A Histopathological Study And Antioxidant Effect Of Ginger To Diminishing Poisoning Lead Acetate-Induced Hepatopathy In Rabbits For Three Months.

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Abstract

Lead acetate is an example of heavy metals that for decades being known for its adverse effects on various body organs and systems such that their functions are compromised. The present study, carried out to evaluate histopathological changes in rabbits liver induced by lead acetate toxicity and to investigate the therapeutic effects of ginger against lead poisoning. Ginger is source of antioxidants was administered orally to prevent the adverse effects of lead acetate. Thirty rabbits, randomized into 3 groups (n = 10), were used for this study. Animals in group (A) served as the control and were drinking distilled water. Animals in groups (B) and (C) were drinking 2% lead acetate. Group (C) animals were, in addition to drinking lead acetate, treated with 100 mg/kg/rabbit of ginger. All treatments were for 3 months. The obtained results showed that lead acetate caused histopathological changes were seen in the liver such as (vacuolation, degeneration, fibrosis and inflammation) and a significant reduction in plasma superoxide dismutase and catalase activity, but a significant increase in plasma malondialdehyde concentration, using ginger cause to modified these harmful effects. These findings lead to the conclusion that ginger significantly decreased the adverse harmful effects of lead acetate exposure on the liver as well as ginger may exert its protective actions against lead induced histopathological changes in liver tissue.

Keywords: Ginger, Lead acetate and hepatopathy.

1. Introduction

Lead (Pb) is an environmental contaminant due to its significant role in modern industry (Shalan et al., 2005). Both occupational and environmental exposures remain a serious problem in many developing and industrializing countries (Yücebilgic et al., 2003). Lead (Pb) is a toxic heavy metal and harmful even in small amounts (Gidlow, 2004). The manifestations of lead poisoning in humans are nonspecific. They may include weight loss, anemia (Khalil-Manesh et al., 1994), nephropathy, infertility, liver, testis and heart damages (Patocka and Cerny, 2003; Gurer-Orhan et al., 2004), etc. Lead is known to produce oxidative damage in the liver tissues by enhancing peroxidation of membrane lipids (Chaurasia and Kar, 1997), a deleterious process solely carried out by free radicals (Halliwell and Gutteridge, 1990). 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 increased the level of lipid peroxides and altered the antioxidant defense system in the hepatic tissues (Sandhir and Gill, 1995). A previous study confirmed the possible involvement of reactive oxygen species (ROS) in lead-induced toxicity (Gurer and Ercal, 2000). Oxidants and antioxidants have attracted widespread interest in nutrition research, biology and medicine. It has become clear that constant generation of prooxidants including oxygen free radicals, is an essential attribute of aerobic life (Sies et al., 1991). ROS are very reactive molecules ranked as free radicals owing to the presence of one unpaired electron such as superoxide ion (O2.-), nitrogen oxide (NO) and hydroxyl radical (OH). Even though naturally present in the organism, they are mainly confined to cell compartments and counterba-lanced by natural antioxidant molecules, such as glutathione, glutathione peroxidase, superoxide dismutase, vitamin E and vitamin C, acting as free radical scavengers (Aruoma et al., 1994). 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). Ginger, which is the underground stem or rhizome of the plant Zingiber officinale Rosco, contains polyphenol compounds (6-gingerol and its derivatives), which have a high antioxidant activity (Chen et al., 1986; Herrman, 1994). There are more than 50 antioxidants isolated from rhizomes of ginger...
(Masuda et al., 2004; Kikuzaki and Nakatani, 2006). Among them, 12 compounds exhibited higher antioxidant activity than α-tocopherol. Ginger and its constituents are stated to have antiemetic, antithrombotic, antihepatotoxic, anti-inflammatory stimulant, chologogue, androgenic and antioxidant effects (Khaki et al., 2009).

