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GINSENOSIDE RB1, A COMPONENT OF PANAX GINSENG, AMELIORATES SELENIUM INDUCED RENAL TOXICITY IN BROILERS

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ABSTRACT

Panax Ginseng (PG) has been widely used as natural product for many years with limited knowledge on its effect on renal dysfunction. Ginsenoside (Rb1) is the most clinically effective derivative of ginseng. The aim of this study was to investigate the protective role of ginsenoside Rb1 on selenium induced renal toxicity in broilers. Forty, one day old chicks acclimatized for 14 days and divided into four groups; negative control, Selenium (Se) treated (0.48 mg Na_2SeO_3/kg b.wt.), Rb1 plus Se treated (100mg Rb_1/kg b.wt. & 0.48 mg Na_2SeO_3/kg b.wt.) and Rb1 treated (100 mg Rb_1/ kg b.wt.) groups. Levels of blood urea nitrogen, uric acid, creatinine, calcium (Ca), phosphorus (P), magnesium (Mg), sodium (Na), potassium (K), catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPx) and malondialdehyde (MDA) were estimated. The pretreatment of chickens with Rb1 significantly ameliorate the degenerative effect of selenium over the biochemical parameters, electrolytes and improves the antioxidant activity. Taken together, it could be concluded that the pretreatment of ginsenoside Rb1 has a protective effects against renal injury induced by sodium selenite toxicosis. The ginsenoside Rb1 is thus can be utilized as a useful natural product tool to protect against selenium toxicosis.

Key words: Panax ginseng, Ginsenoside Rb1, Selenium toxicity, Kidney function, antioxidant, Chicken

INTRODUCTION

Selenium is a natural food supplement in animal's diets, but it can also be a toxin. The difference between an effective or safe dose and a toxic dose of selenium is relatively small (MacFarquhar *et al.*, 2010). Selenium is found in steel and copper alloys, glass, paint manufacturing and nutritional supplements (Barceloux, 1999). A serious problem came from the environmental exposure to selenium due to industrial accidents, poisonings and the dietary abuse of selenium rich plants (Aldosary *et al.*, 2012). The kidneys retain a large amount of absorbed Se along with the liver, cardiac and skeletal muscle. Still, little information is available on the effect of selenium toxicity on the kidney and oxidative stability of reactive oxygen species in broiler birds (Ryu *et al.*, 2005).

Panax ginseng (Korean ginseng) is a medicinal plant thought to be protective against several diseases as cardiovascular disease and diabetes (Han *et al.*, 2006). Most of the pharmacological actions of ginseng are attributed to a variety of ginsenosides (Huang *et al.*, 2005). Ginseng has many medicinal effects on immune response, sexual function, physiological homeostasis, enhance vital energy besides, it possesses anti-stress, anti-tumor properties, anti-apoptotic effects on the kidney (Attele *et al.*, 1999; Kalkan *et al.*, 2012).

Ginsenoside (Rb1) is considered one of the best clinically effective constituent of ginseng as it possesses anti-oxidant, anti-inflammatory and anti-apoptosis effects (Cheng *et al.*, 2005). It has been reported that Rb1 attenuate renal apoptosis and oxidative damage (Xie *et al.*, 2009). Science the Rb1 play a role in oxidative stress, it is expected to have an important roles in the renal protective effects with oxidative damage.

In the present study, we investigated the protective effects of Rb1 against selenium-induced renal dysfunction and explored the antioxidant status. Renal damage was assessed by serum biochemical analysis, renal function tests and examining the potential antioxidant activity. Significant protection from selenium-induced renal injury has been showed by Rb1 with the ability to protect against the induced oxidative damage in broiler chicken.

MATERIALS AND METHODS

Materials

Chickens Forty, one-day-old, commercial boiler chickens (Hubbard strain) were purchased from Al-Kahira Poultry Company. Chickens were maintained at the Laboratory Animal Center, College of Veterinary Medicine, Zagazig University and were kept in clean well ventilated cages under standard managerial, environmental and hygienic conditions. Chickens were divided into 4 main groups, acclimatized for 14 days prior the experiment

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and maintained on a commercial well balanced ration, formulated to meet the nutrient requirement of chickens during the experimental period according to recommendations of the national research council (NRC, 1994), and drinking water was given ad.libitium throughout the experimental period.
Sodium selenite (Na_2SeO_3) was purchased from Sigma Chemicals. It was used as $1/20^{th}$ of LD_{50} of Na_2SeO_3 of broiler chickens, at a dose of 0.48 mg /kg b.wt., in the diet. (Kumar, 2013).
Panax ginseng root extract **(**Ginsenoside Rb_1=saponin of Panax ginseng**)** was obtained from Sigma Chemicals. A dose of 100 mg /kg b.wt., in the diet was used for the experiment (Karakus *et al.*, 2011).

