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Evaluation of biochemical alterations produced by combined exposure of fenvalerate and nitrate in *Bubalus bubalis*

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Abstract

Aim: Evaluation of combined effect of fenvalerate and nitrate on biochemical parameters in buffalo calves.

Materials and Methods: Sixteen male buffalo calves were divided into four groups of four calves each. Group I receiving no treatment served as the control. Group II and III animals were orally administered with fenvalerate (1.0 mg/kg/day) and sodium nitrate (20 mg/kg/day), respectively, for 21 consecutive days and were kept as positive control. Group IV animals were co-administered with fenvalerate and sodium nitrate at the above dose rates for 21 consecutive days. Biochemical parameters including Aspartate aminotransferase (AST), Alkaline phosphatase (ALP), Gamma-glutamyl transpeptidase (GGT), Lactate dehydrogenase (LDH), Glucose, Total protein, Albumin, Cholesterol, Blood urea nitrogen (BUN) and Creatinine were determined on 0, 3, 7, 10, 14, 17 and 21 day of treatment. Estimation of these parameters was also done on 7th day of post-treatment period.

Results: Co-administration of fenvalerate and sodium nitrate produced significant increase in the plasma levels of AST, ALP, GGT, LDH, glucose, BUN, cholesterol and creatinine while significant decrease in the plasma levels of total proteins was observed. No significant alteration was observed in albumin levels. Extent of organ damage as evidenced by biochemical alterations was more pronounced in calves exposed to combination of fenvalerate and sodium nitrate as compared to their individual exposures.

Conclusion: Fenvalerate and sodium nitrate co-administration potentiates the toxicological injury produced, in comparison to their individual exposure.

Keywords: biochemical, buffalo calves, combined exposure, fenvalerate, sodium nitrate

Introduction

Pesticides have played a pivotal role in bringing about green revolution in the world and have evolved as an irreplaceable component for the control of agricultural pests and insects causing public health hazards. Several health ailments and/or outbreaks are reported to occur among animals and humans from insecticide toxicity, which mostly occur either from direct exposure to insecticides or indirectly from contaminated feeds or water. Eventhough the average utilization of pesticides in India is considered to be much lower than many other developed countries, the problem of pesticide residues is relatively high [1]. Pyrethroides, derivatives of carbamic acid, represent a large variety of compounds which have some field applications as insecticides, herbicides and fungicides. Fenvalerate is an insecticide of moderate mammalian toxicity which is used against a broad range of pests [2] and is reported to cause alterations in the biochemical parameters in different tissues of rats [2, 3].

Excessive use of nitrogenous fertilizers subsequently lead to ecosystem pollution by the accumulation of nitrates in vegetables and fodder as well as the contamination of surface and ground water [4]. Therefore, nitrates are a major threat to environment in different agricultural situations and a potential health risk for humans and animals [5]. Ruminants are more susceptible to nitrate poisoning than non-ruminant species. Several clinical studies have documented renal and/or hepatotoxicity in humans associated with excess nitrate intake [6]. However, relatively few studies have assessed the degree of hazard posed by simultaneous exposure to pesticides and nitrate [7].

Humans and animals can be exposed concurrently to more than one chemical in the environment. Such interactions may be deleterious, as both the kinetics and dynamics of the environmental chemicals can be modified by their co-occurrence [8]. Biochemical biomarkers are increasingly used in ecological risk assessment to identify the incidence and effects of environmental pollutants [9]. In domestic animals and humans, simultaneous exposure to fenvalerate and nitrate can lead to various health afflictions and tissue/ organ damage which can be assessed by measuring levels of various biochemical enzymes and other parameters indicative of specific organ damage. In India, buffalo is

Table-1. Effect of sub-acute oral administration of fenvalerate (1mg/kg/day) and sodium nitrate (20mg/kg/day) combination on plasma aspartate aminotransferase, alkaline phosphatase and gamma-glutamyl transpeptidase in buffalo calves.

