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On the Protection of ALP Cardiovascular Toxicity by a Novel Mixed Herbal Medicine; Role of Oxidative Stress and Cellular ATP

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ABSTRACT

Aluminum phosphide (AlP) intoxication is becoming a major concern worldwide due to its high mortality rate (30-100%) besides non-availability of effective antidote till date. The aim of present study was to determine the effect of IMOD™, a novel mixed herbal medicine on energy depletion, oxidative stress and change of electrocardiographic (ECG) parameters in the heart tissue of the rats poisoned by AlP. IMOD™ at doses of 13, 20 and 30 mg kg^{-1} was administered intraperitoneally 30 min after gavage intragastric administration of AlP (0.25 LD_{50}). Sodium bicarbonate was used as the control. After anesthesia, animals were rapidly connected to PowerLab® device for monitoring of ECG, blood pressure and heart rate for 180 min. At 24 h post treatment, rats were decapitated and hearts removed for evaluation of oxidative stress markers and production of energy. AlP ingestion led to significant heart rate and blood pressure decrement as well as ST variation and shortening of PR interval. Administration of IMOD™ normalized AlP-disturbed cardiovascular parameters. IMOD™ also restored heart energy via re-establishment of cellular ATP pool and elimination of oxidative stress markers. These findings confirm the potential benefits of IMOD™ as an effective treatment for acute AlP poisoning that remain to be trialed clinically.

Key words: IMOD™, oxidative stress, ATP phosphorylation, aluminium phosphide, mitochondrial toxicity, sodium bicarbonate

INTRODUCTION

Aluminum phosphide (AlP) is a fumigant pesticide which is used in agriculture for bulk grain preservation presented as pellet, tablet, blister pack, sachet and as dust. AlP exposure to water, moisture or hydrochloric acid of the stomach releases phosphine gas which is highly toxic.

Two main reasons of AlP poisoning in human, respectively ingestion for suicidal attempts and accidental exposure by farmers. Ingestion of AlP mostly results in death in almost all cases especially through refractory hypotension and heart failure. The other common symptoms of toxicity are dysrhythmias, ventricular dysfunction, metabolic acidosis, hypoglycemia and Acute Respiratory Distress Syndrome (ARDS) (Anand *et al*., 2011). Although the exact mechanism of phosphine toxicity is yet to be found, it is believed that oxidative stress plays a pivotal role in phosphine-induced cellular toxicity (Mehrpour *et al*., 2012). It has been shown that phosphine impairs mitochondrial electron transport chain through inhibition of complex IV, the mechanism in which leads to transport of ETC electrons to oxygen molecules and formation of superoxide anions (Dua and Gill, 2004). In addition, phosphine impairs the balance between activities of

enzymes involved in enzymatic antioxidant system and facilitates overproduction of invasive and stable hydrogen peroxide radicals from superoxide anions, resultant damage to cellular structures and subsequently cell death (Yim *et al*., 1998).

Despite large number of clinical and experimental trials that have been conducted for management of AlP poisoning including administration of trimethazidine (Duenas *et al*., 1999), atropine plus pralidoxime (Mittra *et al*., 2001), N-acetylcysteine (NAC) and L-NG-nitroarginine methyl ester (L-NAME) (Azad *et al*., 2001), ascorbic acid plus methylene blue (Soltaninejad *et al*., 2011) and Mg-carrying nanoparticle (Baeeri *et al*., 2013), none of them could completely overcome complications of AlP intoxication and the main problem is still unsolved.

IMOD™ is a multi-herbal formulation containing extracts from *Tanacetum vulgare*, *Urtica dioica* and *Rosa canina* that enriched by selenium, urea and electromagnetic field. The drug is patented is the US and Europe as anti-HIV medicine (Novitsky *et al*., 2007).

IMOD™ was found to possess protective effects against oxidative stress in a variety of clinical and experimental studies (Mohammadirad *et al*., 2011) including immune-based diabetes (Mohseni-Salehi-Monfared *et al*., 2010), Inflammatory Bowel Disease (IBD) (Baghaei *et al*., 2010), hepatotoxicity (Rahimi *et al*., 2012), hypercholesterolemia (Azonov *et al*., 2008), polycystic ovary syndrome (PCOS) (Rezvanfar *et al*., 2011) and Oral Lichen Planus (OLP) (Agha-Hosseini *et al*., 2011).

Since, previous studies failed to address a standard approach to management of metal phosphides poisoning and based on proved benefits of IMOD™ in similar conditions, we aimed to investigate the potential optimistic effects of IMOD™ in an experimental model of AlP poisoning.