Methods

Experimental design

The experiment procedures used in this experiment were carried out and approved by the local institutional of animal care and veterinary committee of Faculty of Veterinary Medicine, Zagazig University, Egypt. Chickens were divided into four groups as following. Group I: (n=10), negative control group, chickens were fed on a commercial basal balanced clean ration, supplemented with clean water, under hygienic measurements without any treatment for 30 days. Group II: (n=10), Se-intoxicated group, chickens were fed on a commercial basal diet, which mixed with Na_2SeO_3 at a concentration of (0.48 mg /kg b.wt.) for 30 days. Group III: (n=10), Rb1 plus Se treated group, chickens were fed with a commercial basal diet, which mixed with Rb1 at a concentration of (100 mg/kg b.wt.) for 10 days, then feeding with diet mixed with Na_2SeO_3 at a concentration of (0.48 mg /kg b.wt.) for 20 days. Group IV: (n=10), Rb1 treated group; chickens were fed on a commercial basal diet, which mixed with Rb1 at a concentration of (100 mg/kg b.wt.) for 30 days.

Blood sampling

The blood samples were collected on the 31^{th} day from the wing vein. 5 ml of blood was collected without anticoagulant into a clean dry centrifuge tube and allowed to clot for 30 min at room temperature. Blood samples were centrifuged at 3000 rpm for 5 min. Clear sera were separated and used for different biochemical analysis.

Estimation of biochemical parameters

All parameters were colorimetrically measured using commercial kits provided by (BioMérieux, Marcy, L'Etoile, France). All analysis was done using Spectrophotometer 5010 $v5^+$, (RIELE GmbH & Co, Berlin, Germany) for biochemical serum analysis. Blood urea nitrogen (Christian *et al.*, 1965), uric acid (Kageyama, 1971) serum creatinine (Heinegard and Tiderstrom, 1973), electrolytes, such as serum calcium (Ca), inorganic phosphorus (P) and magnesium (Mg) (Berti *et al.*, 1988; Ripoll, 1976; Smith, 1955) were measured. Serum sodium and potassium concentrations were assayed using flame photometer (Verzhikovskaia and Popov, 1963).

Antioxidants and (MDA) lipid peroxidation Assay

Kidney was collected from all groups on days 31. One gram of each kidney sample added to 9 ml of normal saline (0.9%) and homogenized using tissue homogenizer, and centrifuged at 3000 rpm for 15 minutes. The supernatant was collected and used for antioxidants estimation (CAT), (SOD), (GPx) and the marker of lipid peroxidation (MDA) according to, (Sidhu *et al.*, 2005). The catalase activity (CAT) (Aebi 1984), superoxide dismutase (SOD) (Weydert and Cullen, 2010), Glutathione peroxidase (GPx) (Weydert and Cullen, 2010) and malondialdehyde (MDA) (Valenzuela, 1991) were determined.

Statistical analysis

Statistical Analysis System software package was used to analyze the data by one-way analysis of variance ANOVA (Bewick *et al.*, 2004). The significant differences between means were determined at a level of ($P<0.05$). All data showed a normal distribution and passed equal variance testing. Differences between means were assessed using Tukey′s honestly significant difference test for post hoc multiple comparisons. Data are expressed as the mean±SEM.

RESULTS

Evaluation of kidney markers

The effects of sodium selenite intoxication as well as the preventive effects of ginsenoside Rb1 on kidney markers are shown in Fig. 1. Significant ($p<0.05$) increases in the level of blood urea nitrogen, uric acid and creatinine levels of chickens were recorded in sodium selenite intoxicated group as compared to negative control group. On the other hand, marked improvement has been detected when treatment with ginsenoside Rb1, significant decrease ($p<0.05$) of blood urea nitrogen, uric acid and creatinine of chickens given Rb1 and Se was detected when compared with the Se intoxicated group. Significant increase ($p<0.05$) in the levels of uric acid and creatinine in the ginsenoside Rb1 treated group when compared with Rb1+Se treated group, but the levels are still not significant when compared with the negative control group.

