CO-ADMINISTRATION OF ALOE VERA WITH PIPERINE AND MORINGA OLEIFERA WITH CURCUMIN ATTENUATE BERYLLIUM INDUCED BLOOD BIOCHEMICAL ALTERATIONS IN RAT

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Abstract:: Female albino rats were administered beryllium nitrate at doses of 1mg/kg i.p. once a day for 90 consecutive days followed by combination therapy of Aloe vera (150mg/kg p.o.) with piperine (2.5mg/kg p.o.) and Moringa oleifera root extract (150mg/kg p.o.) with curcumin (5mg/kg p.o.) once a day for last 15 days to encounter the characteristic blood biochemical alterations. Exposure of beryllium nitrate induced severe alterations in blood biochemical variables. Beryllium nitrate disturbed liver function test as evidenced by induced leakage of AST, ALT in serum whereas activity of SALP was decreased. Exposure of beryllium nitrate disturbed the kidney function test by increased level of urea, uric acid and creatinine. A significant increase in triglyceride, cholesterol and bilirubin was recorded whereas hemoglobin, albumin and blood sugar was decreased as compare to control. Treatment with therapeutic agents maintained the liver and kidney function test and other blood biochemical variables whereas combination therapy of Aloe vera with piperine was found to be most effective in reversal of blood biochemical variables more towards control. By present study, it can be concluded that combination therapy of Aloe vera with piperine is better option in treatment of beryllium toxicity.

Key words: Beryllium, Aloe vera, Piperine, Moringa oleifera, Curcumin

INTRODUCTION

Exposure of toxic metals is unavoidable due to widespread use in human activities such as industry, agriculture and even as medicine. These metals and their compounds accumulate in the environment and contaminate water sources and food chain. The Agency for Toxic Substances and Disease Registry [1] has enlisted 275 most hazardous substances in the environment. Beryllium is one of them, posses a great threat to present day world. General population exposed to it through ambient air, drinking water, diet and tobacco smoking on daily basis. Workers get exposed to beryllium during mining and processing of beryllium containing alloy. Beryllium has many desirable properties such as light weight, enhancing metal hardening capacity, high melting/boiling point and nonmagnetic in nature, thus it gain importance to be used in various industries [2]. In Europe, Be and its compounds are classified as category 2 carcinogens, which are substances to be assimilated with those that are carcinogenic for man [3]. Administration of Beryllium and its compounds provoke oxidative stress and lead to various pathological consequences like apoptosis [4]. A number of diseases have been reported due to beryllium such as bronchitis, pneumonitis, dermatitis, acute pneumonitis, hepatomegaly, chronic pulmonary
granulomatosis, berylliosis and chronic beryllium disease (CBD) [5-7]. A number of synthetic compounds have been tried to encounter the toxic effect of beryllium; however, they have certain limitations. Natural products of plant origin are less toxic and high medicinal values due to rich in antioxidants. Aloe vera is commonly known as Ghee kanwar (Hindi) has been used as traditional medicine from ancient time. It has wide range of medicinal importance such as hepatoprotective [8], antioxidant [9], immunomodulatory [10], anti-diabetic [11] and antimicrobial activity [12]. Moringa oleifera locally known as “Shahjana” is medicinally important as all the parts of plants have biological properties such as antioxidant [13], and hepatoprotective activity [14]. Root juice is employed in cardiac tonic, antiepileptic, used for nervous debility, asthma, enlarged liver and spleen [15]. Piperine is an active principle of Piper longum Linn. and Piper nigrum Linn., and is well known for its biological activities, including hepatoprotective [16], antimitagenic [17], antitumor [18], antidepressant [19] and antioxidant [20]. It has bioavailability enhancing activity for some drugs [21, 22]. Curcumin is a hydrophobic polyphenol derived from the rhizome of the herb Curcuma longa showed antioxidant [23], anti-inflammatory [24], hepatoprotective [25] and nephro-protective [26] properties. Thus, use of natural products of plant origin having polyphenolic constituents, may be a better option in treatment of beryllium toxicity.

