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Attenuation of Beryllium Induced Hepatorenal Dysfunction and Oxidative Stress in Rodents by Combined Effect of Gallic Acid and Piperine

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We determined a minimum effective dose of gallic acid (3,4,5-trihydroxy benzoic acid; 50 mg/kg, i.p.) and piperine (10 mg/kg, p.o.) through their therapeutic potential and further evaluated them individually and in combination against beryllium-induced biochemical alterations and oxidative stress consequences in female albino rats. The administration of beryllium altered blood biochemical variables by significantly depleting hemoglobin, albumin and urea, whereas it enhanced bilirubin and creatinine. The release of serum transaminase, lactate dehydrogenase and γ-glutamyl transpeptidase was significantly greater, and was concomitant with a decrease in serum alkaline phosphatase. A significant increase in lipid peroxidation and a decrease in glutathione, superoxide dismutase and catalase in the liver and kidney was an indication of oxidative stress due to beryllium exposure. Individual administration of gallic acid and piperine moderately reversed the altered biochemical variables, whereas the combination of these was found to completely reverse the beryllium-induced biochemical alterations and oxidative stress consequences. We concluded that gallic acid exerts a synergistic effect when administered with piperine and provides a more pronounced therapeutic potential in reducing beryllium-induced hepatorenal dysfunction and oxidative stress consequences.

Key words: Beryllium toxicity, Gallic acid, 3,4,5-Trihydroxy benzoic acid, Piperine, Biochemical alterations, Hepatorenal dysfunction, Oxidative stress, Combined therapy

INTRODUCTION

Beryllium is one of the most ubiquitous metals in earth's crust, and has unique chemical and physical properties which make this lightweight metal ideally suited for use in a variety of high technology industries including aerospace, ceramics, electronics and nuclear defense (Weston *et al.*, 2005). Exposure to beryllium through various means causes toxic effects to organ systems including the liver, kidney, skeleton, lymph nodes and lungs (ATSDR, 2002). Several synthetic compounds; however, are known to counteract toxic events induced by beryllium, but their therapeutic use at higher concentrations is considered unsatisfactory (Nirala *et al.*, 2007a). Tiferron is a synthetic compound which is well suited to minimize the toxic effects of beryllium due to the presence of hydroxyl moiety (Sharma *et al.*, 2002; Nirala *et al.*, 2007a), and its effectiveness can be enhanced by using certain adjuvants (Nirala *et al.*, 2007a, 2007b). Various naturally occurring phenolic compounds are widely available in plants and their therapeutic ability in reducing toxic metal ion-induced free radical assault is partly attributed to their ability to supply specific chelators which bind to unwanted toxic ions and thus reduce their bioavailability (Hynes and Coinceanainn, 2001). As a result, natural products with hydroxyl or carboxylic acid moieties may serve as alternative medicines or nutritional supplements to attenuate beryllium-induced toxicity. Gallic acid (3,4,5-trihydroxy benzoic acid) is an active constituent which occurs in a variety of plant species such as *Coriandrum sativum* (Bajpai *et al.*, 2005), *Terminalia chebula* Retz., *Rhus chinensis* Mill., *Polygonum aviculare* L. (Cai *et al.*,

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2004), *Terminalia belerica* Roxb. (Anand *et al.,* 1997). In addition to being omnipresent, it possesses hepatoprotective (Anand *et al.,* 1997), anti-cancer (Faried *et al.,* 2007), iron chelating (Hirai *et al.,* 2005; Prasad *et al.,* 2006) and antioxidant activity (Yeh and Yen, 2006). Owing to the presence of three hydroxyl functionalities, this compound may alleviate the beryllium-induced toxicogenic events. Piperine is an active principle of *Piper longum* Linn. and *Piper nigrum* Linn., and is known for its hepatoprotective (Koul and Kapil, 1993) and antioxidant properties (Gulcin, 2005) as well as its ability to enhance the bioavailability and therapeutic effectiveness of various agents (Khajuria *et al.,* 1998; Nirala *et al.*, 2007a, 2007b). Thus, the present study was designed to evaluate the therapeutic potential of gallic acid (GA) in the presence of piperine, against the beryllium-induced biochemical alterations and oxidative stress consequences.

MATERIALS AND METHODS

Animals and chemicals

Female albino Wistar rats (6-8 weeks old; 150 ± 10 g body weight) were maintained in the institutional animal facility under standard husbandry conditions of light (14 h) and dark (10 h) at a temperature of 25°C ± 2°C and relative humidity of 60-70%. Animals were fed dry pellets consisting of a standard animal diet (provided by the animal facility) and given drinking water *ad libitum*. The experimental protocols were approved and carried out according to the guidelines set by the Institutional Animal Ethics Committee.