MATERIALS AND METHODS

Chemicals: Adenosine diphosphate (ADP) sodium salt, adenosine triphosphate (ATP) disodium salt, 1,1,3,3-tetrahydroxypropane (MDA), methanol (High-Performance Liquid Chromatography [HPLC] grade), acetic acid, $FeCl_3$-$6H_2O$, sodium sulfate, trichloroacetic acid (TCA), potassium hydroxide, pentobarbital, diethyl ether, tetrabutyl ammonium hydroxide (TBAHS), n-butanol, 2-thiobarbituric Acid (TBA), KH_2PO_4 (analytical grade), 2,4,6-tripyridyl-s-triazine (TPTZ) from Sigma-Aldrich Chemie (Munich, Germany), AlP from Samiran pesticide formulating Co. (Tehran, Iran), SUPELCOSIL™ LC-18-T HPLC column from Supelco (Antrim, UK) and IMOD™ from Rose Pharmed Research Group (Tehran, Iran) were used.

Animals: Male Wistar rats weighing between 200-220 g were housed single in standard cages in a controlled room temperature and humidity and light-dark cycle. Animals were fed a normal laboratory diet. Rats were deprived of food for 12 h prior to experiments but were allowed free access to tap water. Experiments followed a protocol that was approved by the institute review board and the ethics committee. Fifty-four rats were randomly assigned to nine groups of each six animals. AlP was dispersed in almond oil and administered by gavage. IMOD™ administered intraperitoneally (ip) at doses of 13, 20 and 30 mg kg^{-1}. Prior administration, IMOD™ was diluted 1:9 in saline and injected intraperitoneally. Doses of IMOD™ were picked from our previous study (Baghaei *et al*., 2010). Sodium bicarbonate (Bicarb) was administered intraperitoneally at a dose of 2 mEq kg^{-1}. Pentobarbital was used for induction of anesthesia.

Animals which received almond oil only was named as Almond oil; aluminum phosphide as AlP; AlP+Bicarb as Bicarb; AlP+IMOD (13 mg kg^{-1}) as IMOD-13; AlP+IMOD (20 mg kg^{-1}) as IMOD-20; AlP+IMOD (30 mg kg^{-1}) as IMOD-30; AlP+IMOD (13 mg kg^{-1})+Bicarb as IMOD-13+Bicarb; AlP+IMOD (20 mg kg^{-1})+Bicarb as IMOD-20+Bicarb and AlP+IMOD (30 mg kg^{-1})+Bicarb as IMOD-30+Bicarb.

After 12 h period of fasting, AlP was administered by gavage to all animals except for Almond oil group. Subsequent to 30 min, the animals underwent anesthesia by 50 mg kg^{-1} intraperitoneal injection of pentobarbital, followed by maintenance doses of 25 mg kg^{-1} every 30 min to keep them up anesthetized through cardiovascular examination procedure. Afterward, rats were immediately connected to PowerLab® device through subcutaneous electrocardiographic electrodes and a tail cuff to monitor electrocardiogram (ECG), Blood Pressure (BP) and Heart Rate (HR). All treatments were performed after 30 min to all groups except for Almond oil and AlP group. At 24 h post treatments, animals were decapitated and the heart and blood were removed. Plasma was separated from blood and frozen at -20°C. The heart was rinsed in ice-cold saline, immediately frozen in liquid nitrogen and stored at -80°C. Before analysis of oxidative stress parameters, the heart was homogenized in suitable buffer using a tissue homogenizer at 4°C. The homogenates were centrifuged at 12000 g for 10 min and supernatant was used for analyses.

Determination of AlP LD_{50}: AlP was administered by intragastric gavage of single 8, 10, 12 and 14 mg kg^{-1} doses to 4 groups of each containing 4 rats weighing 200-220 g. From the number of dead animals after 24 h and using Arithmetical method of Karber's, the calculated LD50 equals to 12 mg kg^{-1} (Turner, 1965).

ECG, HR and BP: Subcutaneous electrodes were attached to right arm, left arm and left leg to obtain bipolar ECG leads. Data collection was continued for 180 min and 13 ECG parameters including PR interval, QRS interval and ST height were obtained. Data were analyzed by LabChart® 7 software. Simultaneously, a cuff was connected to the tail of rats in order to record the HR and systolic BP. Systolic BP was measured at 5-min intervals.

Measurement of Ferric reducing antioxidant power (FRAP): Total anti-oxidant capacity of heart tissue was evaluated by measuring the ability to reduce Fe3+ to Fe2+. Interaction of TPTZ with Fe2+ results in formation of a blue color with a maximum absorbance at 593 nm as set up in our lab and described previously (Astaneie *et al*., 2005). Data were expressed as μM ferric ions reduced to ferrous form per g of tissue.