Ginger is a strong anti-oxidant substance and may either mitigate or prevent generation of free radicals. It is considered a safe herbal medicine with only few and insignificant adverse/side effects (Ali et al., 2008). Ginger extracts have been extensively studied for a broad range of biological activities, especially antioxidant activities (Miller et al., 1993). Antioxidant activities of the bark extracts of Garcinia hombroniana and essential oils of the leaves and stem of Tarchonanthus camphoratus have been reported (Nargis et al., 2013; Nanyonga et al., 2013).

Ahmed et al. (2000) found that ginger significantly lowered lipid peroxidation by maintaining the activities of antioxidant enzymes such as superoxide dismutase, catalase and glutathione peroxidase in blood of rats. Effects of Juniperus phoenicea extract on the activity of antioxidant enzymes in liver of oxonate-treated rats have been studied (Gdoura et al., 2013). However, effects of ginger, as a powerful antioxidant, on lead acetate-induced oxidative stress to liver tissue homogenate and liver injury in rats have not yet been studied. Therefore, this research focuses on whether oral administration of ginger prevents lead acetate induced liver toxicity and vacuolation, degeneration of hepatocytes and liver fibrosis in rabbits.

2. Aims of the study:

i. To study the toxicological pathology of lead acetate by using rabbits as experimental model.

ii. To have an idea about the toxicity of lead acetate in different toxic level.

iii. To open the way for further research in toxological pathology of lead acetate, and any heavy metals for the benefits of human and other veterinary uses.

iv. To investigate the antioxidant effects of whole ginger against lead acetate-induced liver damages.

3. Materials & methods

Animals and design:

Thirty rabbits of both sexes weighing between 1200-1750 gm. And average ages between six months to one year were used for the experiment were obtained from the animal house, college of veterinary medicine in Basra. All animals were treated in accordance to the principles of Laboratory Animal Facilities of World Health Organization, Geneva, Switzerland (2003). The animals were fed a standard diet and had free access to water. The rabbits were housed in stainless steel cages in a temperature-controlled room (25 ± 2°C) with constant humidity (40 - 70%) and 12 h light and 12 h dark exposure.

Grouping of animals and treatment: The animals were randomly divided into three groups (groups A, B, and C, n = 10). Animals in group A served as the control group and were drinking distilled water. Animals in groups B and C were drinking 2% lead acetate (LA). Group C animals were, in addition to drinking LA, treated with 100 mg/kg/rabbits of ginger. All treatments were for 3months from (January to March 2014).

Animal sacrifice and collection of samples

Three months after the last treatment, each animal was sacrificed and blood samples were collected via heart puncture. Blood sample obtained from each rabbit was divided into 2: One half in a plain bottle and the other half in an ethyl diamine tetra acetic acid bottle. Liver was excised from each rabbit and fixed in 10% saline formalin and prepared for to histopathological technique procedure and stained using hematoxyline and eosin stains. The samples were prepared for measurements of plasma super oxide dismutase (SOD), catalase (CAT) and Mallon di aldehyde (MDA) were determined using the method described by (Khaki et al., 2009).

Preparation Organs for Histopathological slides:

The liver from the control and experimental groups were rapidly isolated after the previously mentioned duration for experiment, cut into small pieces and dropped in 10% saline formalin for fixation.

After fixation, they were subjected to the normal procedure for paraffin embedding. Sections were cut at the thickness of 5 microns and stained with haematoxyline-eosin, as described by (Drury and Wallington, 1976) before being evaluated by light microscopy.

Microscopic examination:

After sacrificing the experimental animals, tissues samples from various visceral organs were taken fixed in 10% neutral buffered formalin, then paraffin blocks were made and cut on rotary microtome to make slides of five microns which were then stained with H&E. those sections were examined by light microscope (Olympus) to
detect and describe any histopathologic changes induced by the treatment with the lead acetate.

**Collection of data and statistical analysis**

Livers from each rabbit were homogenized for tissue superoxide dismutase (SOD), catalase (CAT) and malondialdehyde (MDA) were determined using the method described by Khaki et al. (2009).

**Statistical analysis**

All values were expressed as mean ± SE. Differences in mean values were compared using SPSS 11.0 by one-way ANOVA test. P < 0.05 was considered as statistically significant.

