African Journal of Biochemistry Research Vol. 5(10), pp. 307-314, 30 September, 2011
Available online at http://www.academicjournals.org/AJBR
ISSN 1996-0778 ©2011 Academic Journals

Full Length Research Paper

Chelating ability of sulbactomax drug in arsenic intoxication

V. K. Dwivedi[1]*, A. Arya[1], H. Gupta[1], A. Bhatnagar[2], P. Kumar[1] and M. Chaudhary[1]

[1]Pre Clinical Division, Venus Medicine Research Centre, Baddi, H.P. India 173205, India.
[2]Analytical Division, Venus Medicine Research Centre, Baddi, H.P. India 173205, India.

Accepted 17 August, 2011

The aim of this study was to determine the chelating ability of Sulbactomax drug in arsenic pre-exposed rats. 24 rats were divided into I to III groups. Group I was control while Groups II to III were arsenic exposed and arsenic plus Sulbactomax treated group. Arsenic toxicity was induced via intraperitoneally administration of arsenic trioxide (As_2O_3) 10 mg/kg body weight/day for three weeks. Toxicity was confirmed by decreased body weight, increased body temperature, loss of appetite and decreased hemoglobin levels in all groups except Group I. After confirmation of these symptoms, drug was administered via intravenous route for three week treatment. At the end of the study, blood samples were collected and the arsenic, zinc concentration, hemoglobin level and δ -aminolevulinic acid dehydrates activity were measured in the blood while other parameters were measured in the plasma sample. Our results revealed that zinc concentration, hemoglobin level, δ -aminolevulinic acid dehydratase, catalase and superoxide dismutase (SOD) enzyme activities significantly increased, while arsenic concentration, lipid peroxidation level, myloperoxidase enzyme activity, tumor necrosis factor TNF-α and IL-6 levels significantly reduced in Group III as compared to Group II. So, the findings concluded that administration of Sulbactomax drug act as an antioxidant and chelating agent that reduce arsenic metal and free radical mediated tissue injury.

Key words: Arsenic, Sulbactomax, antioxidant enzymes, cytokines parameters, arsenic, zinc.

INTRODUCTION

Arsenic (As) is a member of Group V in the periodic table of elements along with nitrogen, phosphorus, antimony and bismuth. The chemistry of arsenic is rather complex, and the compounds it forms are numerous. This is largely because arsenic possesses several different valence or oxidation states, which markedly result in the different biological behavior of its compound. It is a ubiquitous element which is present in low amount, soil and water. Arsenic compounds may represent a concern to environmental and occupational health become concentrated in the environmental as result of natural anthropogenic source. High doses of inorganic arsenic lead to neurologic, muscular, renal and gastrointestinal manifestation which may be responsible for a fatal outcome (Von, 1958). Long term administration of arsenic can cause hepatocellular carcinoma (Weisberger and Williams, 1975) in experimental animals, angiosarcoma of the liver (Ishak, 1976) and carcinoma of skin and lung (Jackson and Grainge, 1975). Arsenic is methylated by alternating reduction of pentavalent arsenic to trivalent and addition of a methyl group from S-adenosylmethionine (Singh and Rana, 2007). Glutathione and possibly other thiols, serve as reducing agents. Arsenic also inhibits several other cellular enzymes, especially those involved in cellular glucose uptake, gluconeogenesis and fatty acid oxidation etc. Exposure of arsenic causes melanosis, depigmentation and various organ failure such as kidney and hepatic. The heme metabolism pathway is known to be highly susceptible to alterations induced by heavy metals and environmental

*Corresponding author. E-mail: vivekdwivedi@venusremedies.com. Tel: 91-1795-302127. Fax: 91-1795 302133.

Abbreviation: **As_2O_3,** Arsenic trioxide; **SOD,** superoxide dismutase; **CAT,** catalase; **MDA,** malonaldialdehyde; **GSH,** reduced glutahione; **MPO,** myloperoxidase, **TNF,** tumor necrosis factor, **IL,** interlukin; **FDC,** fixed dose combination.

chemicals, offering the chance to use these changes as indicator of damage caused by arsenic (Flora et al., 2005).

A fixed-dose combination (FDC) is a formulation of two or more active ingredients combined in a single dosage form available in certain fixed doses. Sulbactomax is a novel fixed dose combination of ceftriaxone and sulbactam antibiotics along with third vector VRP1034. Ceftriaxone is third generation class of antibiotic and sulbactam is β-lacatamase inhibitor. VRP1034 (Trade secrete) is having potent chelating ability which reduce oxidative stress along with removal of heavy metal ions from the body. Dwivedi et al. (2009) reported that a novel fixed dose combination of Sulbactomax drug also play significant role in various bacterial infection. Beside their antibacterial property, authors have tried to determine whether Sulbactomax drug act as an antioxidant and has metal chealtion ability in arsenic pre-exposed rat's model.