Measurement of thiobarbituric acid-reactive substance (TBARS): Lipid peroxidation occurs because of toxic stress and can lead to serious cellular damages. MDA, the main byproduct of PUFs oxidation, is known as a biomarker of lipid peroxidation and its concentration was assessed in cardiac cells using thiobarbituric acid-reactive substance (TBARS) assay. The data was reported as μM g^{-1} of tissue (Amini-Shirazi *et al*., 2009).

Measurement of cardiac ADP and ATP: Isolated hearts were minced and homogenized in 1 mL of 6% ice-cold TCA. The homogenate was centrifuged at 12000 g for 10 min at 4°C. The supernatant was neutralized by 4 molar KOH to pH 6.5, filtered through a Millipore filter (pore size 0.45 μm) and used to determine the quantity of ATP and ADP (μg mL^{-1} mg^{-1} of tissue) using Ion-pair HPLC (Hosseini *et al*., 2010).

Statistical analysis: Data were expressed as Mean±Standard Error of the Mean (SEM). One-way analysis of variance (ANOVA) followed by Tukey's post hoc for multiple comparisons were used. $p<0.05$ were considered statistically significant.

RESULTS

Heart rate (beat min^{-1}): Exposure to AlP resulted in significant HR decline through all time intervals when compared to almond oil ($p<0.05$). Administration of IMOD™ at all doses significantly increased HR as compared to AlP ($p<0.05$). Combination of IMOD™ and bicarb could remarkably augment HR as compared to AlP ($p<0.05$). Bicarb was also able to elevate HR in comparison with AlP ($p<0.05$, Table 1).

Blood pressure (mm Hg): BP was drastically decreased in AlP group through 60-180 min interval compared to almond oil group ($p<0.05$). Administration of IMOD™ at doses of 20 and 30 demonstrated a remarkable increase in BP compared to AlP group ($p<0.05$) while IMOD-13 did not show significant increase. Combination of IMOD™ and Bicarb at all doses raised BP in comparison to AlP ($p<0.05$). Bicarb group showed the elevation only in 120-180 section ($p<0.05$, Table 2).

Table 1: Heart rate

	Time (min)					
	0-30	30-60	60-90	90-120	120-150	150-180
Almond oil	396.12±1.830	409.30±5.164	387.840±2.695	393.340±2.258	390.900±3.728	411.02±2.20
ALP	334.50±14.074^{a}	287.93±4.79^{a}	267.316±0.75^{a}	281.866±4.078^{a}	192.580±16.17^{a}	167.41±7.001^{a}
Bicarb	308.14±4.548^{a}	331.90±2.895^{a,b}	321.480±7.98^{a,b}	286.760±8.377^{a}	293.060±6.72^{a,b}	305.62±4.60^{a,b}
IMOD-13	455.35±2.050^{a,b}	487.45±7.96^{a,b}	523.070±2.83^{a,b}	545.275±2.944^{a,b}	558.200±3.239^{a,b}	460.00±83.959^{b}
IMOD-13+Bicarb	244.52±4.778^{a,b}	242.42±2.38^{a,b}	265.860±11.14^{a}	248.420±7.687^{a,b}	389.580±8.500^{b}	408.40±3.658^{b}
IMOD-20	322.78±2.942^{a}	349.88±8.55^{a,b}	365.400±9.388^{b}	352.880±2.017^{a,b}	364.940±6.048^{b}	372.90±4.319^{b}
IMOD-20+Bicarb	351.26±8.071^{a}	380.76±8.95^{a,b}	431.100±6.234^{a,b}	456.283±1.996^{a,b}	508.266±8.43^{a,b}	543.30±2.804^{a,b}
IMOD-30	368.68±2.848^{b}	354.52±2.33^{a,b}	350.300±3.787^{a,b}	373.720±11.106^{b}	500.360±12.83^{a,b}	549.60±4.376^{a,b}
IMOD-30+Bicarb	350.74±3.530^{a}	396.64±5.36^{b}	413.180±1.932^{b}	415.900±0.459^{b}	409.520±0.990^{b}	411.48±0.99^{b}

Data are Mean±SEM. Control group received almond oil alone; AlP group (0.25 LD50) received only aluminium phosphide; Bicarb group received AlP (0.25 LD_{50})+$NaHCO_3$ (2 mEq kg^{-1}); IMOD 13, 20 and 30 groups received AlP (0.25 LD_{50})+IMOD (0.025, 0.05 and 0.1 LD_{50}), IMOD 13, 20 and 30+Bicarb groups received AlP (0.25 LD_{50})+IMOD (0.025, 0.05, and 0.1 LD_{50}) + $NaHCO_3$(2 mEq kg^{-1}). ^{a}Significantly different from control group at $p<0.05$