4. Results

**Histological and Histopathological Observations/group{A,B}:**

Results of my experiment summarized by different histopathological lesions in liver tissue. Liver sections of control group{G/A} showed normal histological structures of hepatocytes, bile duct and central vein (Fig.1). The most significant treatment-related histopathological toxicologic changes{G/B} were varied from degeneration, vacuolation of hepatocytes with mononuclear cells (Figs.2, 3) / ballooning of hepatocytes (Figs.4). On occasion those changes were associated with advanced degrees of capsular and septal fibrosis (Figs.5, 6) also there were hepatitis with infiltration of inflammatory cells and congestion (Figs.7).

**Histopathological Observations/group{C}:**

Liver sections of {G/C} showed no changes can be detection in structural of liver tissue in rabbits (Fig.8).
Fig (1)/G (A): **Liver**; control, within normal structure. (H & E, 4x)

Fig (2)/G (B): **Liver**; sever diffuse vacuolation and degeneration of hepatocyte. (H & E, 10x)

Fig (3)/G (B): **Liver**; degeneration of hepatocyte with mononuclear cells. (H & E, 40x)

Fig (4)/G (B): **Liver**; an area of marked vacuolation / ballooning like of hepatocytes. (H & E, 10x)
The following results were obtained and are presented as mean ± SEM. Level of significance is taken at “P-value < 0.05” (*).

**Plasma SOD activity**

Group B showed a significant (P-value < 0.05) decrease in plasma SOD activity. Group C was, however, not significantly (P-value > 0.05) different from the control in terms of the plasma SOD activity (Table 1).

**Plasma CAT activity**
Group B showed a significant (P-value < 0.05) decrease in the plasma CAT activity. However, group C showed no significant (P-value > 0.05) difference in the CAT activity from the control (Table 1).

**Plasma MDA concentration**

Group B showed a significant (P-value < 0.05) increase in the plasma MDA concentration whereas Group C showed no significant (P-value > 0.05) difference from the control (Table 1).

**Table 1.** Showed result of plasma SOD, CAT, MDA in whole control and experimental groups.

<table>
<thead>
<tr>
<th>Groups (n=10)</th>
<th>Plasma SOD</th>
<th>Plasma CAT</th>
<th>Smo MDA</th>
</tr>
</thead>
<tbody>
<tr>
<td>(A) Control</td>
<td>1.958 ± 0.05</td>
<td>0.3874 ± 0.03</td>
<td>0.25 ± 0.04</td>
</tr>
<tr>
<td>(B) 2% PB</td>
<td>1.124 ± 0.05*</td>
<td>0.2440 ± 0.02*</td>
<td>4.1 ± 0.06*</td>
</tr>
<tr>
<td>(C) 2% PB +[ginger (100 mg/kg/rabbit/daily)]</td>
<td>1.783 ± 0.06*</td>
<td>0.3692 ± 0.01</td>
<td>2.2 ± 0.06*</td>
</tr>
</tbody>
</table>

Data are presented as mean ± SE. *Significant different at p < 0.05 level, (compared with the control and ginger treated group).

5. DISCUSSION

In this study, lead acetate treatment caused liver tissue damage as evident from increased hepatic MDA concentration and as mentioned by (Attia et al., 2013) who reported that lead acetate treatment caused hepatic injury as evident from increased activities of plasma ALT, AST and ALP and elevated hepatic MDA concentration. Lead-induced oxidative stress in liver was evident from increased levels of lipid peroxidation and reduced level of GSH.

Absorbed lead is stored in soft tissues mainly the liver tissues (Lyn Patrick, 2006). The liver, via the portal vein, is the first organ exposed to internally absorbed nutrients in which the histological analysis can be used to examine the morphological changes to reflect possible effect of lead on the hepatocytes (Abdou and Newairy, 2006).

