation is less, focal necrotic areas were minor with focally seen inflammatory aggregation, together with occasional area of fibrosis (figs. 15&21).

Fig. 10: Liver tissue of the control mice stained with Sirius red.

Fig. 11: Liver tissue stained with Sirius red stain showing the thin fibrous band are remarkably reduced and ameliorated

Fig. 12: Liver tissue of mice treated with 10 μg/kg BV and stained with sirius red showing moderately dense fibrous tissue bands are seen around central and portal venous channel, however the mesh work of thin fibrous bands that exist between hepatocytes are seen more prominently densely stained and widely extending.

Fig. 13: Liver tissue of mice treated with lead showing moderately dense fibrous tissue is seen around central veins and portal venous radicals. Widely prominent fibrocytes are seen focally between hepatocytes in areas and mainly localized in the pericentral zonal areas.
Fig. 14: Liver tissue of mice treated with BV (1μg/kg) + LA showing the fibrous existence disappeared completely.

Fig. 15: Liver tissue of mice treated with BV (10 μg/kg) + LA and stained with sirius red showing moderately dense fibrous tissue. Midely prominent fibrocytes are seen focally between hepatocytes

Fig. 16: Liver tissue of the control group showing central vein (CV) and normal hepatocyte. H&E, x 100.

Fig. 17: Photomicrograph of liver tissue of mice treated with BV (1μg/kg) showing, hepatocyte with mild vacuolation (arrow), normal central vein (CV). H&E, x 200

Fig. 18: Photomicrograph of liver tissue of BV(10μg/kg), showing vacuolated hepatocyte (Arrow), necrotic area (double arrow), H&E, x100.
Fig. 19: photomicrograph of liver tissue of lead treated group showing dilated central vein (CV), inflammatory aggregates (arrow), vaculated cytoplasm (arrow head) H&E:x200.

Fig. 20: Photomicrograph of liver tissue of mice treated with BV (1μg/kg) and LA showing intact hepatocytes (Arrow), central vein (CV), H&E:x200

Fig. 21: Photomicrograph of liver tissue of mice treated with BV (10 μg/kg) and lead showing fibrotic bands extended between hepatocytes cords (Arrow), central vein (CV), H&E:x200.

Discussion:

Bee venom immunotherapy is a highly effective treatment, capable of improving health related quality of life (Bil and Bonifazi, 2007). Animal and human studies, revealed that BV may protect against sequelae of oxidative stress induced by rheumatoid arthritis (Lee et al., 2004&2005a&b, Hong et al., 2005, Luo et al., 2006, Suh et al., 2006, Kim et al., 2008, Nah et al., 2008). It induces apoptosis of human hepatocellular carcinoma (Li et al., 2006), reduces tumor growth (Soman et al., 2009) and suppresses pro-inflammatory response (Yoon et al., 2008, Lee et al., 2009), as well as the cholecystokinin octapeptide induced acute pancreatitis (Seo et al., 2008).

The high utilized doses of BV (10 μg/Kg) induced increase in liver MDA content, a result which is consistent with Stuhlmeier, (2007), who found that BV at concentrations of 5 and 10 μg/ml induce mononuclear and polymorph nuclear cells (MNC and PMN) to produce large quantities of oxygen intermediates (Stuhlmeier, 2007).

In the present study, the found significant increase in hepatic GSH content following administration of BV (1, 2.5 and 5 μg/kg) could be interpreted by the increased hepatic NO, as expressed by the increase in total nitrite/nitrate content. This interpretation stems from the fact that nitric oxide protects cell from oxidative stress where it was indicated that exogenous NO increases the production of GSH (Moellering et al., 1998 & 1999). The present study revealed that the utilized dose levels of BV induced significant increase in liver total nitrite/nitrate content. This result is consistent with the fact that venoms are known to trigger the release of NO (Petricevich 2004). The mater which could be interpreted that BV being composed of mixture of biologically
active substances eg. Enzymes (phospholipases, hialuronidase), peptides (melitin, apamin, mastoparan, bombolitins) and low molecular weight compounds (biogenic amines, acetylcholine, carbohydrates, lipid, free amino acid), then, several types of reactions could produce inflammatory mediators such as cytokines and nitric oxide are possible (Petricevich, 2004).