Table 2: Blood pressure

	Time (min)					
	0-30	30-60	60-90	90-120	120-150	150-180
Almond oil	90.9770±1.082	85.7150±2.429	95.520±1.972	103.30000±1.215	106.190±2.526	100.800±1.175
ALP	92.3800±2.15	77.9200±4.239	62.462±2.613^{a}	53.85000±0.971^{a}	56.392±1.1680^{a}	57.525±1.854^{a}
Bicarb	69.3940±10.505	64.2760±12.257	59.934±6.581^{a}	74.98000±14.178^{a}	77.708±7.7683^{a,b}	95.666±4.845^{b}
IMOD-13	97.1300±7.898	123.7300±10.039^{a,b}	157.782±3.605^{a,b}	164.03000±3.161^{a,b}	159.000±2.135^{a,b}	77.860±16.749
IMOD-13 + Bicarb	64.3375±2.1395	61.5550±5.198^{b}	85.055±5.608	101.14250±1.698^{b}	99.657±4.321^{b}	94.312±2.558^{b}
IMOD-20	126.0560±3.654^{a,b}	123.3860±2.090^{a,b}	149.932±3.103^{a,b}	146.80800±2.775^{a,b}	150.702±2.848^{a,b}	156.320±2.003^{a,b}
IMOD-20 + Bicarb	86.1050±5.649	102.9150±5.505^{a,b}	125.205±4.840^{a,b}	143.10333±1.238^{a,b}	142.891±2.226^{a,b}	135.170±2.957^{a,b}
IMOD-30	71.0870±8.116	77.2625±4.191	82.555±5.129	85.9100±2.053^{b}	105.632±2.952^{b}	116.240±3.579^{b}
IMOD-30+Bicarb	68.4020±6.5509	86.814±3.1452	89.944±5.0339^{b}	87.47800±3.424^{b}	90.984±2.7005^{b}	84.826±3.074^{b}

Data is Mean±SEM. Control group received almond oil alone, AlP group (0.25 LD_{50}) received only aluminium phosphide, Bicarb group received AlP (0.25 LD_{50})+$NaHCO_3$ (2 mEq kg^{-1}); IMOD 13, 20 and 30 groups received AlP (0.25 LD_{50})+IMOD (0.025, 0.05 and 0.1 LD_{50}), IMOD 13, 20 and 30+Bicarb groups received AlP (0.25 LD_{50})+IMOD (0.025, 0.05 and 0.1 LD_{50})+$NaHCO_3$ (2 mEq kg^{-1}). ^{a}Significantly different from control group at $p<0.05$

PR interval (sec): Administration of AlP led to significant increase in PR interval in all time sections compared with almond oil ($p<0.05$). IMOD-13, IMOD-30, IMOD-13+Bicarb and IMOD-20+Bicarb groups showed notable decrease in PR interval compared to AlP ($p<0.05$) while none of IMOD-20, IMOD-30+ Bicarb nor Bicarb group showed significant difference to the Almond oil group (Table 3 and Fig. 1).

QRS interval (sec): Significant increase in QRS interval is evident in AlP group through 30-120 section compared to almond oil group ($p<0.05$). IMOD™ administration at all doses decreased QRS interval compared with AlP group ($p<0.05$). Similar increases were observed in IMOD-13+Bicarb, IMOD-20+Bicarb, IMOD-30+Bicarb and Bicarb groups ($p<0.05$). IMOD-20 could not correct AlP-induced QRS prolongation (Table 4 and Fig. 1).

Table 3: PR interval (sec)

	Time (min)					
	0-30	30-60	60-90	90-120	120-150	150-180
Almond oil	0.042±0.0002	0.042±0.0009	0.043±0.0004	0.042±0.0005	0.043±0.0008	0.038±0.0005
ALP	0.050±0.0021[a]	0.051±0.002[a]	0.056±0.0017[a]	0.050±0.002[a]	0.058±0.002[a]	0.053±0.0011[a]
Bicarb	0.048±0.0006	0.049±0.0005	0.040±0.0002[a,b]	0.049±0.0006[a]	0.050±0.0006	0.050±0.0005[a]
IMOD-13	0.042±0.001[b]	0.037±0.0014[b]	0.041±0.0005[b]	0.037±0.0004[b]	0.036±0.0003[b]	0.043±0.005[b]
IMOD-13+Bicarb	0.045±0.0007	0.045±0.0005	0.045±0.0005[b]	0.046±0.0011	0.043±0.001[b]	0.041±0.0001[b]
IMOD-20	0.045±0.0005	0.053±0.002[a,b]	0.054±0.0017[a]	0.059±0.0006[a,b]	0.056±0.002[a]	0.054±0.0006[a]
IMOD-20+Bicarb	0.049±0.0017[a]	0.049±0.0017	0.044±0.0008[b]	0.042±0.0003[b]	0.042±0.003[b]	0.038±0.0002[b]
IMOD-30	0.048±0.002	0.043±0.0019	0.042±0.0003[b]	0.042±0.001[b]	0.040±0.0007[b]	0.039±0.0007[b]
IMOD-30+Bicarb	0.051±0.0008[a]	0.052±0.0006[a]	0.052±0.0002[a]	0.052±0.00004[a]	0.053±0.0001[a]	0.053±0.0003[a]