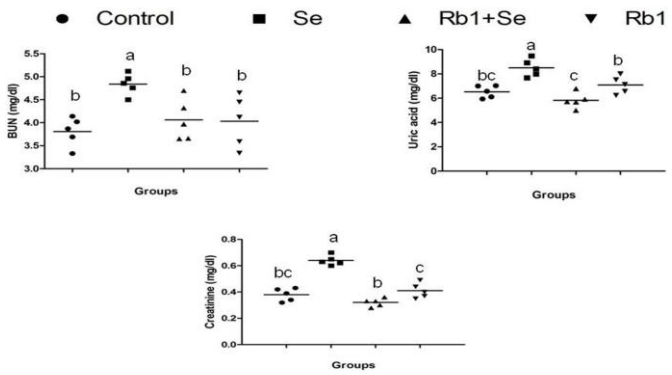

Fig. 1. Effect of selenium and ginsenoside Rb1 on the levels of kidney markers. Changes in the levels of blood urea nitrogen, uric acid and creatinine (mg/dl) were observed. Values were significantly different at ($P<0.05$) when compared with the negative group. Data are expressed as means±SEM. Bars showing the same letter (a, b, c) are not significantly different.

Evaluation of serum electrolytes

The effects of sodium selenite intoxication as well as the preventive effects of ginsenoside Rb1 on the serum electrolyte are shown in Fig. 2. Significant ($p<0.05$) decrease in calcium and phosphorus, magnesium, sodium was recorded in sodium selenite intoxicated group. Significant increase ($p<0.05$) in serum potassium level was also reported in the sodium selenite intoxicated group. Treatment with ginsenoside Rb1 showed significant improvement. Significant increase ($p<0.05$) was observed in calcium, phosphorus, magnesium, sodium towards the normal level. Similarly, significant decrease ($p<0.05$) in serum potassium level was observed. Meanwhile, significant decrease ($p<0.05$) in the level of potassium in the ginsenoside Rb1 treated group when compared with Rb1+Se treated group, but the levels are still non-significant when compared with the negative control group.

Evaluation of renal antioxidant and lipid peroxidation profile

Effects of sodium selenite intoxication and the preventive effects of ginsenoside Rb1 on the renal antioxidant and lipid peroxidation profile are shown in figure 3. Significant decrease ($p<0.05$) in the levels of renal CAT, SOD and GPx sodium was reported in the sodium selenite intoxicated group. On the other hand, significant increase ($p<0.05$) in the activity of CAT, SOD and GPx was detected towards the normal control level. Meanwhile, the level of renal CAT in the ginsenoside Rb1 treated group was significantly decrease ($p<0.05$) when compared with Rb1+Se treated group, but the levels are still non-significant when compared with the negative control group. Significant increase ($p<0.05$) in MDA level was observed in sodium selenite intoxicated group. Significant decrease in MDA level was detected in other treated groups.

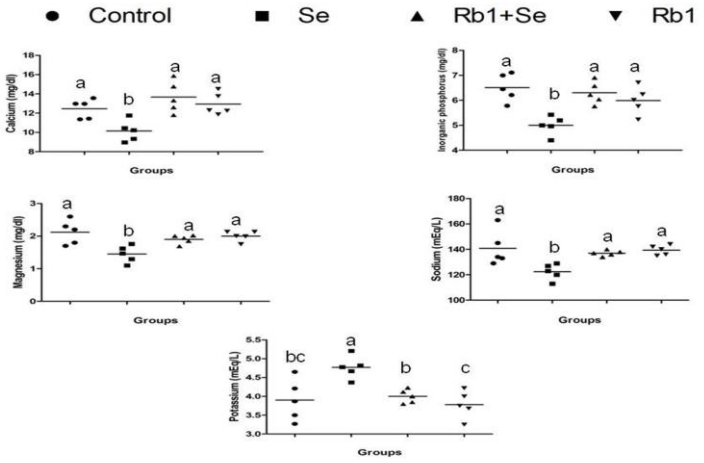

Fig. 2. Effect of selenium and ginsenoside Rb1 on the levels of serum electrolytes. Changes in Ca (mg/dl), P (mg/dl), Mg (mg/dl), Na (mEq/l) and K (mEq/l) were reported. Values were significantly different at ($P<0.05$) when compared with the control group. Data are expressed as means ±SEM. Bars showing the same letter (a, b, c) are not significantly different.