Beryllium (in the form of beryllium nitrate [$Be(NO_3)_2$]) was purchased from Fluka (Bochs, Switzerland). Gallic acid and piperine were procured from the Sigma-Aldrich company (St. Louis, MO, U.S.A.). All the other chemicals used in the study were of highest purity and AR grade.

Preparation and administration of doses

$Be(NO_3)_2$ was dissolved in triple distilled water (1 mg/2 mL/kg body weight) and was administered intraperitoneally. Gallic acid (25, 50 and 75 mg/2 mL/kg body weight) was prepared in normal saline which was adjusted to a pH of 7.4 and administered intraperitoneally. An aqueous suspension, which was administered orally, consisted of piperine (5, 10 and 15 mg/5 mL/kg body weight) and was prepared in 1% gum acacia since it does not significantly alter the biochemical variables (Bhadauria *et al.,* 2007).

Experimental protocol 1: Determination of minimum effective dose

Sixty rats were assigned into 10 groups of 6 animals each. **Group 1:** received sodium nitrate once daily for 28 days (1 mg/kg, i.p.) followed by saline (2 mL/kg, i.p.) for 5 days and served as the normal control. **Groups 2 and 3:** received sodium nitrate (as in group 1) followed by GA *per se* (75 mg/kg, i.p.) and piperine *per se* (15 mg/kg, p.o.), respectively for 5 days. **Groups 4-10:** received beryllium nitrate once daily for 28 days (1 mg/kg, i.p.). **Group 4:** served as the experimental control and received saline (2 mL/kg, i.p.) for 5 days after toxicant administration. **Groups 5-7:** treated with different doses of GA (25, 50 and 75 mg/kg, i.p.) for 5 consecutive days after toxicant administration. **Groups 8-10:** treated with different doses of piperine (5, 10 and 15 mg/kg, p.o.) for 5 consecutive days after toxicant administration.

Twenty-four hours after the final administration, the animals were euthanized under a mild ether anesthesia and blood was drawn immediately by puncturing the retro-orbital venous sinus for isolation of serum, which was used to determine the leakage of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) (Reitman and Frankel, 1957), as well as lactate dehydrogenase (LDH) (Wroblewski and La Due, 1955) and serum alkaline phosphatase (SALP) (Fiske and Subbarow, 1925). Following this step, γ-glutamyl transpeptidase (γ-GT) (in serum) was measured using the Merck kit as per the manufacturer's instructions.

Experimental protocol 2: Evaluation of the combined effect of GA and piperine

An additional 30 rats were assigned to 5 groups of 6 animals each and treated as follows:

Group 1: sodium nitrate (28 days) + saline (5 days).
Group 2: beryllium nitrate (28 days) + saline (5 days).
Group 3: beryllium nitrate (28 days) + GA (50 mg/kg, i.p.; 5 days).
Group 4: beryllium nitrate (28 days) + piperine (10 mg/kg, p.o.; 5 days).
Group 5: beryllium nitrate (28 days) + combination of GA and piperine for 5 days (as in group 3 and 4).

Twenty-four hours after the final administration, the animals were euthanized under mild ether anesthesia and blood was drawn for isolation of serum. Their livers and kidneys were promptly excised, blotted and processed in order to estimate the markers for oxidative stress and antioxidant status. Few of the findings in group 4 are not original (Nirala *et al.*, 2007a, 2007b), however we repeated the presentation of piperine to justify the direct comparison between GA and piperine.

Blood biochemical assay

Hemoglobin was estimated in the blood using Sahli's apparatus (Swarup *et al.*, 1992). The serum was used to determine the AST, ALT, LDH and SALP as described above. As well, total bilirubin, albumin, γ-GT, urea and cre-

atinine in serum were measured using the Merck kits.

Oxidative stress and antioxidant status

Reduced glutathione (GSH) (Brehe and Burch, 1976), lipid peroxidation (LPO) (Sharma and Krishna Murti, 1968), total superoxide dismutase (SOD) (Mishra and Fridovich, 1972), catalase (Aebi, 1984) and total protein (Lowry *et al.*, 1951) were estimated in the livers and kidneys of each rat.

Statistical analysis

Results were expressed as the mean ± SE of 6 animals and belonging to each particular group. The data were subjected to statistical analysis through a one-way analysis of variance (ANOVA) and statistical significance was set *a priori* at $P \leq 0.05$. Next, the data were also subjected to a student's *t*-test with a statistical significance set *a priori* at $P \leq 0.01$ and $P \leq 0.05$ (Snedecor and Cochran, 1994). We also performed a Tukey's honestly significant difference *post hoc* test to compare the efficacy of the different treatments with statistical significance set *a priori* at ($P \leq 0.05$).