Data is Mean±SEM. Control group received almond oil alone, AlP group (0.25 LD_{50}) received only aluminium phosphide, Bicarb group received AlP (0.25 LD_{50})+$NaHCO_3$ (2 mEq kg^{-1}), IMOD 13, 20 and 30 groups received AlP (0.25 LD_{50})+IMOD (0.025, 0.05 and 0.1 LD_{50}), IMOD 13, 20 and 30+Bicarb groups received AlP (0.25 LD_{50})+IMOD (0.025, 0.05 and 0.1 LD_{50})+$NaHCO_3$ (2 mEq kg^{-1}). [a]Significantly different from control group at $p<0.05$

Table 4: QRS interval (sec)

	Time (min)					
	0-30	30-60	60-90	90-120	120-150	150-180
Almond oil	0.016±0.0002	0.016±0.0002	0.016±0.0002	0.016±0.0002	0.016±0.0002	0.017±0.00
ALP	0.016±0.0009	0.019±0.0002[a]	0.019±0.0001[a]	0.020±0.001[a]	0.013±0.002	0.013±0.001
Bicarb	0.013±0.0002[b]	0.013±0.00[a,b]	0.013±0.00[a,b]	0.013±0.0002[a,b]	0.013±0.00	0.013±0.0002[a]
IMOD-13	0.017±0.0006	0.017±0.001	0.015±0.0002[b]	0.017±0.0004	0.019±0.0006[b]	0.025±0.001[a,b]
IMOD-13+Bicarb	0.015±0.0002	0.015±0.00[b]	0.015±0.00[a,b]	0.015±0.00[b]	0.015±0.00	0.014±0.0002
IMOD-20	0.014±0.0000	0.014±0.00[b]	0.014±0.00[a,b]	0.014±0.00[b]	0.014±0.00	0.014±0.00
IMOD-20+Bicarb	0.017±0.0003	0.017±0.0001	0.017±0.0002[b]	0.017±0.0002	0.020±0.001[b]	0.020±0.0001[b]
IMOD-30	0.018±0.0010	0.019±0.001[a,b]	0.017±0.0002[a,b]	0.016±0.0002[b]	0.020±0.002[b]	0.028±0.0007[a,b]
IMOD-30+Bicarb	0.015±0.0002	0.016±0.0002	0.015±0.0003[b]	0.015±0.0002[b]	0.010±0.0003	0.017±0.0002[b]

Data is Mean±SEM of six animals in each group. Control group received almond oil alone, AlP group (0.25 LD_{50}) received only aluminium phosphide, Bicarb group received AlP (0.25 LD_{50})+$NaHCO_3$ (2 mEq kg^{-1}), IMOD 13, 20 and 30 groups received AlP (0.25 LD_{50})+IMOD (0.025, 0.05 and 0.1 LD_{50}), IMOD 13, 20 and 30+Bicarb groups received AlP (0.25 LD_{50})+IMOD (0.025, 0.05 and 0.1 LD_{50})+$NaHCO_3$ (2 mEq kg^{-1}), [a]Significantly different from control group at $p<0.05$

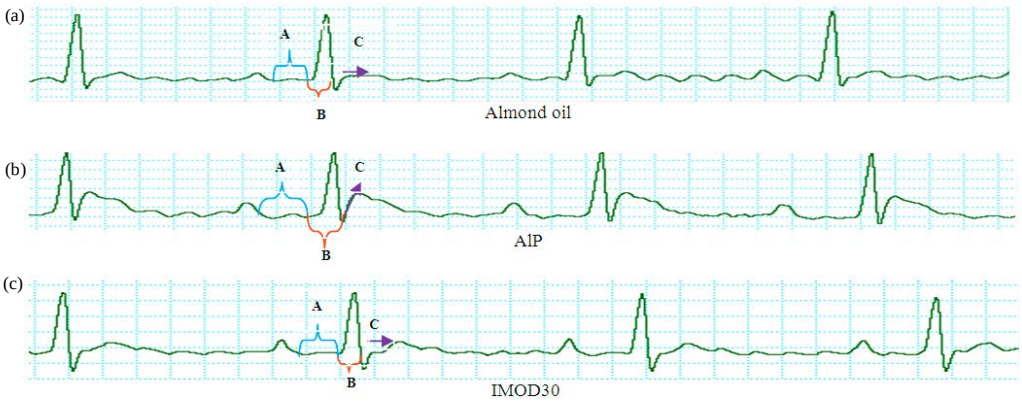

Fig. 1(a-c): Changes in ECG parameters (a) PR interval, (b) QRS interval and (c) ST height