MATERIALS AND METHODS

Chemicals

All the chemicals and biochemicals used in the present study were procured from Sigma, St. Louis, MO, USA. Δ-aminolevulinic acid (δ -ALAD) was purchased from Sigma Chemical, St Louis, MO, USA. Arsenic trioxide was purchased from CDH laboratories Ltd, New Delhi. Other chemicals, purchased locally, were of analytical grade. Ketamine hydrochloride was purchased from Samarth Life Science Pvt. Ltd. Mumbai. Other commercial kits were procured from Erba diagnostics Mannheim Gmb, Germanny. ELISA kits were procured from *invitrogen* for the measurment of cytokines parameters.

Drugs

Sulbactomax (ceftriaxone plus sulbactam + VRP1034), was obtained from Venus Medicine Research Centre, Baddi, H.P. The ratio of ceftriaxone and sulbactam in Sulbactomax is 2:1 respectively, along with VRP1034.

Animal groups and treatments

The animals were obtained from the animal house facility of Venus Medicine Research Centre, Baddi, and H.P. The experiment was carried out after approval from Institutional ethics committee. The study was performed on male Wistar rats weighing 145 ± 10 g housed in polypropelene cages in an air-conditioned room with temperature maintained at 25 ± 2°C and 12 h alternating day and night cycles. Animals were allowed standard rat chow diet and sterile distilled water. Twenty four rats were selected and divided into three groups of eight rats each which is given as follow:

Group I: Control normal saline treated group;
Group II: Arsenic trioxide induced group (10.0 mg/kg body weight/day);
Group III: Arsenic trioxide + Sulbactomax treated group (1.5 mg/kg body weight/day).

Arsenic trioxide was administered by *i.p* route to Groups II and III for 21 days except Group I. Body weight, body temperature, food intake and water intake were measured per day for 21 days. After clear symptoms, such as loss of appetite, increased body temperature and decreased body weight, appeared in all arsenic induced groups, treatment was started for 21 days. Sulbactomax drug were given to Group III according to their body weight via intravenous route for 21 days. At the end of experiment, blood samples were collected from each group in 3.8% sodium citrate containing vials. Immediately 1.5 ml blood samples were transferred into other tubes for measurement of hemoglobin, δ-ALAD enzyme activity and arsenic and zinc metal estimation.

Plasma preparation

Blood was centrifuged at 6000 rpm for 15 min and supernatant was carefully taken into other polypropelene tube and stored at 2°C to 8°C for the measurement of antioxidant enzymatic and biochemical parameters.

Determination of hemoglobin level

Hemoglobin level was measured according to Sahli haemoglobinometer.

Blood δ -aminolevulinic acid dehydratase (ALAD) activity

The ALAD activity in blood was assayed according to method of Berlin and Schaller (1974). For assay δ-ALAD activity, take 0.2 ml of blood sample and mixed with 1.3 ml double distilled water and incubate the test tubes for 10 min at 37°C for complete hemolysis. After incubation of all test tubes, added 1.0 ml of δ-ALA standard solution and further incubate all test tubes for one hr at 37°C. The reaction was stopped after one hr by adding 1.0 ml of 10% TCA solution. All test tubes were centrifuged at 600 g for 5 to 10 min. After centrifuge, 1.5 ml of the supernatant was taken in clean test tubes and equal amount of Ehrlich reagent was added and absorbance was recorded at 555 nm wavelength after 5 min. The molar extension coefficient 6.1×10^4 was used for calculation.

Measurement of intracellular and extracellular antioxidant enzymes activities

Superoxide dismutase (SOD) assay

SOD activity was determined by the method of Misra and Fradovich (1974). The reaction mixture consisted of 1.0 ml carbonate buffer (0.2 M, pH 10.2), 0.8 ml KCl (0.015 M), 0.1 ml of plasma sample and water to make the final volume to 3.0 ml. The reaction was started by adding 0.2 ml of epinephrine (0.025 M). The change in absorbance was recorded at 480 nm at 15 second interval for one min at 25°C. Suitable control lacking enzyme preparation was run simultaneously.

One unit of enzyme activity is defined as the amount of enzyme causing 50% inhibition of auto oxidation of epinephrine.

Catalase (CAT) activity

Catalase activity was measured according to procedure of Aebi (1984) at room temperature with slight modification. 100 µl plasma samples were placed on ice bath for 30 min at room temperature. 10 µl Triton-X was added in each plasma containing test tube. In a cuvette, 200 µl phosphate buffer (0.2 M; pH 6.8), 20 µl of sample and 2.53 ml distilled water was added. The reaction was started by adding 250 µl of H_2O_2 (0.066 M in phosphate buffer) and decrease in optical density was recorded at 240 nm wave length at every 15 second for one min. The molar extinction coefficient of 43.6 M Cm^{-1} was used for determination of catalase activity.

One unit of enzyme activity was defined as the amount of

enzyme that liberates half of the peroxide oxygen from H_2O_2 in one minute at 25°C.