RESULTS

The minimum effective doses of GA and piperine were determined using important biochemical endpoints. Moreover, the study was further extended to evaluate the combined effects of GA and piperine against beryllium-induced toxicogenic consequences.

Experimental protocol 1: Determination of minimum effective dose

Fig. 1(A-E) demonstrate the dose response effect of GA and piperine against beryllium toxicity. The release of AST, ALT, LDH and γ-GT significantly increased ($P \leq 0.01$), whereas SALP significantly decreased after beryllium administration ($P \leq 0.01$). Individual administration of GA and piperine at different doses demonstrated dose-dependent therapeutic effects. The lowermost dose of GA (25 mg/kg) and piperine (5 mg/kg) did not protect AST and γ-GT in a significant manner, whereas the higher doses showed significant protection ($P \leq 0.05$; $P \leq 0.01$). The activities of ALT, LDH and SALP were reversed more prominently with the highest doses of GA and piperine ($P \leq 0.01$) whereas 25 mg/kg dose of GA and 5 mg/kg dose of piperine restored these variables ($P \leq 0.01$). The administration of GA and piperine *per se*, at maximum doses, did not significantly alter any serum parameter. On the basis of these findings, a 50 mg/kg dose of GA and 10 mg/kg dose of piperine were considered to be minimum effective doses and were to be processed for further evaluation of their synergistic effects.