MATERIALS AND METHODS

Chemicals: Beryllium nitrate, piperine and curcumin were purchased from Sigma Aldrich Company (USA). Aloe vera was collected from botanical garden of Jiwaji University, Gwalior and processed [27]. The roots of Moringa oleifera were collected from university campus and identified by the department of botany, Jiwaji University, Gwalior. Moringa oleifera root extract was prepared [28]. All the therapeutic agents were stored, refrigerated in a desiccator to avoid oxidation and thermal decomposition. All other chemicals used in this study were of pure and analytical grade and procured from standard chemical dealers.

Maintenance of animals and their feeding: Adult female albino rats of Wistar strain (8-10 weeks old having 160 ± 10 g body weight) were randomly selected from departmental animal facility. Animals were housed under standard conditions (25 ± 2°C temp., 60%-70% relative humidity and 14 h light and 10 h dark). Animals were fed on standard commercially available pellets of animal (Pranav Agro Industries Ltd. New Delhi, India) and drinking water ad libitum. Animals used in this study were treated and cared for in accordance with the guidelines recommended by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) Chennai, India and experimental protocols were approved by Institutional Ethics Committee (CPCSEA/501/01/A) of Jiwaji University, Gwalior, India.

Preparation of doses and treatments: Beryllium nitrate (35% w/v; Sigma Aldrich, USA) was diluted in triple distilled water making up doses of 1mg/2ml/kg and administered intraperitoneally [21]. The doses of Aloe vera (150mg/kg/5ml) was prepared in triple distill water, Moringa oleifera root extract (150mg/kg/5ml) was prepared in 1% gum acacia, doses of piperine (2.5 mg/kg/3ml) and curcumin (5mg/kg/3ml) were prepared in olive oil and administered orally with the help of an intragastric rubber catheter. Twenty four adult female rats were divided into 4 groups of six each as follows:

Group 1: Normal control Na(NO₃)₂ at a dose of 1 mg/kg i.p. daily for 90 days

Group 2: Be(NO₃)₂ (1 mg/kg i.p. daily ) for 90 days

Group 3: Be(NO₃)₂ (as in group II) + Aloe vera (150 mg/kg p.o.) + Piperine (2.5 mg/kg p.o.) daily for 15 days.

Group 4: Be(NO₃)₂ (as in group II) + Moringa oleifera (150 mg/kg p.o.) + Curcumin (5 mg/kg p.o.) daily for 15 days.

After 24 hours of final administration, the animals were sacrificed under mild ether anaesthesia, drawing blood by puncturing retro-orbital venous sinus.

Blood biochemical study: Hemoglobin content was determined by Sahli’s apparatus [29]. The activities of aspartate amino-transferase (AST) and alanine aminotransferase (ALT) in serum were estimated [30]. Serum alkaline phosphatase, blood sugar, albumin, bilirubin, urea, uric acid, creatinine, triglyceride and total cholesterol in serum were assessed by kit methods as per instructions provided by the company (E-Merck, Germany).
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Statistical analysis: Results are presented as mean ± S.E. of six animals used in each group. Data were subjected to statistical analysis through one-way analysis of variance (ANOVA) taking significant at 5% level of probability followed by Student’s t-test taking significant at P > 0.05 [31]. Percent protection was calculated by the following formula: % Protection = 1-(D-N/T-N) X 10 [D=Drug, N=Normal, T=Toxicant]