Reduced glutathione (GSH) estimation

Reduced glutathione was estimated by the method of Eillman (1959). 0.5 ml plasma sample was mixed with equal amount of 5% (w/v) TCA reagent and kept for 10 min at room temperature, proteins were precipitated and filterate was removed carefully after centrifuge at 3500 rpm for 15 min. 0.25 ml filtrate was taken and added to 2.0 ml of Na_2HPO_4 (4.25%) and 0.04 ml of DTNB (0.04%). A blank sample was prepared in similar manner using double distilled water in place of the filtrate. The pale yellow color was developed and optical density was measured at 412 nm by spectrophotometer.

Estimation of total thiol content

Total thiol content was analyzed by the method of Hu (1994). 0.2 ml plasma sample was taken in test tubes and added 0.6 ml of Tris EDTA buffer (Tris 0.25 M , EDTA 20 mM; pH 8.2) followed by addition of 40 µl of 10 mM of 5,5' dithionitrobis 2-nitrobenzoic acid (DTNB in methanol) and make the total reaction volume up to 4.0 ml by adding 3.16 ml of methanol. All test tubes were sealed and the color was developed for 15 to 20 min, followed by centrifugation at 3000 g for 10 to 15 min at room temperature. The absorbance of the supernatant was measured at 412 nm wavelength.

Measurement of myleoperoxidase enzyme

Myeloperoxidase enzyme was determined by O-dianisidine method with slight modification (Kurutas et al., 2005). The assay mixture consisted of 0.3 ml of sodium phosphate buffer (0.1 M; pH 6.0), 0.3 ml of H_2O_2 (0.01 M), 0.2 ml of O-dianisidine (0.02 M) (freshly prepared in distilled water) and make final volume up to 3.0 ml with distilled water. The reaction was started by the addition of 0.025 ml plasma. The change in absorbance was recorded at 460 nm wavelength. All measurements were carried out in duplicate.

One unit of enzyme activity is defined as that giving an increase in absorbance of 0.001 min^{-1}.

Measurement of lipid per-oxidation level

Free radical mediated damage was assessed by the measurement of the extent of lipid peroxidation in the term of malonaldialdehyde (MDA) formed, essentially according to method of Ohkawa et al. (1979). It was determined by thio barbituric reaction. The reaction mixture consisted of 0.25 ml of plasma preparation, 0.20 ml of 8.1% sodium dodecyl sulphate (SDS), 1.5 ml of (20%, pH 3.5) acetic acid, 1.5 ml of 0.8% thio barbituric acid (TBA) and 0.6 ml distilled water and make the volume upto 4.0 ml. The tubes were kept in boiled water at 95°C for one hour and cooled immediately under running tap water. Added 1.0 ml of water and 5.0 ml of mixture of n-butanol and pyridine (15:1 v/v) and was vortexed. The tubes were centrifuged at 3500 rpm for 15 to 20 min. The upper layer was aspirated out and optical density was measured at 532 nm. The molar extension coefficient 1.56×10^5 was used for calculation.

Determination of biochemicals and cytokines parameters

The hepatic and renal enzymes serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT), Creatinine and alkaline phosphatase (ALP) levels) were measured in the plasma sample by standard kit method. Cytokine parameters (TNF α and Interlukin-6) were assayed by ELISA Reader, (Merck, Serial No- 21041098, MIOS -junior) according to manufacturer's instruction.

Metal estimation

For estimation of arsenic and zinc concentration in the blood, 0.5 ml of blood samples were directly mixed with 4.5 ml of acidic glycerol (1% HNO_3 and 5% glycerol mixture). Arsenic and zinc metal estimation were measured by using flame atomic absorption spectrophotometer (Analytikjena, model No contra A300, Germany) with hallow cathode lamp at wave length 193.7 and 213.9 nm respectively. The direct absorption of solution was determined by atomic absorption spectrophotometer and suitable standard curves of each metal were prepared using 20 to 100 µg/ml. All chemical used for metal estimation were Merck grade.

Statistical analysis

The resulting data were analyzed statistically. All values were expressed as mean ± SD. One-way analysis of variance (ANOVA) followed by Newman-Keuls comparison test was used to determine statistical difference between control vs. arsenic pre-exposed group and arsenic pre-exposed group vs. Sulbactomax treated groups. P values < 0.05 were considered statistically significant.

RESULTS

There was no mortality found during the experimental period. The clear symptoms such as anemia, loss of hair, loss of body weight and diarrhea were observed in arsenic pre-exposed group.

There was statistically significant decreased body weight ($P<0.01$), food intake ($P<0.001$) and water intake ($P<0.01$), along with significant ($P<0.001$) increase in body temperature in arsenic pre-exposed group after 21 days of intraperitoneally administering arsenic trioxide as compared to the control group. After treatment with Sulbactomax drug for 21 days, these physiological parameters were significantly improved in treated group as compared to the arsenic pre-exposed group and these physiological parameters almost reached near to the control group (Table 1). The arsenic concentration and δ-ALAD enzyme activity are major parameters in the arsenic toxicity. These parameters were affected by arsenic which is involved in the heme synthesis path way, due to inhibition of this enzyme heme synthesis is inhibited. So in the present study, the concentartion of arsenic ($P<0.001$) was significantly increased along with significantly ($P<0.001$) decreased zinc concentration in the blood of pre-exposed group as compared with control normal saline group. These metal concentration were significantly ($P<0.001$) improved in the blood of Sulbactomax treated group as compared with arsenic pre-exposed group after 21 days treatment.