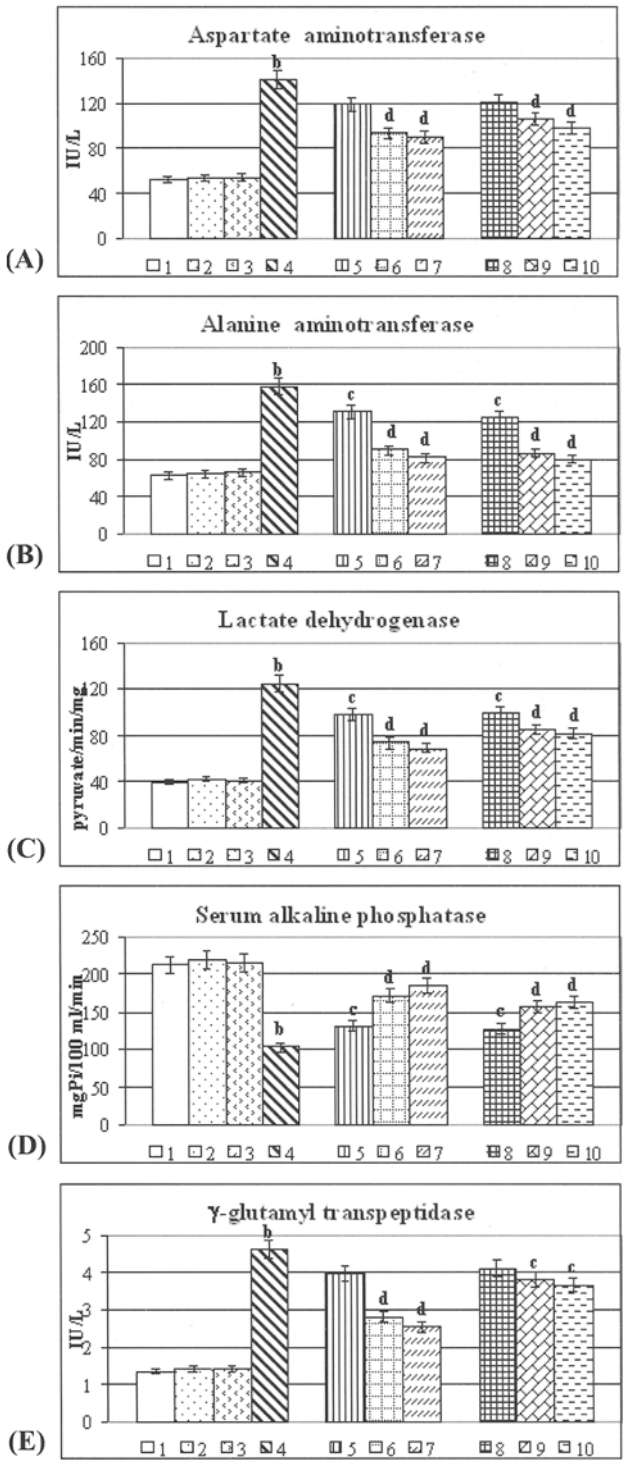

Fig. 1 (A-E). The therapeutic influence of GA and piperine at different doses against beryllium- induced serological alterations. The results are expressed as the mean ± SE for the n = 6 of each group. P-value [b] ≤ 0.01 (Be vs. control); P-value [c] ≤ 0.05 and [d] ≤ 0.01 (treatments vs. Be). *Significant for ANOVA of AST = 40.00*, ALT = 42.01*, LDH = 49.45*, SALP = 21.40* and γ-GT = 60.00*. **Abbreviations:** Control (1); GA *per se* (2); Piperine *per se* (3); Beryllium (4); Be + GA 25 mg/kg (5); Be + GA 50 mg/kg (6); Be + GA 75 mg/kg (7); Be + Piperine 5 mg/kg (8) Be + Piperine 10 mg/kg (9) and Be + Piperine 15 mg/kg (10).

Experimental protocol 2: Evaluation of the combined effect of GA and piperine

Blood biochemical assay

Table I represents the beryllium-induced alterations in blood biochemical variables. Exposure to beryllium for 28 days significantly decreased hemoglobin, albumin and urea ($P \leq 0.01$). Moreover, treatment with GA at a dose of 50 mg/kg significantly reversed these parameters (P 0.05). The combined therapy of GA and piperine demonstrated synergistic effects and the values were found to be close to control levels ($P \leq 0.01$). In addition, a significant raise in serum bilirubin and creatinine levels was observed after beryllium exposure ($P \leq 0.01$). Therapy with GA alone as well as the combination with piperine showed a significant decrease in both parameters ($P \leq 0.01$), whereas piperine treatment was found to be effective only for creatinine ($P \leq 0.01$). A Tukey's HSD *post hoc* test found significant differences between the monotherapy and combined therapy ($P \leq 0.05$).

Beryllium exposure significantly altered the liver cytoplasmic enzymes which include AST, ALT, LDH, SALP and γ-GT (Table II). The individually administered GA and piperine showed a significant reversal for the liver function tests, however the co-treatment of GA and piperine presented a more pronounced protective effect ($P \leq 0.01$) and reversed the altered parameters closer to control levels.

We found a significant difference between monotherapy and combined therapy for all the variables when statistically analyzed by the Tukey's HSD *post hoc* test ($P \leq 0.05$).

Table I. Effectiveness of gallic acid along with piperine against beryllium induced alterations in blood biochemical variables [Values are expressed as the mean ± SE from six rats in each group]

Treatments	Hemoglobin (mg/100 mL)	Bilirubin (mg/dl)	Albumin (mg/dl)	Urea (mg/dl)	Creatinine (mg/dl)
Control	15.6 ± 0.86	0.29 ± 0.016	5.94 ± 0.32	33.8 ± 1.86	0.308 ± 0.017
Be	11.7 ± 0.64[b]	0.80 ± 0.044[b]	3.81 ± 0.21[b]	24.1 ± 1.33[b]	0.893 ± 0.049[b]
Be + GA	14.7 ± 0.81[c]	0.42 ± 0.023[d]	4.88 ± 0.26[c]	30.5 ± 1.68[c]	0.513 ± 0.028[d]
% Protection	***76.92%***	***74.50%***	***50.23%***	***65.97%***	***64.95%***
Be + Pip	13.9 ± 0.76	0.68 ± 0.037	4.22 ± 0.23	28.2 ± 1.55	0.607 ± 0.033[d]
% Protection	***56.41%***	***23.52%***	***19.24%***	***42.26%***	***48.88%***
Be + GA+Pip	15.1 ± 0.83[d]	0.35 ± 0.19[d§†]	5.24 ± 0.28[d†]	32.6 ± 1.80[d†]	0.348 ± 0.019[d§†]
% Protection	***87.17%***	***88.23%***	***67.13%***	***87.62%***	***93.16%***
F Variance	4.514*	64.426*	11.620*	6.459*	65.738*

Anova at 5 % level: * Significant; P-value Be vs. control at [b] ≤ 0.01, P value treatments vs. Be at [c] ≤ 0.05, [d] ≤ 0.01
[§]significant difference in GA vs. GA + Pip, [†]significant difference in Pip vs. GA + Pip for Tukey's HSD *post hoc* test ($P \leq 0.05$).
Abbreviations: Be = beryllium; GA = gallic acid; Pip = piperine.