	Treatment							Post-treatment
Days	**0**	**3**	**7**	**10**	**14**	**17**	**21**	**7**
Aspartate aminotransferase (U/L)								
Group I	129.0 ± 5.02^{a}	133.0 ± 5.15^{a}	131.5 ± 6.20^{a}	124.0 ± 3.81^{a}	127.3 ± 4.39^{a}	131.5 ± 4.23^{a}	129.5 ± 4.41^{a}	131.0 ± 2.86^{a}
Group II	130.3 ± 3.50^{a}	134.0 ± 4.06^{a}	141.8 ± 3.71^{a}	154.0 ± 2.27^{bc}	155.5 ± 4.25^{b}	158.3 ± 3.71^{b}	155.3 ± 4.55^{b}	140.5 ± 1.85^{a}
Group III	135.3 ± 3.15^{a}	141.3 ± 3.79^{a}	147.5 ± 5.17^{a}	146.8 ± 3.90^{b}	156.8 ± 3.90^{b}	167.9 ± 4.23^{bc}	179.0 ± 5.02^{c}	159.0 ± 5.02^{b}
Group IV	134.5 ± 3.66^{a}	144.5 ± 3.66^{a}	153.3 ± 2.70^{a}	162.0 ± 4.53^{c}	171.0 ± 4.80^{c}	180.7 ± 4.93^{c}	192.0 ± 4.38^{c}	168.0 ± 3.49^{b}
Alkaline phosphatase (U/L)								
Group I	192.5 ± 5.89^{a}	190.3 ± 4.96^{a}	184.3 ± 4.00^{a}	190.8 ± 3.25^{a}	192.3 ± 3.57^{a}	194.3 ± 2.50^{a}	197.8 ± 4.25^{a}	198.8 ± 2.69^{a}
Group II	193.5 ± 3.80^{a}	188.5 ± 3.28^{a}	187.3 ± 2.63^{a}	197.3 ± 3.97^{a}	199.8 ± 4.09^{a}	206.5 ± 4.09^{a}	210.0 ± 5.49^{a}	203.5 ± 3.10^{a}
Group III	189.0 ± 4.04^{a}	190.8 ± 3.84^{a}	192.8 ± 5.12^{a}	209.9 ± 8.29^{ab}	232.0 ± 8.29^{b}	237.8 ± 9.19^{b}	246.3 ± 8.51^{b}	208.8 ± 9.66^{ab}
Group IV	188.3 ± 4.77^{a}	187.0 ± 6.56^{a}	190.5 ± 7.12^{a}	217.0 ± 7.15^{b}	250.0 ± 11.4^{b}	262.0 ± 7.74^{c}	274.0 ± 9.27^{c}	226.0 ± 8.91^{b}
Gamma-glutamyl transpeptidase (U/L)								
Group I	15.8 ± 0.85^{a}	14.5 ± 0.65^{a}	14.3 ± 0.85^{a}	12.5 ± 1.04^{a}	14.0 ± 1.22^{a}	15.8 ± 1.25^{a}	13.3 ± 0.85^{a}	14.8 ± 1.70^{a}
Group II	14.8 ± 1.11^{a}	15.8 ± 0.85^{a}	16.0 ± 0.71^{a}	15.5 ± 0.96^{ab}	19.5 ± 0.65^{b}	20.8 ± 1.75^{b}	21.3 ± 0.85^{b}	17.3 ± 1.11^{ab}
Group III	13.3 ± 0.85^{a}	15.0 ± 0.91^{a}	13.0 ± 1.08^{a}	16.0 ± 1.08^{ab}	18.3 ± 0.63^{b}	21.5 ± 1.44^{b}	22.3 ± 1.25^{b}	19.5 ± 1.04^{b}
Group IV	14.0 ± 1.08^{a}	12.5 ± 0.65^{a}	15.5 ± 1.04^{a}	17.8 ± 1.31^{b}	19.5± 1.19^{b}	22.5± 1.55^{b}	24.5± 1.55^{b}	20.0 ± 1.29^{b}

Values given are mean ± SE, n= 4*, *Means with at least one common superscript do not differ significantly ($p<0.05$). Group I - Control, II - Fenvalerate, III - Sodium nitrate, IV - Fenvalerate and Sodium nitrate combination.

an important dairy animal which contributes about 70% of the total milk production.

The present research investigation was therefore conducted with the primary aim to elucidate the interactive effect of fenvalerate and sodium nitrate on biochemical parameters in buffalo calves.

Materials and Methods

Ethical approval: The experimental protocol followed the ethical guidelines on the proper care and use of animals and had been approved by the Institutional Animal Ethics Committee.

Animals: The experiment was performed on sixteen healthy male buffalo calves of 6-12 months age and weighing between 100-130 kg, kept under normal ambient conditions in the experimental animal shed of the department. During this period, all animals were dewormed and subjected to regular clinical examination. The animals were maintained on green fodder, concentrates and wheat straw. Water was provided *ad libitum*.

Experimental design: The animals were randomly divided into four groups of 4 animals each and their baseline biochemical values were determined. Group I serving as control, was administered with 50 ml distilled water. Group II animals were orally administered with fenvalerate at 1.0 mg/kg/day for 21 consecutive days, whereas sodium nitrate at 20 mg/kg/day was administered to group III animals. Group IV animals were administered both fenvalerate and sodium nitrate at the same dosages and for the same duration as group II and III, respectively. The requisite amount of insecticide and nitrate was suspended in 50 ml of distilled water and drenched to animals of subsequent groups. The daily oral dose of fenvalerate was selected on the basis of the recommended concentrations of fenvalerate used for crop protection, per acre yield of fodder and average daily consumption of fodder by buffalo calves to resemble the levels of the insecticide to which dairy animals are likely to be exposed. The dosage of sodium nitrate was selected on the basis of previous literature as similar dose was used in a study conducted by Shahid Mahboob et al [10] in rabbits for a period of 40 days to study haematological parameters.

