reserves have estimated in 12 million tonnes of ore at an average grade of 1.16% REE with average thickness of 113 m. This includes 136,800 tonnes of REE at an average grade of 1.14% and 3200 tonnes of HREE at an average grade of 0.02%. The explored area is also estimated to contain 10,000 tonnes of Nb_2O_5 at an average grade of 0.08% and 3600 tonnes of Th at an average grade of 0.03%. In addition, the area also shows significant concentration of Ba (average 26,560 ppm), Sr (4960 ppm) and V (467 ppm). These results substantiate the fact that Ambadongar carbonatite complex is a potential target for locating large resources for REE. In addition, Nb, Th and associated elements may be useful by-products. Potential of REE and Nb of Ambadongar area can be compared with the Iron Hill (Powderhorn) carbonatite complex, Colorado, USA[8].

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B. NAGABHUSHANAM[1,*]
S. DURAI RAJU[2]
K. L. MUNDRA[3]
S. D. RAI[1]
R. K. PUROHIT[1]
M. B. VERMA[1]
L. K. NANDA[1]

[1]*Atomic Minerals Directorate for Exploration and Research, Hyderabad 500 016, India*
[2]*Atomic Minerals Directorate for Exploration and Research, Beach Sand and Offshore Investigations, Thiruvananthapuram 695 012, India*
[3]*Atomic Minerals Directorate for Exploration and Research, Western Region, Jaipur 302 033, India*
**For correspondence*
e-mail: bnagabhushanam.amd@gov.in

Chronic flubendiamide exposure induces oxidative stress in water buffalo (*Bubalus bubalis*) calves

Flubendiamide is a recently introduced, fast-acting insecticide with an excellent residual effect[1]. In an unpublished study, it was reported not to cause any genotoxic, carcinogenic and neurotoxic effects in mammals. However, recent studies suggest that it is toxic for fish[2] and Chinese tiger frog[3]. Alterations in leukogram[4], erythrocytic indices[5] and aspartic acid concentration in cerebrospinal fluid[6] in water buffalo following its chronic exposure to flubendiamide have also been recorded in our laboratory.

The knowledge about toxicopathology of flubendiamide in mammals is limited at present. Recently, oxidative stress has been reported as an important biomarker of flubendiamide toxicity in soil-dwelling bacteria[7] and in a freshwater invertebrate, *Daphnia magna*[8]. The present study, therefore, aimed to examine changes in oxidative stress indices in blood of buffalo calves exposed to flubendiamide.

Eight healthy, 8–12-month-old, male water buffalo calves (*Bubalus bubalis*) with body weight between 120 and 180 kg, were provided a balanced ration, dewormed and acclimatized for two weeks at experimental animal shed of the department. The experiment was conducted after approval from the University Animal Ethics Committee. The animals were divided into two equal groups. Group I (healthy control) did not receive any treatment and group II animals received flubendiamide (Fame, Bayer Cropscience Limited, Gujarat) @ 0.024 mg/kg/day orally for 90 consecutive days. Blood samples were collected on 0, 30th, 60th and 90th days of treatment and day-30 post-treatment.

Lipid peroxides (LPO) in erythrocytes, blood glutathione (GSH) concentration, plasma total antioxidant activity (TAA) and activities of superoxide dismutase (SOD), catalase, glutathione peroxidase (GP_X), glutathione reductase, glutahione-S-transferase and glucose-6-phosphate dehydrogenase (G6PD) in erythrocytes were estimated by standard protocols as described elsewhere[9]. The data obtained were analysed by Student's *t*-test and one-way ANOVA with Turkey's post-hoc test using SPSS 16.0 software package. The significance was assessed at $P < 0.05$.

Flubendiamide exposure resulted in a significant increase in LPO to a level higher than the control group by 29.78% on day-60 and by 49.38% on day-90 (Table 1). LPO level declined on day-30 post-treatment, but it remained significantly higher than control. Blood glutathione level was significantly lower (29.32%) than control on day-90. However, the level on day-30 post-treatment increased to become statistically comparable to control. TAA did not show any significant difference up to day-60, but on day-90 it was significantly lower (24.87%) than control. Post-treatment the value increased on day-30 to become statistically comparable to control.

SOD activity in erythrocytes declined on day-90 to become significantly lower (17.94%) than control. However, activity increased on day-30 post-treatment to become comparable to control (Table 2).