Table 5: ST height

	Time (min)					
	0-30	30-60	60-90	90-120	120-150	150-180
Almond oil	0.0366±0.001	0.028±0.002	0.0430±0.003	0.032±0.003	0.029±0.003	0.0190±0.002
ALP	0.004±0.006[a]	-0.008±0.001[a]	-0.0130±0.001[a]	-0.045±0.027[a]	0.150±0.403	0.0420±0.265
Bicarb	0.173±0.002[a,b]	0.166±0.003[a,b]	0.1680±0.004[a,b]	0.160±0.001[a,b]	0.153±0.002	0.1440±0.002
IMOD-13	0.021±0.007	0.013±0.008[b]	0.0310±0.003[b]	0.013±0.003[b]	-0.010±0.006	-0.0550±0.024
IMOD-13+Bicarb	0.127±0.0006[a,b]	0.120±0.001[a,b]	0.1120±0.003[a,b]	0.119±0.006[a,b]	0.085±0.004	0.0720±0.0029
IMOD-20	0.192±0.001[a,b]	0.197±0.001[a,b]	0.2002±0.002[a,b]	0.204±0.003[a,b]	0.205±0.0008	0.1990±0.003
IMOD-20+Bicarb	0.044±0.002[b]	0.036±0.005[b]	0.0370±0.003[b]	0.041±0.005[b]	0.040±0.005	0.0197±0.007
IMOD-30	0.004±0.003[a]	0.003±0.001[a]	0.0040±0.002[a,b]	0.007±0.001	0.040±0.020	-0.0110±0.004
IMOD-30+Bicarb	0.060±0.003[a,b]	0.046±0.004[b]	0.0320±0.002[b]	0.026±0.00[b]	0.017±0.001	0.0170±0.00

Data is Mean±SEM. Control group received almond oil alone, AlP group (0.25 LD_{50}) received only aluminium phosphide; Bicarb group received AlP (0.25 LD_{50})+$NaHCO_3$ (2 mEq kg^{-1}), IMOD 13, 20 and 30 groups received AlP (0.25 LD_{50})+IMOD (0.025, 0.05 and 0.1 LD_{50}); IMOD 13, 20 and 30+ Bicarb groups received AlP (0.25 LD_{50}) +IMOD (0.025, 0.05 and 0.1 LD_{50})+$NaHCO_3$ (2 mEq kg^{-1}). [a]Significantly different from control group at $p<0.05$, [b]Significantly different from AlP group at $p<0.05$

ST height: AlP gavage caused significant ST height depression in first 120 min as compared to Almond oil group ($p<0.05$). Remarkable rise in ST height was observed in IMOD-13 and IMOD-20 groups compared with AlP ($p<0.05$). IMOD™ plus bicarb at all doses increased ST depression when compared to AlP ($p<0.05$). Bicarb increased ST height in comparison to AlP ($p<0.05$, Table 5 and Fig. 1).

TBARS: AlP group showed significant higher values than Almond oil group ($p<0.05$). Bicarb administration, alone or in combination with 3 doses of IMOD™, significantly reduced TBARS in heart tissue compared to AlP ($p<0.05$). However, none of IMOD-13, IMOD-20 and IMOD-30 groups showed significant reduction of TBARS values as compared to AlP (Fig. 2).

FRAP: AlP exposure led to significant decreased values comparing to almond oil group ($p<0.05$). All treatment groups demonstrated noteworthy increase in tissue antioxidant power when compared to AlP ($p<0.05$, Fig. 3).