There was statistically ($P<0.001$) significant decreased hemoglobin level and δ-ALAD enzyme activity in arsenic pre-exposed group as compared with control group. After

Table 1. Status of body weight, body temperature, food and water intake in arsenic pre-exposed group and Sulbactomax treated group.

Groups	Body weight (g)	Body temperature (°C)	Food intake (g)	Water intake (ml)
Control normal saline group	145.5 ± 3.5	35.53 ± 0.28	74.6 ± 2.68	75.2 ± 6.88
As pre-exposed group	120 ± 17.8**	38.01± 0.96***	53.1± 8.49***	60.0± 5.37**
Sulbactomax treated group	134.4 ± 12.7*	36.72± 0.59**	68.1± 6.64***	72.5± 9.45**

All data are expressed as mean± SD of each group. Newman keuls test was performed for statistical significance between control group vs. arsenic pre exposed group and arsenic exposed group vs. Sulbactomax treated group. Where ***p<0.001 (highly significant), **p<0.01(significant), *p<0.05 (significant), ^{Ns}p>0.05 (non significant).

Table 2. Status of biochemical parameters, arsenic and zinc levels in pre-exposed group and Sulbactomax treated group.

Groups	Hemoglobin level (mg/dl)	δ-ALAD activity (nmole/min/ml blood)	Blood arsenic (µgm/ml)	Blood zinc (µgm/ml)
Control group	11.8 ± 0.67	7.46 ± 0.62	0.065 ± 0.01	5.53± 0.98
As pre-exposed group	8.7 ± 0.71***	5.07± 0.11***	2.56± 0.42***	3.45± 0.20***
Sulbactomax treated group	9.9 ± 0.52**	6.61± 0.81***	1.01± 0.10***	4.57± 0.44***

All data are expressed as mean± SD of each group was performed for statistical significance between control group vs. arsenic pre exposed group and arsenic exposed group vs. Sulbactomax treated group. Where ***p<0.001 (highly significant), **p<0.01(significant), *p<0.05 (significant), ^{Ns}p>0.05 (non significant).

intravenous treatment of Sulbactomax drug (ceftriaxone plus sulbactam with VRP1034) for 3 weeks, the level of hemoglobin (P<0.01) and δ-ALAD enzyme activity (P<0.001) were significantly increased in drug treated group as compared to arsenic pre-exposed group and these parameters were significantly increased and reached near to control level when compared with Sulbactomax treated group (Table 2). There was significant increase in the endogenous antioxidant enzymes activities (SOD, P<0.001; CAT, P<0.001; total thiol, P<0.001; and GSH, P<0.001) along with significant decrease MDA level (P<0.001) and myloperoxidase enzyme activity (P<0.01) in the plasma of arsenic plus Sulbactomax treated group when compared with the arsenic pre-exposed group, in that these parameters almost reached those of the control group after treatment with Sulbactomax drug for 21 days (Table 3).

The hepatic enzymes (serum glutamic oxaloacetic transaminase, serum glutamic pyruvic transaminase and alkaline phosphatase) and renal enzymes (Creatinine) were (P<0.001) significantly reduced in the plasma of the Sulbactomax treated group after 21 days treatment as compared to the arsenic pre-exposed group. These enzymatic parameters almost reacted like those of the control group after treatment with the drug (Table 4).

The levels of cytokines (TNF– α and IL-6) were (P<0.001) significantly increased in the plasma of arsenic pre-exposed group as compared with control group. These levels were found to be significantly (P<0.001) lowered in the plasma of Sulbactomax treated group after 21 days treatment as compared with arsenic pre-exposed group (Figures 1 and 2).

DISCUSSION

Arsenic (As) is a well known heavy metal that causes tissues damage, including the immune system. Arsenic is an uncoupler of mitochondrial oxidative phosphorylation that induces generation of reactive oxygen species. Various studies have reported that arsenic is immunotoxic (Fuente et al., 2002; Hornhardt et al., 2006). Arsenic metal interferes with the antigen-presenting function of splenic macrophages that is able to alter the response of IgM and IgG antibody-forming cells to sheep erythrocytes, and the proliferative response of lymphocytes to phytohaemagglutinin (Sikorski et al., 1989; Sikorski et al., 1991; Savabieasfahani

Table 3. Status of antioxidant enzymatic and free radical mediated damage parameters in pre-exposed group and Sulbactomax treated group.