Table 1. Changes in lipid peroxides, glutathione and total antioxidant capacity (mean ± SE) in blood of buffalo calves after repeated oral flubendiamide administration

Parameter	Group	Days of observation				
		0	30	60	90	30, post-treatment
Lipid peroxides	Control	6.19 ± 0.69^{aA}	6.67 ± 0.33^{aA}	6.45 ± 0.29^{aA}	6.42 ± 0.30^{aA}	6.05 ± 0.43^{aA}
	Treatment	6.60 ± 0.48^{aA}	7.24 ± 0.27^{abA}	8.37 ± 0.34^{cB}	9.59 ± 0.27^{dB}	8.08 ± 0.21^{bcB}
Glutathione	Control	258.10 ± 11.12^{aA}	254.53 ± 19.22^{aA}	259.06 ± 7.86^{aA}	262.87 ± 6.14^{aB}	260.20 ± 11.70^{aA}
	Treatment	265.55 ± 4.75^{bA}	256.35 ± 8.97^{bA}	237.64 ± 17.73^{bA}	185.79 ± 7.23^{aA}	247.79 ± 5.42^{bA}
Total antioxidant capacity	Control	1.87 ± 0.05^{aA}	1.91 ± 0.12^{aA}	1.92 ± 0.05^{aA}	1.97 ± 0.14^{aB}	2.00 ± 0.05^{aA}
	Treatment	2.00 ± 0.08^{cA}	1.87 ± 0.06^{bcA}	1.84 ± 0.06^{bcA}	1.48 ± 0.06^{aA}	1.75 ± 0.02^{bA}

Values are expressed as nmol MDA produced/g Hb/h for lipid peroxides, μmol/ml for glutathione and mmol/l for total antioxidant activity.
For a given parameter, values bearing different superscripts in small letters in a row and capital letters in a column differ significantly ($P < 0.05$).

Table 2. Changes in activity of superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase, glutathione-S-transferase and glucose-6-phosphate dehydrogenase (mean ± SE) in blood of buffalo calves after repeated oral flubendiamide administration

Parameter	Group	Days of observation				
		0	30	60	90	30, post-treatment
Superoxide dismutase	Control	6.52 ± 0.41^{aA}	6.34 ± 0.16^{aA}	6.22 ± 0.18^{aA}	6.52 ± 0.39^{aB}	6.75 ± 0.15^{aA}
	Treatment	6.54 ± 0.25^{bA}	6.34 ± 0.44^{abA}	6.14 ± 0.23^{abA}	5.35 ± 0.37^{aA}	6.18 ± 0.13^{abA}
Catalase	Control	3667.04 ± 54.13^{aA}	3688.90 ± 71.06^{aA}	3795.02 ± 70.26^{aA}	3626.59 ± 72.34^{aA}	3669.64 ± 60.25^{aA}
	Treatment	3595.03 ± 76.01^{aA}	3681.70 ± 87.38^{aA}	3706.36 ± 64.33^{aA}	3995.55 ± 45.23^{bB}	3661.20 ± 63.71^{aA}
Glutathione peroxidase	Control	8.04 ± 0.17^{aA}	7.90 ± 0.31^{aA}	8.05 ± 0.29^{aA}	7.88 ± 0.39^{aB}	7.77 ± 0.24^{aB}
	Treatment	8.14 ± 0.37^{bA}	7.49 ± 0.32^{bA}	7.14 ± 0.39^{bA}	5.63 ± 0.34^{aA}	6.07 ± 0.08^{aA}
Glutathione reductase	Control	8.20 ± 0.37^{aA}	8.12 ± 0.46^{aA}	8.30 ± 0.39^{aA}	8.09 ± 0.18^{aB}	8.05 ± 0.27^{aA}
	Treatment	8.09 ± 0.29^{bcA}	8.11 ± 0.32^{bcA}	7.50 ± 0.44^{abA}	7.02 ± 0.20^{aA}	8.78 ± 0.24^{cA}
Glutathione-S-transferase	Control	4491.54 ± 44.79^{aA}	4489.90 ± 95.07^{aA}	4435.02 ± 78.90^{aA}	4393.09 ± 70.08^{aA}	4444.64 ± 32.67^{aA}
	Treatment	4595.03 ± 76.01^{aA}	4637.45 ± 29.96^{aA}	4746.36 ± 45.23^{abB}	4913.05 ± 50.10^{bB}	4636.45 ± 69.82^{aA}
Glucose-6-phosphate dehydrogenase	Control	5.81 ± 0.10^{aA}	5.84 ± 0.14^{aA}	5.79 ± 0.16^{aA}	5.83 ± 0.26^{aB}	5.67 ± 0.21^{aA}
	Treatment	5.87 ± 0.29^{bA}	6.09 ± 0.17^{bA}	5.52 ± 0.26^{abA}	4.83 ± 0.23^{aA}	5.34 ± 0.19^{abA}

Values are expressed as the amount of enzyme inhibiting 50% auto-oxidation of pyrogallol for SOD, as μmol H_2O_2 decomposed/min/mg Hb for catalase, EU/mg Hb for GPX, oxidation of 1 μmol of NADPH/min for glutathione reductase, μmol of conjugate of GSH and CDNB/min/g Hb for glutathione-S-transferase and EU for G6PD.
For a given parameter, values bearing different superscripts in small letters in a row and capital letters in a column differ significantly ($P < 0.05$).