Table II. Efficacy of gallic acid in combination with piperine against beryllium induced alterations in liver function tests [Values are mean ± SE from six rats in each group]

Treatments	AST (IU/L)	ALT (IU/L)	LDH (pyruvate/min/mg)	SALP (mgPi/100ml/min)	γ-GT (IU/L)
Control	54.7 ± 3.02	61.3 ± 3.38	38.2 ± 2.11	210 ± 11.6	1.45 ± 0.080
Be	138 ± 7.62[b]	162 ± 8.95[b]	120 ± 6.63[b]	104 ± 5.74[b]	4.57 ± 0.252[b]
Be + GA	92.7 ± 5.12[d]	89.3 ± 4.93[d]	73.2 ± 4.04[d]	174 ± 9.61[d]	2.87 ± 0.158[d]
% Protection	***54.38%***	***72.19%***	***57.21%***	***66.03%***	***54.48%***
Be + Pip	105 ± 5.80[d]	85.7 ± 4.73[d]	85.1 ± 4.70[d]	158 ± 8.73[d]	3.77 ± 0.200[c]
% Protection	***39.61%***	***75.76%***	***42.66%***	***50.94%***	***25.64%***
Be + GA+Pip	85.0 ± 4.69[d†]	73.1 ± 4.04[d§†]	53.6 ± 2.96[d§†]	193 ± 10.6[d†]	1.98 ± 0.109[d§†]
% Protection	***63.62%***	***88.28%***	***81.17%***	***83.96%***	***83.01%***
F Variance	36.937*	60.266*	61.685*	22.046*	64.556*

Anova at 5 % level: *Significant; P-value Be vs. control at [b] ≤ 0.01, P value treatments vs. Be at [c] ≤ 0.05, [d] ≤ 0.01.
§significant difference in GA vs. GA + Pip, [†]significant difference in Pip vs. GA + Pip for Tukey's HSD *post hoc* test ($P \leq 0.05$).
Abbreviations: AST = aspartate aminotransferase; ALT = alanine aminotransferase; LDH = lactate dehydrogenase; SALP = serum alkaline phosphatase; γ-GT = γ-glutamyl transpeptidase; Be = beryllium; GA = gallic acid; Pip = piperine.

Oxidative stress and antioxidant status

Hepatorenal oxidative stress was assessed by measuring LPO, which was found to increase significantly after exposure to beryllium ($P \leq 0.01$) (Figs. 2A, B). In addition, the monotherapy of GA and piperine significantly inhibited LPO ($P \leq 0.01$); however, the combined treatment of GA and piperine diminished LPO more profoundly ($P \leq 0.01$) with a 79.69% and 70.84% protection against oxidative stress, respectively. Tukey's HSD *post hoc* test revealed a significant difference between monotherapy and combined therapy against the hepatic and renal LPO.

Cellular antioxidant status was measured by estimating GSH, total SOD and catalase. Moreover, beryllium exposure significantly reduced hepatic and renal GSH (Figs. 2C, D) ($P \leq 0.01$). The combined therapy of GA and piperine improved GSH content ($P < 0.01$) and a significant difference was found between the monotherapy and co-treatment of GA and piperine by Tukey's HSD *post hoc* test ($P \leq 0.05$).

Hepatic and renal total SOD (Figs. 3A, B) as well as catalase activity (Figs. 3C, D) were significantly decreased after beryllium intoxication ($P \leq 0.01$). In addition, the individual administration of GA and piperine showed a significant improvement in the hepatorenal SOD activity