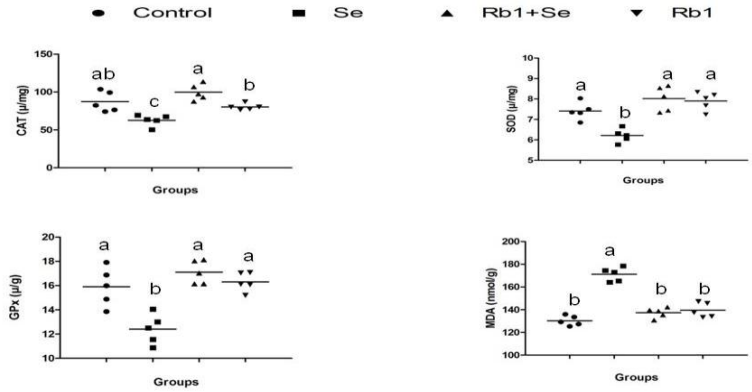

Fig. 3. Effect of selenium and ginsenoside Rb1 on the levels of serum antioxidant activity and lipid peroxidation profile. Changes in the levels of CAT (µl/mg), SOD (µl/mg), GPx (µl/g) and MDA (nmol/g) were observed. Values were significantly different at ($P<0.05$) when compared with the control group. Data are expressed as means ±SEM. Bars showing the same letter (a, b, c) are not significantly different.

Discussion

Selenium toxicity is found in poultry flocks causing tissue lesions, oxidative damage, apoptosis of splenocytes, and deaths (Peng *et al.*, 2012). Excess selenium can cause growth depression, anemia, impaired immune function and reduced egg production (Zwolak and Zaporowska, 2012). Recently, high concentrations of selenium is used as hepatic toxins in the research studies (Xu *et al.*, 2014). Several studies previously reported the markers to be included in kidney function tests. One group stated that uremia, hyperuricemia, increase in the serum creatinine level with calcium and phosphorus imbalance were detected in renal dysfunction (Chandra *et al.*, 1985). Others stated that blood urea nitrogen, uric acid and serum creatinine levels are considered significant markers of renal

dysfunction (Gowda *et al.*, 2010). The production of reactive oxygen species by renal cortical mitochondria and the involvement of oxidative stress to renal toxicity could be the reason for the kidney dysfunction (Hoffman, 2002).

Blood urea nitrogen (BUN) has little value in the configuration of avian renal disease but it is a sensitive detector for hydration state. The decrease in BUN associated with Se toxicity may be attributed to dehydration. About 99% of BUN is reabsorbed in the dehydrated bird (Lumeij, 1987). Excretion of uric acid depends on the urine flow and therefore is unaffected by moderate changes in glomerular filtration (Chandra *et al.*, 1985). Hyperuricemia may be seen with severe dehydration, due to hyperglycemia causing increased urination and fluid loss, associated with selenium toxicity (Jones, 1999; Lumeij, 1987). The uric acid significantly increases with renal disease if there is extensive tubular damage (Chandra *et al.*, 1985). Ginsenoside Rb_1 significantly improves the elevated levels of blood urea nitrogen, serum uric acid and creatinine levels. The mechanism by which it prevents Se-induced nephrotoxicity is not well studied. However the saponins in the ginsenoside Rb_1 may have improved the kidney function through several antioxidant properties such as free radical scavenging activity or the inhibition of the formation of oxidized product. Panax Ginseng in general has been shown to have antioxidant and anti-inflammatory effects (Lee and Son, 2011). Also recent studies have provided a great support for evidencing the protective effects of ginseng on kidney damage (Kang *et al.*, 2013). In our investigation, ginsenoside Rb1+Se and ginsenoside Rb1 treated groups showed an improvement in kidney function tests when compared with control. Changes in uric acid and creatinine between those treated groups have been detected, but these changes were not significant when compared with control. There is no clear reason to describe those changes other than the ginsenoside Rb1-Se interaction, which might play a role in this case.