Groups	SOD (nmole/min/ml plasma)	CAT (nmole/min/ml Plasma)	Total thiol (mg/dL)	GSH (mg/dL)	MDA (µmole/ml plasma)	MPO (µmole/min/ml plasma)
Control group	3.47 ± 0.22	61.93 ± 8.49	0.487 ± 0.07	7.95± 0.64	7.30 ± 0.42	8.45± 1.23
As pre-exposed group	0.985 ± 0.011***	36.31± 3.71***	0.167± 0.04***	3.28± 0.98***	11.37± 1.61***	21.56± 3.11***
Sulbactomax treated group	2.23 ± 0.10***	53.0± 6.22***	0.342± 0.01***	4.59± 1.02***	7.75±0.35***	12.99± 2.85***

All data are expressed as mean± SD of each group. Newman keuls test was performed for statistical significance between control group vs arsenic pre exposed group and arsenic exposed group vs. Sulbactomax treated group. SOD; superoxide dismutase, CAT; catalase, GSH; reduced gluathione, MDA; malonaldialdehyde, MPO; myloperoxidase. Where ***p<0.001 (highly significant), **p<0.01(significant), *p<0.05 (significant), ^{Ns}p>0.05 (non significant).

Table 4. Status of hepatic and renal enzymetic parameters in pre-exposed group and Sulbactomax treated group.

Groups	SGOT (mg/dL)	SGPT (mg/dL)	Creatinine (mg/dL)	ALP (mg/dL)
Control group	26.12 ± 2.10	32.48 ± 2.8	0.63 ± 0.03	37.95± 6.4
As pre-exposed group	72.5 ± 1.5***	86.12± 5.41***	1.98± 0.15***	113.41± 8.41***
Sulbactomax treated group	45.37± 3.12***	39.5± 3.33***	0.86± 0.23***	68.24 ± 6.09***

All data are expressed as mean± SD of each group. Newman keuls test was performed for statistical significance between control group vs. arsenic pre exposed group and arsenic exposed group vs. Sulbactomax treated group. Where ***p<0.001 (highly significant),**p<0.01(significant), p<0.05 (significant), ^{Ns}p>0.05 (non significant).

et al., 1998). The heme metabolic path way is highly susceptible to alterations induced by metal ions. So in the present investigation, the δ-aminolevulinic acid dehydratase enzyme activity and hemoglobin level were significantly (P<0.001) inhibited in the arsenic pre-exposed group as compared to control group after intraperitoneal administration of arsenic trioxide (AS_2O_3) for 21 days. The level of arsenic concentration was significantly increased along with significant decreased the zinc level in arsenic pre-exposed group as compared to control group. After intravenous administration of a novel fixed dose combination drug (ceftriaxone plus sulbactam with VRP1034) for 21 days treatment, the level of arsenic was decreased along with increased hemoglobin, zinc levels and δ-aminolevulinic acid dehydratase enzyme activity in the treated group.

Therefore, it is interpreted that arsenic metal interferes with heme synthesis path way that inhibits the δ-aminolevulinic acid dehydratase enzyme and these enzymes are responsible for the synthesis of heme. Due to decrease of δ ALAD enzyme activity, the heme synthesis path way is inhibited. Kannan and Flora (2004) suggested that the hemoglobin level and δ-aminolevulinic acid dehydratase enzyme activity were inhibited during arsenic exposure in rats. The principal biochemical mechanism in acute arsenic intoxication is the reversible combination of arsenic with susceptible sulfhydryl-containing enzymes.

Oxidative stress has also been identified as an important mechanism of arsenic toxicity (Flora and Gupta, 2005; Kitchin, 2001). Arsenic induces oxidative DNA damage and increased lipid peroxidation due to excessive generation of free radicals. A number of various studies have showed that arsenic-induced the formation of reactive oxygen and nitrogen species as well as elevated DNA oxidation. Various reports have been suggested that inorganic arsenic inhibits several of the antioxidant systems in the body, such as glutathione, glutathione peroxidase, thioredoxin reductase, and superoxide dismutase (Shen et al., 2003; Shila et al., 2005; Wu et al., 2001). In our study, reduced glutathione, total thiol levels and SOD, catalase enzyme activites were significantly reduced along with significantly increased malonaldialdehyde and myloperoxidase levels (free radical mediated damage) in arsenic