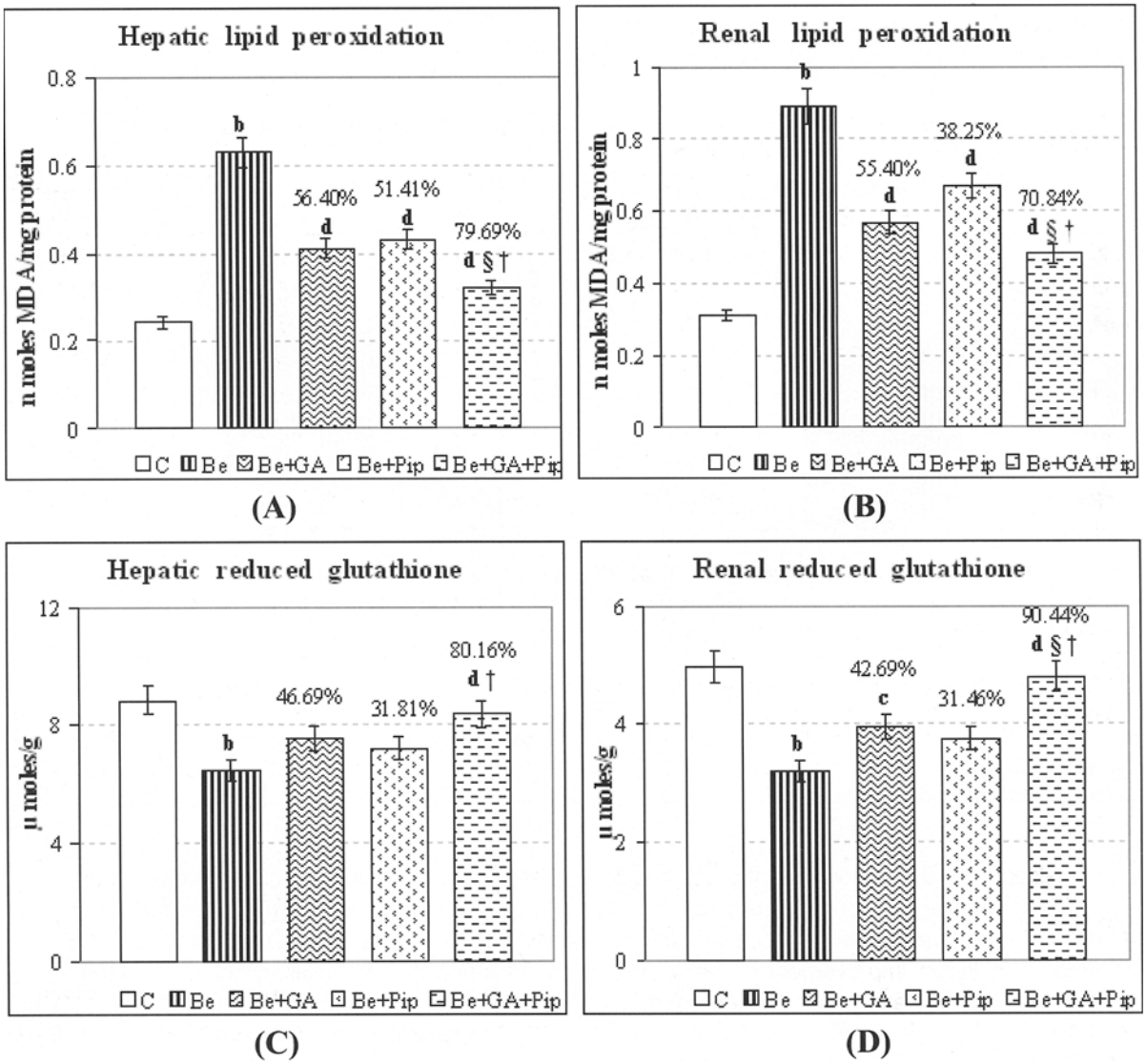

Fig. 2(A-D). The therapeutic influence of GA, piperine and a combination of GA and Pip against beryllium-induced oxidative stress. The results are expressed as the mean ± SE for the n = 6 of each group. Statistical significance was set *a priori* at P-value [b] ≤ 0.01 (Be vs. control); P-value [c] ≤ 0.05 and [d] ≤ 0.01 (treatments vs. Be). A significant difference was observed in the comparison of GA vs. GA + Pip[§], **A** Significant difference observed for Pip vs. GA + Pip for Tukey's HSD *post hoc* test (P 0.05) [†]. *Significant for ANOVA of hepatic LPO = 46.037*, renal LPO = 48.873*; hepatic GSH = 6.032* and renal GSH = 12.471*. The treatments are represented by their % protection assigned to each bar. **Abbreviations:** Control (C); Beryllium (Be); Be + Gallic acid (Be + GA); Be + Piperine (Be + Pip) and Be+GA+Piperine (Be + GA + Pip).