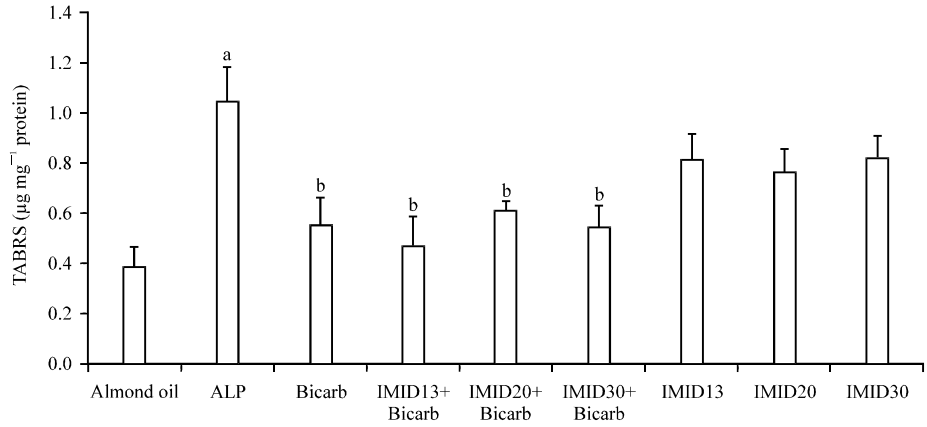

Fig. 2: Lipid peroxidation as TBARS in heart. Data are Mean±SEM. Control group received almond oil alone; AlP group (0.25 LD_{50}) received only aluminium phosphide; Bicarb group received AlP (0.25 LD_{50})+$NaHCO_3$ (2 mEq kg^{-1}); IMOD 13, 20 and 30 groups received AlP (0.25 LD_{50})+IMOD (0.025, 0.05 and 0.1 LD_{50});IMOD 13, 20 and 30+Bicarb groups received AlP (0.25 LD_{50})+IMOD (0.025, 0.05 and 0.1 LD_{50})+NaHCO3 (2 mEq kg^{-1}). [a]Significantly different from control group at $p<0.05$. [b]Significantly different from AlP group at $p<0.05$

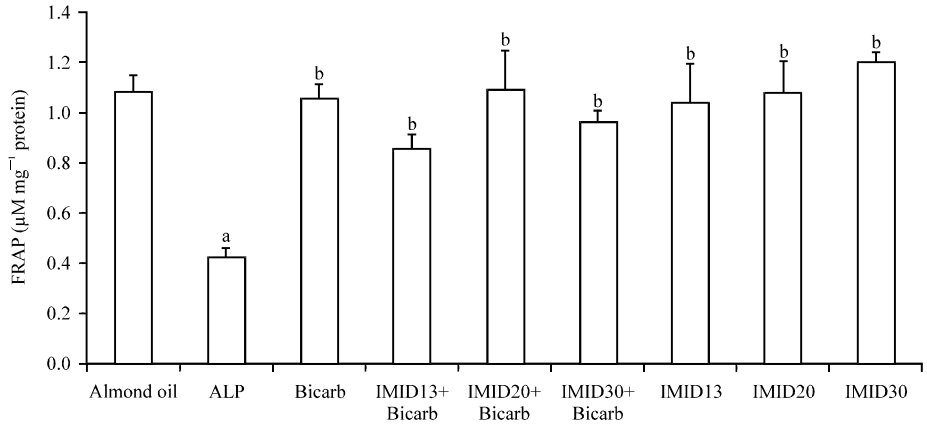

Fig. 3: Ferric reducing anti-oxidant power of heart. Data are Mean±SEM. Control group received almond oil alone; AlP group (0.25 LD_{50}) received only aluminium phosphide; Bicarb group received AlP (0.25 LD_{50})+NaHCO3 (2 mEq kg^{-1}); IMOD 13, 20 and 30 groups received AlP (0.25 LD_{50})+IMOD (0.025, 0.05 and 0.1 LD_{50});IMOD 13, 20 and 30+Bicarb groups received AlP (0.25 LD_{50})+IMOD (0.025, 0.05 and 0.1 LD_{50})+NaHCO3 (2 mEq kg^{-1}). [a]Significantly different from control group at $p<0.05$, [b]Significantly different from AlP group at $p<0.05$

Cardiac energy as ATP/ADP: Administration of AlP caused significant reduction in cellular ATP level (increase in ADP/ATP ratio) compared to almond oil ($p<0.05$). Administration of IMOD™ could increase cellular ATP levels in a dose-dependent manner in comparison to AlP ($p<0.05$). IMOD™ plus Bicarb and Bicarb groups were also able to enhance cellular ATP values when compared to AlP ($p<0.05$, Fig. 4).