According to my experiment results; microscopic findings showed prominent changes in the liver such as degeneration, vacuolation of hepatocytes with advanced degrees fibrosis and hepatitis with infiltration of inflammatory cells and congestion. All the above results agree with (Abdou and Newairy, 2006) who reported that lead poisoning via drinking water caused dilation of hepatic vein and congestion of blood vessels with degeneration and necrosis. Similar hepatotoxicity lesions were also reported by (Suradkar et al., 2010; Lyn Patrick, 2006; King et al., 1983) who indicated that the histopathological changes in liver exposed to lead acetate include enlargement of blood vessels along with sinusoids hemorrhage and dilation of central veins.

On the other hand; several researches regarded that lead acetate more dangerous substance on liver tissue because lead acetate induce, different degrees of inflammation and fibrosis of hepatocytes as showed (Al-Bideri, 2011) who found that the varieties in the histological alterations of the liver tissues due to lead intoxication obtained by different investigators could be due to the variations in the level of exposure, duration, route of administration and animal species used in the experiments.

**Treatment with antioxidants:**

The histological examination of liver tissue in rabbits groups treated with daily doses of ginger revealed that most of the histological alterations induced in lead acetate treated groups were markedly reduced. (Fig.8/G.C) of these rabbits showed, no alterations as compared to the lead acetate treated rabbits (G.B). These findings confirmed the protective effect of ginger against the histological changes in lead acetate hepatotoxicity. This finding reinforce those of (Khaki, 2010; Al-Bideri, 2011; Vitalis et al., 2007) who said that ginger is effective in preventing the hepatotoxic effects of lead. On the other hand; these results consistent with (Attia et al., 2013; Masuda et al., 2004; Kikuzaki and Nakatani, 2006; Miller et al., 1993; Ahmed et al., 2000) who found that
treatment of rats with whole ginger at a dose of 160 mg/kg body weight prevented the levels of lipid peroxidation to rise when the animals were challenged with lead acetate, indicating that this dose of ginger is fully capable of mitigating the oxidative stress induced following treatment of the animals with lead acetate.

A number of recent studies confirmed the possible involvement of reactive oxygen species (ROS) in lead-induced toxicity (Gurer et al., 2000; Gurer-Orhan et al., 2004). Several antioxidant enzymes and molecules have been used to evaluate lead-induced oxidative damage in animal and human studies. Reduced glutathione (GSH) and glutathione disulfide (GSSG) concentrations, as well as modifications in superoxide dismutase (SOD) activity are the most frequently used markers in tissues or in blood (Khaki, 2010). Based on the observation that free radical was generated during the pathogenesis processes induced by lead exposure, it was presumed that supplementation of antioxidants could be an alternative method for chelation therapy (Khaki, 2010; Flora et al., 2003; Flora and Tandon, 1986). Specifically, ascorbic acid, the known chelating agent with antioxidant features, was widely reported with the capability of protecting cells from oxidative stress (Patra et al., 2004). More importantly, due to the presence of health-protective antioxidants such as lycopene, vitamin C, and vitamin A in TP (Patra et al., 2004, Upasani et al., 2001 ). There was no significant (P-value > 0.05) difference in the SOD activity of the plasma of the control and that of the animals treated with tomato along with Pb. But, there was a significant (P-value < 0.05) decrease in the plasma SOD activity in animals treated with Pb only compared with the control. This finding is in agreement with (Pinon-Lataillade et al., 1995). There was a significant (P-value < 0.05) decrease in plasma CAT activity of animals treated with Pb only relative to the control. There was, however no significant (P-value > 0.05) difference between the control and the animals treated with ginger along with Pb in this respect. This further establishes that ginger must have reduced the oxidative stress that Pb could cause. Finally, there was no significant (P-value > 0.05) difference in both the plasma and the tissue MDA concentration of the control and those of the animals treated with ginger along with Pb, whereas animals treated with Pb only showed a significant (P-value < 0.05) increase in plasma MDA concentration. This confirms that it was ginger, the source of antioxidants, (Lisa, 2002; khaki et al., 2009) that reduced the oxidative stress that Pb exposure could have caused in the ginger-treated animals. In summary, ginger can decreased the damage to liver tissue from oxidative damage induces by lead, and it is dependent on their antioxidant effects.

References


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