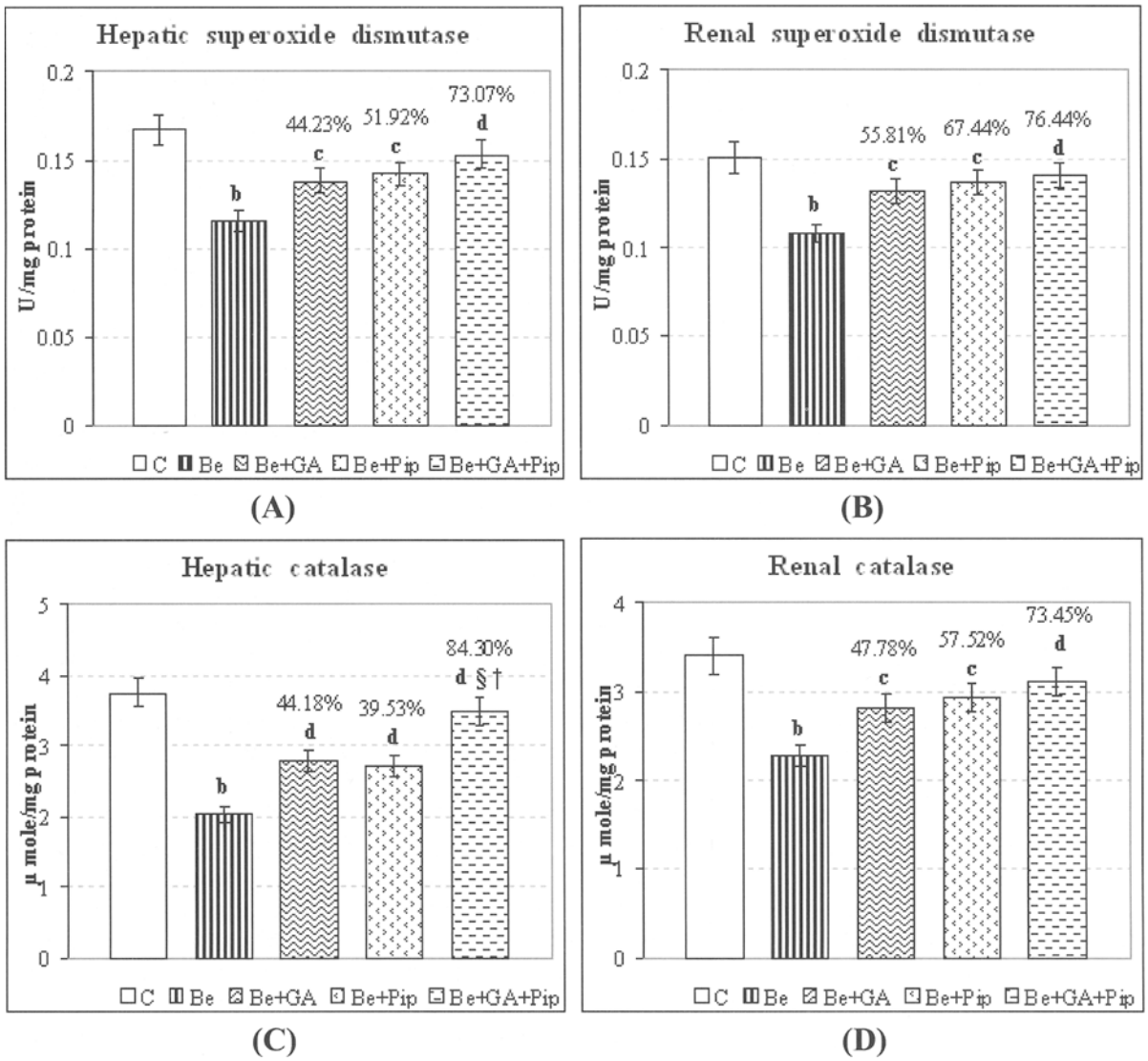

Fig. 3(A-D). The therapeutic influence of GA, piperine and a combination of GA and Pip against beryllium-induced alterations in the enzymatic antioxidant status. The results are expressed as the mean ± SE with n = 6 for each group. Statistical significance was set *a priori* at P-value $^{b}\leq$ 0.01 (Be vs. control taking); P-value $^{d} \leq 0.01$ (treatments vs. Be). §Significant difference observed for GA vs. GA + Pip, †Significant difference observed for Pip vs. GA + Pip for Tukey's HSD *post hoc* test (P 0.05). *Significant observed for ANOVA of hepatic SOD = 7.031*, renal SOD = 5.556*, hepatic catalase = 19.996* and renal catalase = 7.931*. The treatments are represented by their % protection assigned to each bar. **Abbreviations:** Control (C); Beryllium (Be); Be + Gallic acid (Be + GA); Be + Piperine (Be + Pip) and Be + GA + Piperine (Be + GA + Pip).

and renal catalase ($P \leq 0.05$); however, the combination of GA and piperine showed a more profound recovery for both variables ($P \leq 0.01$). A Tukey's HSD *post hoc* test ($P \leq 0.05$) found a significant improvement in liver catalase as a result of combined therapy rather than monotherapy.