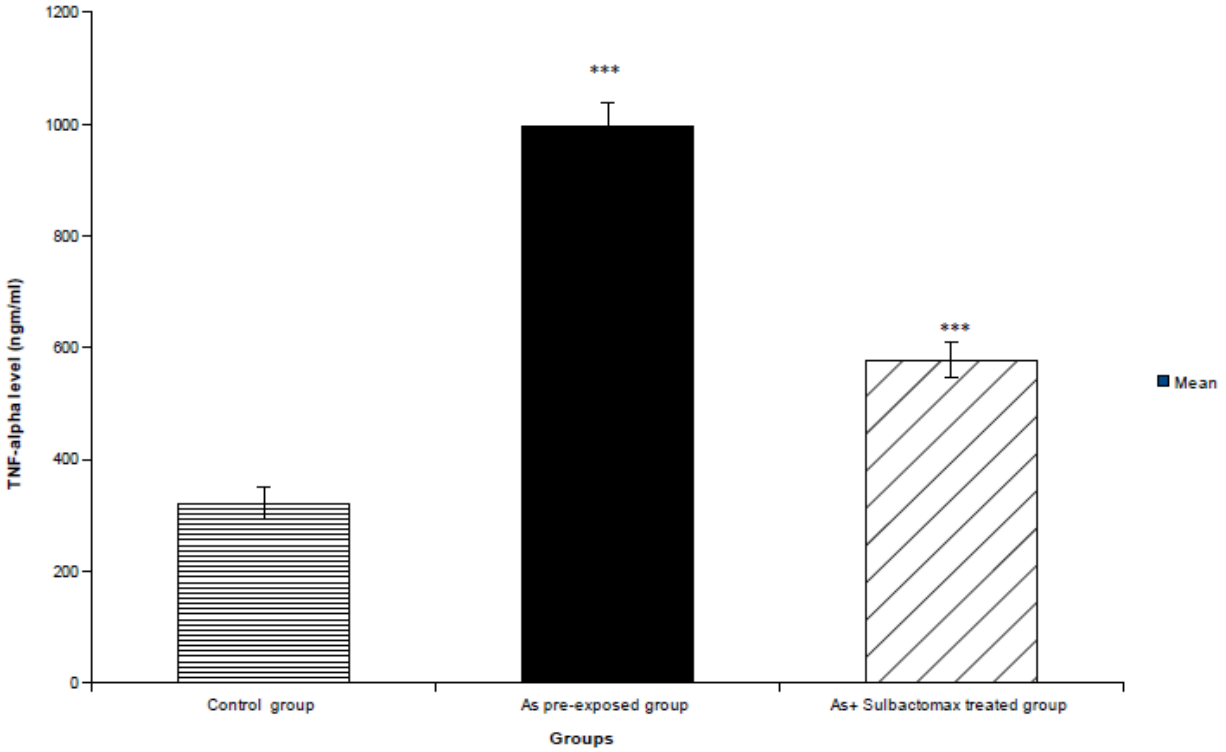

Figure 1. Status of TNF-α level in pre-exposed group and Sulbactomax treated group. Data are expressed as mean± SD of each group. Newman keuls test was performed for statistical significance between control group vs. arsenic exposed group and arsenic exposed group vs. Sulbactomax treated group. Where, ***p<0.001 (highly significant), **p<0.01(significant), *p<0.05 (significant) and ^{Ns}p>0.05 (non significant).

pre-exposed groups as compared with control group. After treatment with Sulbactomax drug, these parameters were significantly improved along with reduced free radical mediated damage levels in arsenic pre-exposed plus Sulbactomax treated group. Besides these parameters, the hepatic, renal and cytokinine (IL-6 and TNF-α) parameters were also increased in arsenic pre-exposed group as compared to control group. After treatment Sulbactomax drug for 21 days, these parameters were improved in arsenic induced plus drug treated group. Mazumdar (2005) has reported that hepatic damage occurred in experimental animals by chronic arsenic toxicity. Increased oxidative stress and cytokine response are associated with increased accumulation of collagen in the liver due to prolonged arsenic exposure. Das et al. (2005) suggested that implications of oxidative stress and hepatic cytokine (TNF-alpha and IL-6) response in the pathogenesis of hepatic collagenesis in chronic arsenic toxicity. Arsenic toxicity may cause nutritional deficit which leads to anemia and generate oxy free radicals. Thus, Sulbactomax drug may increases the antioxidant levels in the body may protect against arsenic-induced toxicity. Indeed, the administration of ascorbic acid, α-tocopherol or selenium has been shown to decrease arsenic-induced toxicity. Besides these exogenous antioxidant compounds, various chelating agents may be useful for the prevention of arsenic toxicity. Sulbactomax drug is a novel fixed dose combination of ceftriaxone plus sulbactam along with VRP1034. VRP1034 has chelating, antioxidant properties which are protected by patent.

Various studies have been reported that ceftriaxone and sulbactam individually showed the free radical scavenging property (Lapenna et al., 1995; Gunther et al., 1993). Cephalosporins are known as thioether containing class of antibiotics which are more effective in preventing the free radical-mediated oxidation of sulfhydryl groups in the antibiotics. Beside free radical scavenging property and antimicrobial effect, ceftriaxone and sulbactam individually interact with arsenic ions and other heavy metals and form complex which chelate out from sulfhydryl group of antibiotics. Combination of ceftriaxone plus sulbactam with VRP 1034 (Sulbactomax) is the most active drug which enhanced the free radical scavenging property and also enhanced the removal of heavy metal ions. Chemical vector mediated technology was provided compatibility of cephalosporin and beta lacatmase inhibitor with VRP 1034 without interfering in the pharmacokinetic property of drug component and their role was to prevent the oxidation of methionine group, thiazolidine and dihyrothiazine present in antibiotics. There was no evidence regarding a novel