RESULTS

Combination therapy of Aloe vera with piperine and Moringa oleifera with curcumin, were evaluated against beryllium induced blood biochemical variables in rats. Administration of beryllium nitrate at doses of 1mg/kg i.p. (once a day for 90 days) disturbed the liver function test by increase in the activities of serum aspartate aminotransferase (AST), alanine aminotransferase (ALT) and decreased activity of serum glutamate oxaloacetate transaminase (SGOT) and serum glutamate pyruvate transaminase (SGPT) together with significant increase in serum bilirubin, increased level of urea, uric acid and creatinine. Nitrate also disturbed the normal kidney function by increased level of urea, uric acid and creatinine together with significant increase in serum bilirubin, triglyceride and cholesterol and significant decrease in albumin, blood sugar and hemoglobin in blood (Tables 1,2). Therapy with Aloe vera with piperine and Moringa oleifera with curcumin showed recovery in retention of liver and kidney function test with other blood biochemical variables whereas combination of Aloe vera with piperine was found to be most effective in prevention of beryllium induced biochemical alterations more significantly (P < 0.05) which was confirmed by one way analysis variance using ANOVA.

DISCUSSION

In previous study, we screened aqueous doses of Aloe vera and hydroalcoholic root extract of Moringa oleifera at four different doses (50, 100, 150 and 200mg/kg, p.o.) against beryllium (1mg/kg/2ml i.p.) induced systemic toxicity and we found that Aloe vera and Moringa oleifera at 150 mg/kg was found to be more effective in these doses in prevention of beryllium toxicity [27,28] and further combination study of Aloe vera and Moringa oleifera with piperine and curcumin was evaluated against beryllium toxicity. Among these combinations, Aloe vera with piperine and Moringa oleifera with curcumin was found to be most effective separately in attenuation of beryllium toxicity after subchronic exposure of beryllium nitrate [32]. In present study, Aloe vera with piperine and Moringa oleifera with curcumin were administered after 90 days chronic exposure of beryllium nitrate. The study reports better

Table 1: Effect of Aloe vera with piperine and Moringa oleifera with curcumin against beryllium induced hepatorenal dysfunction. Values are mean ± S.E. N=6, # PdH0.05 vs. control group, *PdH 0.05 vs. beryllium, ANOVA® =Significant.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>ALT (IU/dl)</th>
<th>AST (IU/dl)</th>
<th>SALP (mgP/h/100ml)</th>
<th>Urea (mg/dl)</th>
<th>Uric acid (mg/dl)</th>
<th>Creatinine (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>47 ± 3.31</td>
<td>68 ± 3.68</td>
<td>205 ± 11.12</td>
<td>32 ± 2.08</td>
<td>1.1 ± 0.10</td>
<td>0.4 ± 0.02</td>
</tr>
<tr>
<td>Be per se</td>
<td>155.5 ± 8.95*</td>
<td>180 ± 9.66*</td>
<td>105 ± 5.47*</td>
<td>66 ± 5.03*</td>
<td>4.2 ± 0.27*</td>
<td>0.7 ± 0.05*</td>
</tr>
<tr>
<td>Be+ AV+ Pip</td>
<td>77.2 ± 3.90*</td>
<td>110.4 ± 5.89*</td>
<td>174 ± 9.86*</td>
<td>37 ± 3.30*</td>
<td>1.8 ± 0.16*</td>
<td>0.5 ± 0.04*</td>
</tr>
<tr>
<td>(Protection)</td>
<td>72.1 (%)</td>
<td>62.1 (%)</td>
<td>69.0 (%)</td>
<td>85.2 (%)</td>
<td>77.4 (%)</td>
<td>66.6 (%)</td>
</tr>
<tr>
<td>Be+ MO+ Cur</td>
<td>87.6 ± 4.41*</td>
<td>118.5 ± 6.17*</td>
<td>152 ± 8.71*</td>
<td>42 ± 3.47*</td>
<td>2.5 ± 0.13*</td>
<td>0.6 ± 0.03*</td>
</tr>
<tr>
<td>(%Protection)</td>
<td>62.5 (%)</td>
<td>54.9 (%)</td>
<td>47.0 (%)</td>
<td>70.5 (%)</td>
<td>54.8 (%)</td>
<td>50.0 (%)</td>
</tr>
<tr>
<td>ANOVA (F Values)</td>
<td>20.6*</td>
<td>47.4*</td>
<td>21.6*</td>
<td>17.3*</td>
<td>53.2*</td>
<td>9.6*</td>
</tr>
</tbody>
</table>