During the course of an inflammatory response, large amounts of NO surpass its physiological amount (Xie and Nathan 1994). Nitric oxide may inhibit expression of numerous cytokines such as, IL-1β, TNF-α (Marcinkiewicz and Chaim, 1993, Marcinkiewicz, 1997, Guzik et al, 2003) as well as of IL-6 and IFN-γ (Bogdan, 2001, Guzik et al., 2003). These effects are exerted also by S-nitrosoylation of transcription factors including NF-kB/IKB (Bogdan, 2001, Guzik et al., 2003). In this concern, it was found that NO has a biphasic effect on NF-kB activity in murine macrophage. This mechanism depends on the local concentration of NO and enables it to up- and down-regulate the expression of host defense protein, including iNOS, cyclooxygenase-2 and IL-6 (Connelly et al., 2001). Local NO produces a concentration-dependent influence on NF-kB, which comprised enhancement at low concentration followed by inhibition at high concentration (Connelly et al., 2001). On the other hand, it was suggested that NO positively affects NF-kB activity at the earlier time points after addition of stimuli, however at later time points, the NF-kB activity was moderately reduced (Diaz et al., 2004). Local NO may induce marked enhancement of NF-kB activity at low concentration followed by inhibition at high concentration (Connelly et al., 2001). The contribution of acute phase inducer IL-6 in the pathogenesis of liver disease is yet unclear (Streetz et al., 2003). It was found that IL-6 plays a critical role in the accompanying liver inflammation. The increased hepatic IL-6 production may be correlated with non-alcoholic steatohepatitis development, where hepatocyte IL-6 expression, degree of inflammation and stage of fibrosis were positively correlated (Wieckowska et al., 2008). In addition, others showed an involvement of IL-6 in the pathogenesis of liver diseases and a protective role of IL-6/gp130-dependent pathway in non-parenchymal liver cells during progress of hepatic fibrosis (Streetz et al., 2003). It was also found that IL-6 alleviates hepatic steatosis and ischemia/reperfusion injury in mice with fatty liver disease (Hong et al., 2004), and that IL-6-deficient mice are susceptible to ethanol-induced hepatic steatosis, as IL-6 protects against ethanol-induced oxidative stress and mitochondrial permeability transition in the liver (El-Assal et al 2004). Moreover, IL-6 is considered as a critical component of the regenerative response, where the disruption of IL-6 gene was found to impair liver regeneration (Cressman et al., 1996), while the absence of IL-6 was found to increase the hepatic dysfunction and mortality in sepsis (Deutschman et al., 2006).

In the present work, the hepatic histopathological changes that accompanied treatment with the BV at dose levels 2.5, 5 and 10 μg/kg, namely vacuolar degenerative hepatocytes, prominent apoptotic cells and scattered areas of necrosis, may be interrelated with the BV parallel induced decrease of the hepatic IL-6 content. Moreover, the changes in the hepatic IL-6 content and the accompanied hepatic histopathological findings, in the present study, may confirm the findings of El-Assal et al., (2004), that IL-6 deficient mice are prone to ethanol-induced lipid peroxidation and steatosis and may also confirm the findings that BV down-regulates the gene of IL-6 receptor and reverses the LPS-induced up-regulation of IL-6 receptor (Yu et al., 2009). The preference of using Lead acetate salt in this study refers to its bioavailability which is 100% (Smith et al., 2008).

In the present study, the every other day administration of 100 mg/kg LA along one month has induced reduction in relative liver weight, increase in ALT activity and liver nitrite contents and insignificant decrement in liver GSH and SOD contents and increase in MDA and total nitrite/nitrate content, which did not also reach statistical significance. These results could be interpreted as LA induced oxidative stress (Annabi-Berrahal et al., 2007, Wang et al., 2007, El Nekeety et al., 2009, Newairy and Abdou, 2009). As hepatic GSH level depends largely on food intake, thus the observed decrease in hepatic GSG content could be interpreted as a consequence of lead acetate decreased food intake (El-Nekeety et al., 2009) the matter which is indicated in the present study by the decreased body weight gain (data not shown).
The current findings concerning the changes in liver MDA content and SOD activity in LA exposed mice being statistically insignificant, are consistence with those of Pillai and Gupta, (2005) and Annabi-Berrahal et al., (2007), for MDA content and with those of Marques et al., (2006) for SOD activity. In addition, the present study showed that plasma ALT activity was increased after administration of LA, the matter which also confirms the findings of Shalan et al., (2005) and Rahman and Sultana, (2006).