Significantly higher catalase activity (10.17%) was noted on day-90, but it declined on day-30 post-treatment, to become comparable to control. The GPX activity was lower (11.30%) than control on day-60 and it further declined on day-90 to become 31.98% lower than control. GPX activity in the treatment group on day-30 increased, but was still lower than control. In treatment group, glutathione reductase activity on day-90 was significantly lower (13.23%), but it increased again on day-30 after treatment to become comparable to control. Glutathione-S-transferase activity was significantly higher on day-60 and the difference between the two groups further increased on day-90. G6PD activity in flubendiamide-treated animals declined on day-90 to become significantly (17.15%) lower than control. On day-30 post-treatment, it increased again to become comparable to control.

Water buffalo (*Bubalus bubalis*) is an economically important livestock species in many Asian, European and Mediterranean countries, including India. A majority of buffaloes under Indian conditions graze in areas where exposure to environmental pollutants, particularly agricultural pesticides is frequent[10]. Pesticide-induced oxidative stress is the final manifestation of the multi-step pathway, resulting in an imbalance between pro-oxidant and antioxidant defence mechanisms[11]. Level of lipid peroxidation reflects the degree to which cellular lipid membranes are attacked by free radicals. Therefore, an increase in LPO level could be attributed to either enhanced formation of free radicals or compromise in cellular antioxidant defence system. Recently, an increase in LPO level along with degenerative changes in testicular tissues was recorded after flubendiamide exposure of Wistar rats[12]. Flubendiamide exposure is also reported to alter hematological profile in water buffalo[4,5].

The alteration in glutathione-S-transferase is a sensitive indicator of oxidative stress[13]. Lower levels of GSH in flubendiamide-exposed animals may be due to either inhibition of GSH synthesis or its increased utilization by intracellular antioxidants. Significant depletion of GSH level in buffalo calves was also observed after repeated oral and dermal exposure to cypermethrin[14]. Likewise, a decrease in GSH levels in testicular tissue of rats was recorded after repeated flubendiamide administration[11]. The total antioxidant capacity reflects combined capacity of different components of antioxidant defence of the body to counteract the effects of a pro-oxidant. The results of the present study suggest compromise in antioxidant defence system in flubendiamide-treated animals. Return of TAA

towards control levels on day-30 after treatment has further strengthened this hypothesis. Decrease in TAA along with elevated blood lead levels in pregnant buffaloes reared nearby the highways was also reported earlier[14]. SOD, GPX and catalase are important components of enzymatic antioxidant defence system[15]. In the present study, an increase in catalase and glutathione-S-transferase activities in flubendiamide-exposed animals can be attributed to upregulation of their synthesis in order to counteract enhanced free-radical production. Upregulation of catalase gene has been recorded after experimental induction of oxidative stress in anaerobic bacteria *Methanosarcina barkeri*[16]. A dose-dependent increase in catalase activity in flubendiamide toxicity was also recorded in insect larvae[17]. However, a generalized declining trend in activities of other antioxidant enzymes, including GPX, glutathione reductase and G6PD was recorded in flubendiamide-treated animals after day-60, with further decrease in activities on day-90. This may be due to excess utilization of these enzymes to counteract the excess free-radical generation in treatment group. In a recent study on flubendiamide exposure in *Daphnia magna*, increase in catalase activity and decrease in SOD and GPX were recorded, which further corroborated our findings[8]. The authors further recorded down-regulation of SOD and GPX genes during oxidative stress induced by diamide pesticides.

Thus, it can be concluded that chronic flubendiamide toxicity induces oxidative stress.

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AMITA RANJAN[1,*]
V. K. DUMKA[2]
RAKESH RANJAN[3]

[1]*Department of Veterinary Pharmacology and Toxicology, Rajasthan University of Veterinary and Animal Sciences, Bikaner 334 001, India*
[2]*Department of Veterinary Pharmacology and Toxicology, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana 141 004, India*
[3]*National Research Centre on Camel, Bikaner 334 022, India*
**For correspondence.*
e-mail: amita_pharma@rediffmail.com