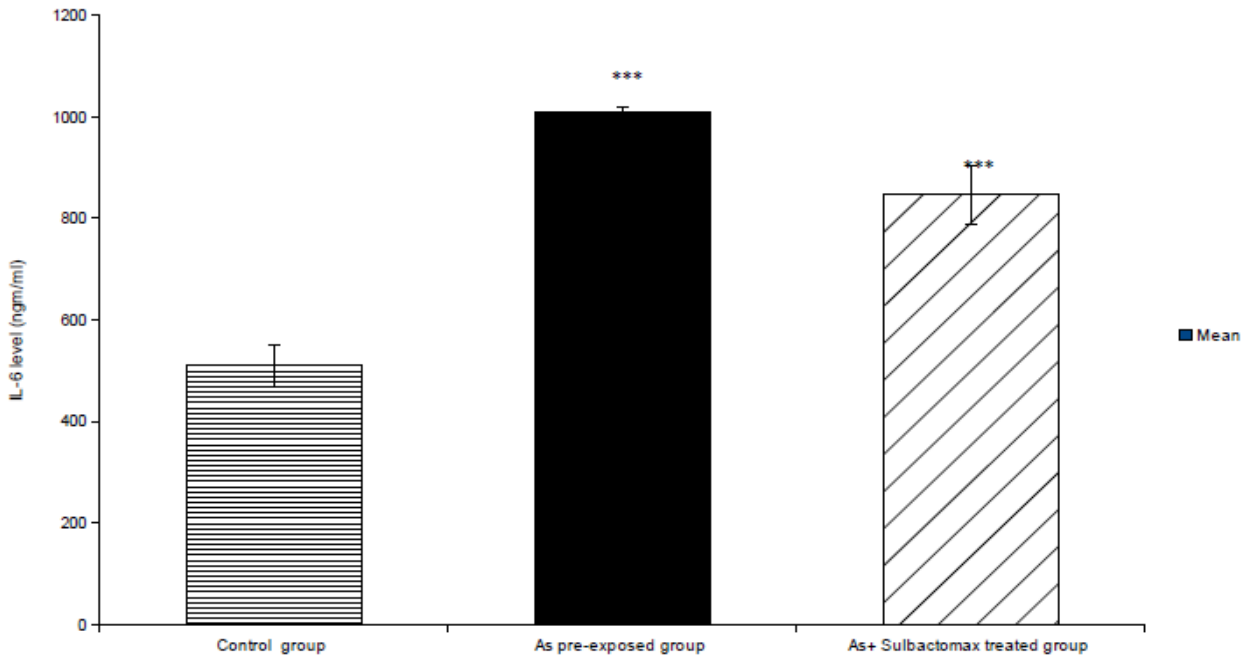

Figure 2. Status of IL-6 levels in pre-exposed group and Sulbactomax treated group. Data are expressed as mean± SD of each group. Newman keuls test was performed for statistical significance between control group vs. arsenic exposed group and arsenic exposed group vs. Sulbactomax treated group. Where ***p<0.001 (highly significant), **p<0.01(significant), *p<0.05 (significant) and ^{Ns}p>0.05 (non significant).

fixed dose combination of two antibiotic (beta lactam and betalactamase inhibitor) along with VRP1034 (Sulbactomax) has metal chelator and free radical scavenging properties. These results suggested that the novel fixed dose combination of Sulbactomax showed free radical scavenging and metal chelating properties which removes heavy metal and decreases free radical mediate damage tissue injury along with increase intracellular and extracellular antioxidant defense system and prevent hepatic and renal toxicity.

Conclusion

The conclusion of present study showed that Sulbactomax is the most and safe effective drug for the treatment of arsenic toxicity due to removal of arsenic metal along with increased δ-aminolevulinic acid dehydratase enzyme activity and also prevents free radical mediate damage tissue injury due to free radical scavenger's property and chelation ability property of drug.

ACKNOWLEDGEMENT

The authors are thankful to Management of Venus Medicine Research Centre to provide infrastructure to carry out this experiment.