In addition, the histopathological examination of the liver of mice exposed to LA revealed mild to moderate toxicity confirms the other obtained biochemical changes. Although the utilized dose of LA in the present study, which is 100 mg/kg, p.o, every other day, was followed according to Xu et al., (2008), these results indicate that this dosage regimen is not a good model for lead hepatotoxicity. Actually, it is a matter of long-term chronic exposure to lead, i.e. it needs more than 30 days to get significant alterations in the above-mentioned parameters. In other works, lead acetate exposure was continued for longer periods of 42 day, 8 weeks, and 3 months (El-Ashmawy et al., 2006, Marques et al., 2006, Wang et al., 2007).

In the present study, LA induced significant increase in liver nitrite content and insignificant increase in liver total nitrate/nitrite content. This can be interpreted as LA increases ROS, which enhances inactivation of nitric oxide (Vaziri et al., 1999). In this concern, Vaziri et al., (1999), determined plasma and tissue abundance of nitrotyrosine, which is the footprint of nitric oxide oxidation by ROS. Nitric oxide avidly reacts with superoxide and other ROS to produce peroxynitrite, a highly cytotoxic reactive nitrogen species. It can react with tyrosine residue of protein to produce nitrotyrosine. At the same time, the ROS can react with tyrosine to produce tyrosyl radicals that can, in turn, oxidize NO and, again, generate nitrotyrosine (Eiserich et al., 1995, Halliwell, 1997, Vaziri et al., 1999). It is noteworthy that, liver nitrite content of mice exposed to LA, exhibits significant increase, while the total liver nitrate/nitrite content showed insignificant increase, the matter that could be interpreted on basis of the fact that lead abolishes heme synthesis and decreases hemoglobin (El-Missiry, 2000, Wang et al., 2007). As hemoglobin converts nitrite to nitrate, the decrease in hemoglobin due to lead toxicity prevented the conversion of nitrite to nitrate (Doyle and Hoekstra, 1981, Doyle et al., 1985, Miranda et al., 2001).

Pre-administration of BV at the different utilized doses, before LA, could ameliorate the increase in liver nitrite content induced by LA. These results may be explained on the fact that BV could prevent the effects of LA on heme synthesis and hemoglobin. Pre-administration of BV at different utilized doses, before LA, could ameliorate the insignificant increase in liver total nitrate/nitrite content induced by LA. These results may be interpreted as BV reduces pro-inflammatory mediators. In this concern, BV suppresses LPS-induced NO and inducible NO synthase expression in a dose-dependent manner in LPS-stimulated BV2 microglia (Moon et al., 2007). It exerts a potent suppressive effect on pro-inflammatory responses as it suppresses LPS-induced NOS activation in rat C6 glioma cells (Lee et al., 2009). In addition, apamin, which is one of the BV components, could abolish stimulated nitric oxide synthesis (Sheng and Braun, 2007).

The present data revealed that pre-administration of BV prior to LA could ameliorate the insignificant increase in liver IL-6 content induced by LA. This could be explained by the ability of BV to reduce proinflammatory mediators and consequently confirms the results of Moon et al., (2007) and Kim et al., (2008) on the effect of BV on IL-6 production. It could also be explained on basis of the findings that BV downregulate the gene of IL-6 receptor and reverses the LPS-induced up-regulation of IL-6 receptor (Yu et al., 2009).

In conclusion, the present study shows optimal potential for the lowest used BV dose level (1 μg/kg) in protection against LA toxicity. In addition, it points to a significant diversity of the changes in biochemical toxicity markers, as a consequence of BV administration. While this kind of diversity complicates the interpretation of results of such toxicity interaction experiments, it may also point to the needs for series of investigations on the possible interactive potentials of different BV fractions, with the xenobiotics inflammatory toxic mechanisms.

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