Table 2: Effect of Aloe vera with piperine and Moringa oleifera with curcumin against beryllium induced blood biochemical alteration. Values are mean ±S.E. N=6, # PdH0.05 vs. control group, *PdH 0.05 vs. beryllium, ANOVA® =Significant.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Hemoglobin (g/dl)</th>
<th>Bilirubin (mg/dl)</th>
<th>Albumin (mg/dl)</th>
<th>Blood Sugar (mg/dl)</th>
<th>Triglyceride (mg/dl)</th>
<th>Cholesterol (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>15.4 ± 0.81</td>
<td>0.4 ± 0.03</td>
<td>5.9 ± 0.47</td>
<td>105.0 ± 5.47</td>
<td>24.0 ± 1.78</td>
<td>42.0 ± 3.78</td>
</tr>
<tr>
<td>Be per se</td>
<td>10.3 ± 0.58*</td>
<td>0.9 ± 0.05*</td>
<td>3.0 ± 0.28*</td>
<td>51.4 ± 3.26*</td>
<td>72.0 ± 3.78*</td>
<td>84.0 ± 4.87*</td>
</tr>
<tr>
<td>Be+ AV+ Pip</td>
<td>13.4 ± 0.98*</td>
<td>0.5 ± 0.02*</td>
<td>5.0 ± 0.46*</td>
<td>94.6 ± 5.07*</td>
<td>39.0 ± 3.06*</td>
<td>48.0 ± 2.88*</td>
</tr>
<tr>
<td>(%Protection)</td>
<td>60.7 (%)</td>
<td>80.0 (%)</td>
<td>68.9 (%)</td>
<td>80.5 (%)</td>
<td>60.7 (%)</td>
<td>85.7 (%)</td>
</tr>
<tr>
<td>Be+ MO+ Cur</td>
<td>12.8 ± 0.73*</td>
<td>0.55 ± 0.02*</td>
<td>4.8 ± 0.47*</td>
<td>77.8 ± 4.64*</td>
<td>43.0 ± 3.59*</td>
<td>58.0 ± 3.59*</td>
</tr>
<tr>
<td>(%Protection)</td>
<td>49.0 (%)</td>
<td>70.0 (%)</td>
<td>62.0 (%)</td>
<td>49.2 (%)</td>
<td>60.4 (%)</td>
<td>61.9 (%)</td>
</tr>
<tr>
<td>ANOVA (F Values)</td>
<td>6.9*</td>
<td>29.8*</td>
<td>7.8*</td>
<td>24.8*</td>
<td>40.4*</td>
<td>23.1*</td>
</tr>
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antitoxic potential of Aloe vera with piperine in attenuation of beryllium induced blood biochemical variables.

A significant fall in hemoglobin was observed in present study that might be due to diminishment in synthesis of heme and globin proteins. Beryllium suppressed the activity of δ-amino levulinic acid dehydratase (ALAD) in blood and increased the activity of δ-amino levulinic acid synthetase (ALAS) in liver which act as the key regulatory enzymes in heme biosynthesis. ALAS, the first key regulatory enzymes catalyze the formation of delta aminolevulinate by succinyl CoA with glycine. ALAS undergoes feedback inhibition when the end product “heme” is plenty enough, the expression of ALAS stopped while low level of heme increases ALAS expression. ALAD catalyze the condensation of two molecules of delta aminolevulinate and lead to the formation of porphobilinogen. ALAD requires zinc as divalent metal ion as cofactor for it’s activity. Be\(^{++}\) is also a divalent metal ion and quite toxic, it may bind with enzymatic (ALAD) active site and inhibited its action thus the level of heme is not maintained. Decreased heme content induced the expression of ALAS for more synthesis of heme but due to inhibition of ALAD, the amount of heme/ hemoglobin decreased in blood. Aloe vera rich source of antioxidants and piperine act as antioxidants may bind with beryllium and inhibit the binding of beryllium to ALAD thus the level of ALAD is reversed towards normal and due to formation of heme the activity of ALAS is maintained towards normal. Thus the level of hemoglobin is reversed towards normal.