REFERENCES

Aebi H (1984). Catalase. In Methods in Enzymol, Packer L (ed.). Academic Press: Orlando, FL, 125-126.
Berlin A, Schaller KH (1974). European standardized method for the determination of delta aminolevulinic acid dehydratase activity in blood. Zeit. Klin. Chem. Klin Biochem., 12: 389-390.
Das S, Santra A, Lahiri S, Mazumder DNG (2005).Implications of oxidative stress and hepatic cytokine (TNF-α and IL-6) response in the pathogenesis of hepatic collagenesis in chronic arsenic toxicity. Toxicol. Appl. Pharmacol., 204: 18-26.
Dwivedi VK, Chaudhary M, Soni A, Yadav J, Tariq A (2009). Diffusion of Sulbactomax and ceftriaxone into cerebrospinal fluid of meningitis induced rat model. Int. J. Pharmacol., 5: 307-312.
Ellman GL (1959). Tissue sulfhydrl groups. Arch. Biochem. Biophys., 82: 70-77.
Flora SJS, Bhadauria S, Pant SC, Dhaked RK (2005). Arsenic induced blood and brain oxidative stress and its response to some thiol chelators in rats. Life Sci., 77: 2324-2337.
Flora SJS, Gupta R (2005). Protective Value of Aloe vera against Some Toxic Effects of Arsenic in Rats. Phytother. Res., 19: 23-28.
Fuente HDL, Portales-pérez D, Baranda L, Barriga FD, Alanís VS, Layseca X, Amaro RG (2002).Effect of arsenic, cadmium and lead on the induction of apoptosis of normal human mononuclear cells. Clin. Exp. Immunol., 129 (1): 69-77.
Gunther MR, Mao J, Cohen MS (1993). Oxidant-scavenging activities of ampicillin and sulbactam and their effects on neutrophil functions. Antimicrob. Agents Chemother., 37(5): 950-956.
Hornhardt S, Gomolka M, Walsh L, Jung T (2006). Comparative investigations of sodium arsenite, arsenic trioxide and cadmium sulphate in combination with gamma-radiation on apoptosis, micronuclei induction and DNA damage in a human lymphoblastoid cell line. Mutat. Res., 600: 165-176.
Hu M (1994). Measurement of protein thiol groups and glutathione in plasma. Method Enzymol., 233: 380-382.
Ishak KG (1976). Mesencymal tumors of the liver. In Hepatocellular

Carcinoma (Okuda K, Rogers RL, eds.) John Wiley, New York, pp. 247-307.
Jackson R, Grainge JW (1975). Arsenic and Cancer. Can. Med. Assoc. J., 113: 396-401.
Kannan GM, Flora SJS (2004). Chronic arsenic poisoning in the rat: treatment with combined administration of succimers and an antioxidant.Ecotoxicol.Environ. Safe, 58: 37-43.
Kitchin KT (2001). Recent advances in arsenic carcinogenesis: mode of action, animal model system and methylated arsenic metabolites. Toxicol. Appl. Pharmacol., 172: 249-261.
Kurutas EB, Arican O, Sasmaz S (2005). Superoxide dismutase and myeloperoxidase activities in polymorphonuclear leukocytes in acne vulgaris. Acta Dermatoven., 14: 39-42.
Lapenna D, Cellini L, Gioiaa S De, Mezzettia A, Ciofania G (1995) Cephalosporins are scavengers of hypochlorous acid. Biochem Pharmacol., 49(9): 1249-1254.
Mamzudar DN (2005). Effect of chronic intake of arsenic- contaminated water on liver. Toxicol. Appl. Pharmacol., 206: 169-175.
Misra HP, Fridovich I (1974). The role of superoxide anion in the auto-oxidation of epinephrine and a sample assay for Super-oxide dismutase. J. Biol. Chem., 247: 3170-3175.
Ohkawa H, Ohishi N, Yagi (1979). Assay of lipid per-oxidation in animal tissue by thio barbutric acid reaction. Anal. Biochem., 95: 351-358.
Savabieasfahani M, Lochmiller RL, Rafferty DP, Sinclair JA (1998). Sensitivity of wild cotton rats (Sigmodon hispidus) to the immunotoxic effects of low-level arsenic exposure. Arch. Environ. Contam. Toxicol., 34: 289-296.
Shen ZY, Shen WY, Chen MH, Shen J, Zing Y (2003). Reactivre oxygen species and antioxidants in apoptosis of esopheal acncer cells induced by AS_2O_3. Int. J. Mol. Med., 11: 479-484.
Shila S, Subthara M, Devi MA Paneerselvam C (2005). Arsenic intoxication induced reduction of gluthione level and of the activity of releated enzymes in rat brain region: reversal by DL-alpha lipoic acid. Arch Toxicol., 208: 357-365.
Sikorski EE, Burns LA, McCoy KL, Stern M, Munson A (1991). Suppression of splenic accesory cell function in mice exposed to gallium arsenide. Toxicol. Appl. Pharmacol., 110: 143-156.
Sikorski EE, McCay JA, White KL, Bradley SG, Munson AE (1989). Immunotoxicity of the semiconductor gallium arsenide in female B6C3F1 mice. Fundam. Appl. Toxicol., 13: 843-158.
Singh S, Rana SVS (2007). Amelioration of arsenic toxicity by L-Ascorbic acid in laboratory rat. J. Environ. Biol., 28: 377-387.
Von OWF (1958). Poisoning. A Guide to Clinical Diagnoses and Treatment. W.B. Saunders, Philadelphia.
Weisberger JH, Williams GM (1975). Metabolism of chemical carcinogens. in Cancer (Becker FF, ed.). Plenum Press, Ney York, pp. 185-234.
Wu MM, Chiou HY, Wang TW, Hsuch YM, Wang IH, Chen CJ, Lee TC (2001). Association of blood arsenic level with increased reactive oxidant and decreased antioxidant capacity in human population northeastern Taiwan. Environ, Health. Prospect, 109: 1011-1017.