Avian kidney cannot concentrate the electrolytes much above normal levels (Chandra *et al.*, 1985). The renal dysfunction in birds is associated with hypocalcemia, hypomagnesemia, hyponatremia and hyperkalemia (Lierz, 2003). Electrolyte imbalance such as hypocalcemia, Hypophosphatemia, hypomagnesemia, hyponatremia with hyperkalemia was reported in our study. Selenium intoxication is associated with osmotic diuresis due to hyperglycemia which cause marked urinary loses of water, electrolytes and aggravated urinary excretion of ketones with additional electrolyte loss (Steinbrenner *et al.*, 2011). Hypocalcemia was a result of metabolic changes such as metabolic acidosis, and impaired parathormone hormone (Desta *et al.*, 2011). Hypomagnesemia may be due to the hyperglycemia and glycosuria-related hypermagnesuria (Steinbrenner *et al.*, 2011). Hyponatremia may be due to the alteration in the sodium channel proteins in the collecting ducts and distal convoluted tubules leading to increased fractional excretion of sodium in urine. Translocation of Na+, K+-ATPase pumps from the baso-lateral membrane of proximal convoluted tubules to the cytosol leads to a decrease in sodium pumping from renal tubules to the blood (Kamble *et al.*, 2009). On the other hand, hyperkalemia may be attributed to the condition of acidosis with extracellular migration of potassium (Nuttall, 2006). Ginsenoside Rb_1 resulted in amerilation of electrolyte imbalance. This may have resulted from a decrease in urine volume, which resulted in the improvement of the metabolic abnormalities (Kang *et al.*, 2008).

Electrolytes levels in ginsenoside Rb1+Se and ginsenoside Rb1 treated groups where significantly improved when compared with control. A change in potassium level between those treated groups has been detected, but the change was not significant when compared with control. It is expected that Se toxicity might play a role in releasing potassium from the body cells to the blood stream, the maldistribution of potassium between intra- and extracellular space predisposed to hyperkalemia (Lehnhardt and Kemper, 2011). This could describe the change in potassium level between the two groups.

Selenium has a role in triggering oxidative stress in hepatic and renal tissues (Elgaml, 2014; Kamble *et al.*, 2009; Padmaja and Raju, 2005). Selenium triggering kidney diseases, primarily by free radical generation and the depletion of antioxidant status, causes damage in the cell membrane and the organelles of the hepatocyte and kidney (Manikandan *et al.*, 2010). It induces oxidative damage by increasing the production of ROS (Maraldi *et al.*, 2011) and decreasing the biological activities of some antioxidants, such as CAT, SOD and GPx (Zikic *et al.*, 1998). Se intoxication increases lipid peroxidation and suppresses the antioxidant defense mechanisms in kidney tissue (Agarwal and Behari, 2007; Atencio *et al.*, 2009). The increased activity of CAT, SOD and GPx were detected in kidney tissues of Rb1 treated groups when compared with Se intoxicated group. These antioxidants can prevent or decrease the harmful effects and ROS in kidney tissue. Ginsenoside Rb1 decreased the MDA concentration in the Se+ Rb1 and Rb1 groups. The role of Rb1 in preventing LPO, protecting the integrity and functioning of kidney tissues is of important reflection in protection against selenium toxicity. Ginsenoside Rb1

could help for the treatment of kidney diseases (Kalkan *et al.*, 2012; Karakus *et al.*, 2011), so it plays an important role in antioxidant defense and in the elimination of free radicals. Rb1 may exert its protective effect by significantly decreasing Se redistribution or accumulation in organs (Hassan *et al.*, 2014; Ramesh *et al.*, 2012). Ginsenoside Rb1+Se and ginsenoside Rb1 treated groups showed an increase in the antioxidant activity when compared with Se treated group. Changes in catalase between those treated groups have been detected; little information is known to describe the difference in CAT activity between ginsenoside Rb1+Se and ginsenoside Rb1 treated groups, but these changes still not significant when compared with control.

In conclusion, the result of this study recommended the use of the herbal panax Ginseng extract, ginsenoside Rb1, as feed additive. Ginsenoside Rb1 can be beneficially used for future animal and poultry feed formulation. Additional studies on the other active components of panax Ginseng might be needed; biochemical and functional importance of those extracts could provide an important approach for improving the future animal and poultry health.

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