Beryllium administration decreased blood sugar level and provides the signal for glycolgenolysis thus the level of hepatic glycogen decreases [27]. Beryllium inhibits the key regulatory enzymes of carbohydrate metabolism [22] thus glucose and its derived product (glucose-6-phosphate) enter into pentose phosphate pathway for body energy. Therapy with Aloe vera with piperine showed more pronounced therapeutic effect by maintaining the activity of key regulatory enzymes of carbohydrate metabolism thus the level of blood sugar is maintained. Whereas Moringa oleifera showed hypoglycemic effect and even in combination with curcumin was not capable to maintain the glucose level up to the normal.

Beryllium nitrate induced oxidative stress and leads to membrane damage [22]. Exposure of beryllium nitrate elevated leakage of serum transaminases indicating severe hepatic cell necrosis. Treatment with Aloe vera with piperine, provide antioxidants which prevented cellular injury and organ dysfunction prominently and subsequently inhibited rapid leakage of these enzymes into blood circulation. Whereas therapy with Moringa oleifera with curcumin showed less protective effect in protection of membrane damage. SALP is a membrane bound enzyme, decreased in beryllium toxicity it is due to binding of beryllium to active site of enzyme. Therapy with Aloe vera in combination with piperine acted as chelator that binds with soluble beryllium salt and inhibits binding of beryllium to SALP. Hyperbilirubinemia indicated the severity of necrosis [33], rose significantly (P<0.05) with beryllium intoxication.

Beryllium nitrate also disturbed normal kidney function and increased serum level of urea, uric acid and creatinine. Combination of Moringa oleifera and curcumin significantly reversed these variables and helped in maintaining the normal kidney function but combination of Aloe vera with piperine was more effective in reversal of kidney function test towards normal, this is due to more synergistic action of piperine along with Aloe vera as compare to curcumin with Moringa oleifera. Aloe vera rich in minerals, vitamins, phenolics and flavonoids act as antioxidants, neutralize the beryllium induced reactive oxygen species. Various compound present in Aloe vera may compete with beryllium ion for binding to enzymes of major metabolic pathway and helps in restoring the activity of enzymes. 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 Aloe vera with piperine might stimulate protein synthesis as a contributory hepatoprotective mechanism by accelerating the regeneration process of liver cells.

Exposure to beryllium also interferes with lipid metabolism causing increase in the serum level of cholesterol and triglyceride. Significantly increased lipid profile indicated severe lipid peroxidation and physiological defects in the organs after beryllium administration. Oxidative stress increases the supply of non-essential fatty acids, which in turn increases triglycerides and cholesterol levels in serum. Therapy with Moringa oleifera in combination with curcumin reduced the elevated level of triglycerides and total cholesterol after toxicant exposure this is due to
reduction in lipid peroxidation. But *Aloe vera* with piperine is shown to be better effect in reversal of lipid peroxidation and reduction of induced lipid profile after beryllium toxicity. Thus, *Aloe vera* with piperine have better antioxidant and antitoxic potential as compared to *Moringa oleifera* with curcumin which is confirmed by present study as evidenced by the reversal of blood biochemical variables and maintaining the liver and kidney function towards normal indicating mix therapy is much more useful than individual application of therapeutic agents [34].

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Abbreviations used in text and tables: Be= Beryllium nitrate, AV= *Aloe vera*; MO= *Moringa oleifera*, Pip= Piperine; Cur = Curcumin.

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