DISCUSSION

Severe alterations in the biochemical variables and hepatorenal oxidative stress consequences were observed after a subchronic exposure to beryllium. A reduction in hemoglobin content was consistent with the fall in synthesis of the heme and globin proteins (Venugopal and Luckey, 1978) or the suppression in the activity of δ-amino levulinic acid synthetase (ALAS) and -amino levulinic acid dehydratase (ALAD) (Sakaguchi *et al.*, 1997). An increase in bilirubin was an indication that hepatotoxicity (Zimmerman, 1973) and erythrocyte degradation rate (Edmondson and Peters, 1985) increased, which may have also been the cause of hemoglobin depletion. The efficacy of GA as an iron ion chelator is well reported (Hirai *et al.*, 2005) and its chelating effect against toxic beryllium ions cannot be ruled out due to the presence of an active hydroxyl moiety in its structure. Therefore, subsequent decreases in beryl-

lium burden might be helpful in the recovery of hemoglobin in erythrocytes and the down-regulating of the bilirubin level. Piperine appears to play a role in increasing the effectiveness of GA by increasing its bioavailability through various processes (Nirala *et al.*, 2007b). A decreased concentration in the serum albumin level indicated a functional abnormality in the liver and represents a toxic response to beryllium exposure. The synergistic therapeutic potential of GA and piperine might stimulate protein synthesis as a contributory hepatoprotective mechanism by accelerating the regeneration process of liver cells (Nirala *et al.*, 2007b).

Elevated levels of AST, ALT and LDH in the circulation were indicative of a hepatic injury after beryllium exposure. Gallic acid might act as an indirect or direct antioxidant by combining with toxic beryllium ions or reactive metabolites to inactivate them in parallel to the antioxidant action of piperine. Thus, the combined therapy prevented cellular injury and organ dysfunction more prominently and the subsequently inhibited rapid leakage of these enzymes into the blood circulation. The displacement of magnesium ions (Mg^{++}) by beryllium ions (Be^{++}) is one of the major causes of inhibition for the activity of SALP during beryllium toxicity (Boukhalfa *et al.*, 2004). The combination of GA and piperine increased the activity of SALP likely by mobilizing the beryllium ions from the site and by up-regulating the magnesium metabolism. A decrease in the serum urea content indicated a perturbed deammination ability of the liver, whereas increased serum creatinine reflected impairment in the kidneys, particularly for glomerulus filtration rate due to beryllium intoxication. A decrease in the beryllium burden due to combined treatment might the variables back towards control levels, thereby improving hepatorenal functions.

A change in the cellular antioxidant status and MDA level for this study was regarded as an indicator of increased ROS production, which is attributed to the oxidative damage in cellular macromolecules such as lipids, proteins and nucleic acids of tissues. As well, they reflect the pathological process of toxic exposure. A higher intracellular GSH concentration reduces damage and promotes better survival under the conditions of oxidative stress (Dickinson *et al.,* 2003). The γ-GT was found to be involved in the metabolism of glutathione and the transport processes of the proximal renal tubules and bile ductules (Meister and Tate, 1976). An elevated γ-GT due to beryllium intoxication might increase the breakdown of GSH into its constituents, thereby causing impairment in the cellular oxidative defense mechanism, which could lead to hepatorenal injury. The SOD is a specific antioxidant enzyme which dismutates O^2 and forms H_2O_2 that is eventually scavenged by peroxisomal catalase or glutathione peroxidase (Irmak *et al.*, 2002; Ilhan *et al.*, 2004). Under the toxic circumstances, these endogenous antioxidant defense mechanisms might be perturbed as a result of an overproduction of ROS, inactivation of detoxification systems, consumption of antioxidants or failure to adequately replenish the antioxidants of tissues. The status of antioxidant enzymes were markedly increased after the co-treatment of GA and piperine, and may be attributed to the dual function of combined therapy. These functions include the indirect antioxidant effect of GA since it would scavenge toxic beryllium ions from the tissue and the direct antioxidant effects as it scavenges free radicals (Bajpai *et al.*, 2005) and suppresses the overproduction of ROS (Yeh and Yen, 2006). Simultaneously, the antioxidative properties of piperine were found to inhibit the formation of free radicals as well as enhance the therapeutic potential of GA via increasing its bioavailability by either increasing its absorption or decreasing its biotransformation in the liver. Therefore, GA and Piperine synergistically decreased the utilization of GSH, as well as increased SOD and catalase, which in turn decreased oxidative stress.

The therapeutic index (TI) is a ratio of the median lethal dose (LD_{50}) to the median effective dose (ED_{50}), and this helps in the assessment of safety for a particular drug. Piperine has been reported to increase the TI in a variety of drugs by increasing their bioavailability (Gupta *et al.,* 2000; Pattanaik *et al.*, 2006) It was assumed that the co-administration of piperine might enhance the bioavailability of GA, which would enhance its subsequent effectiveness. The LD_{50} of gallic acid and piperine are 5000 mg/kg and 514 mg/kg, respectively. The doses used in this study indicate a wider therapeutic index of these compounds. Thus, it can be concluded that the combined administration of gallic acid and piperine provides a more pronounced therapeutic potential, compared to the individual treatment in the regulation of beryllium-induced hepatorenal dysfunction and oxidative stress.

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