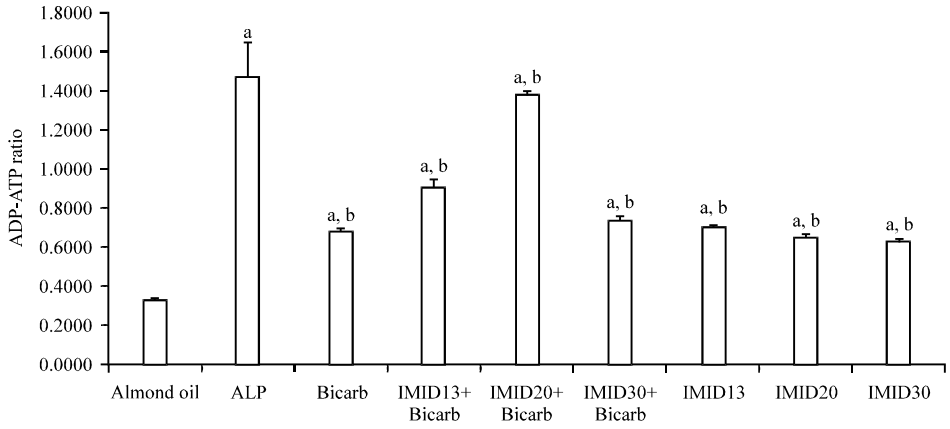

Fig. 4: ADP/ATP ratio in heart tissue. Data are Mean±SEM. Control group received almond oil alone; AlP group (0.25 LD_{50}) received only aluminium phosphide; Bicarb group received AlP (0.25 LD_{50})+NaHCO3 (2 mEq kg^{-1}); IMOD 13, 20 and 30 groups received AlP (0.25 LD_{50})+IMOD (0.025, 0.05 and 0.1 LD_{50});IMOD 13, 20 and 30+Bicarb groups received AlP (0.25 LD_{50})+IMOD (0.025, 0.05 and 0.1 LD_{50})+NaHCO3 (2 mEq kg^{-1}). [a]Significantly different from control group at $p<0.05$, [b]Significantly different from AlP group at $p<0.05$

DISCUSSION

This study set out with the aim of assessing the positive role of IMOD™ in ALP poisoning through protection of cardiovascular system, prevention of oxidative stress and restoration of cellular ATP reserve.

Cardiac toxicity remains the major cause of mortality by AlP poisoning, where refractory hypotension and heart failure occur in most cases. Other reported cardiovascular abnormalities include tachycardia, bradycardia, atrial flutter and fibrillation, ST and T wave changes (Mehrpour *et al*., 2012). In our study, simultaneous administration of IMOD™ alleviated AlP-induced hypotension and bradycardia.

PR interval is one of electrical conduction indicators in the heart which represents the atrial impulse through the AV node to right and left bundle branches. Prolonged PR interval in AlP group indicates a conduction delay due to heart block or ischemia related tissue injury (Oh *et al*., 2009). Co-administration of IMOD™ and sodium bicarbonate could successfully lessen this disorder.

QRS complex which is next to P wave in ECG, tracks depolarization of ventricles (Kashani and Barold, 2005). Widened QRS complex in AlP group is consistent with right or left Bundle Branch Block (BBB). IMOD™, alone or in combination with Bicarb could normalize QRS complex width and prevent conduction block induced by AlP.

ST segment is indicator of ending ventricular depolarization and beginning of repolarization. Both elevation and depression in ST height in AlP group are results of myocardial damage (Sandau and Smith, 2009). IMOD™ was found to mitigate ST changes in a dose-dependent manner.

Phosphine is a potent inhibitor of Electron Transport Chain (ETC). *In vitro* studies showed that this inhibition occurs at complex IV mitochondrial (cytochrome c oxidase) level (Dua and Gill, 2004). The former outcome of such inhibition is decline in ATP production (rise in ADP/ATP ratio) which is evident in AlP group as compared to Almond oil. Interestingly administration of IMOD™ at all doses made a significant increase in ATP production that could be due to removal of inhibition or

increment in activity of not inhibited complexes. The latter consequence of complex IV inhibition is production of Reactive Oxygen Species (ROS) via transport of ETC electrons to oxygen molecules, formation of superoxide anions and subsequent production of more stable and invasive ROS. Accumulation of ROS leads to oxidation of cellular macromolecules and ultimately cell death. Lipid peroxidation is a well-established marker of oxidative stress, often measured by TBARS assay. As expected, ingestion of AlP caused remarkable increase in lipid peroxidation. Although combination of IMOD™ and Bicarb successfully reduced amount of lipid peroxidation products, IMOD-only groups did not exhibit such effect.

FRAP assay is a suitable method for measurement of antioxidant power of body tissues and fluids. As expected, IMOD™ was able to increase heart tissue antioxidant levels via free radical scavenging potential so that FRAP level in IMOD-30 group was even higher than sham.

Taken together, these findings support protective cardiovascular effect of IMOD™ in an experimental model of AlP poisoning. Hence, further study investigating clinical safety and efficacy of IMOD™ on AlP poisoning would be very interesting.

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