Chemicals: Fenvalerate (Reagent®, 20% EC) insecticide was commercially obtained from Bharat Insecticides Limited, New Delhi. Sodium nitrate (analytical grade) was procured from Merck Specialiaties Private Limited, Mumbai.

Estimation of biochemical parameters: To study the biochemical parameters, blood samples were collected in heparinized vials from the jugular vein of animals on 0, 3, 7, 10, 14, 17, 21 day of treatment and 7 day post treatment. Plasma was separated by centrifugation at 3000 rpm for 15 min. Biochemical parameters including Aspartate aminotransferase (AST), Alkaline phosphatase (ALP), Gamma-glutamyl transpeptidase (GGT), Lactate dehydrogenase (LDH), Glucose, Total protein, Albumin, Cholesterol, Blood urea nitrogen (BUN) and Creatinine were estimated using Bayer Autopack kits (Bayer Diagnostics India Ltd., India).

Statistical analysis: All results were subjected to analysis of variance carried in completely randomized design and the significance was tested using Duncan's multiple range test [11]. The significance was assayed at 5 % ($p<0.05$) levels. These statistical calculations were carried out with SPSS 16.0 software.

Results and Discussion

The results for the combined effect of fenvalerate and sodium nitrate on AST, ALP and GGTP are presented in Table-1. Combined exposure to fenvalerate and sodium nitrate produced significant elevation in plasma AST and ALP levels from 10th day onwards. Maximum increase of 42.8% was observed in AST

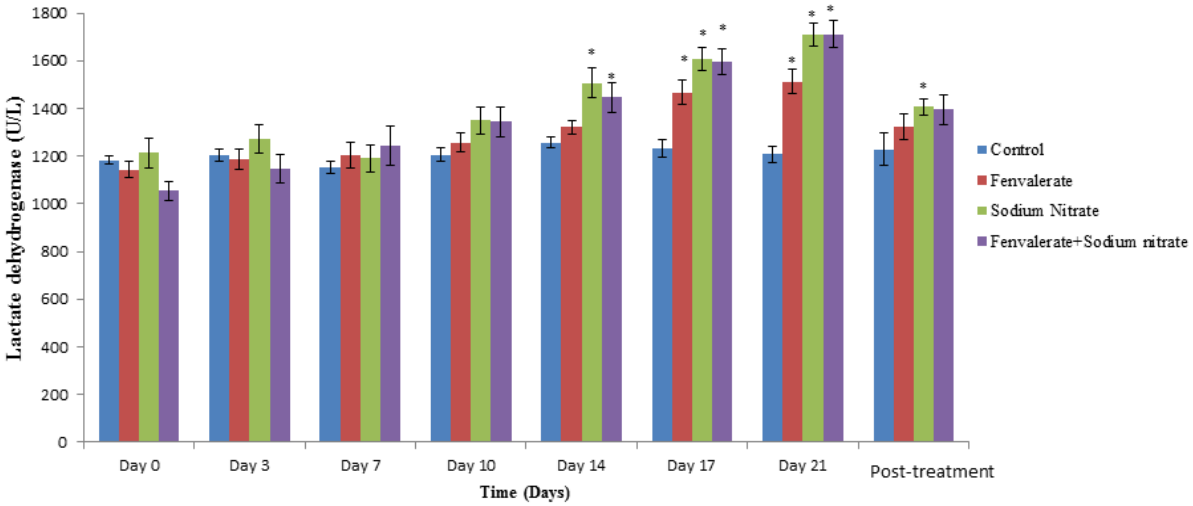

Figure- 1. Effect of sub-acute oral administration of fenvalerate (1 mg/kg/day) and sodium nitrate (20 mg/kg/day) combination on plasma lactate dehydrogenase in buffalo calves.

levels on 21st day of treatment. Although there was significant increase in AST levels in animal groups treated individually with either fenvalerate or nitrate, the percent increase following combined exposure of toxicants was comparatively greater than the corresponding values for individual exposures. The levels of plasma AST in fenvalerate-nitrate interaction group varied significantly from those of fenvalerate and nitrate treated groups on 17th day of treatment. Maximum increase of 45.5% was observed in plasma ALP levels in the fenvalerate and sodium nitrate co-administered group on 21st day of treatment which was comparatively higher than that observed in nitrate (30.3%) treated group. Combined exposure of fenvalerate and sodium nitrate produced significant elevation in plasma GGT levels from 10th day onwards and a maximum increase of 75.0% was observed on 21st day of treatment in buffalo calves. The percent increase in enzyme levels in this group was comparatively higher as compared to the individual exposures of fenvalerate (43.9%) and sodium nitrate (67.7%).

Several studies have documented the biochemical alterations on fenvalerate [2,3,12-14] and nitrate exposure [15-18] in different species of animals. Increases in the activity of phosphatases and aminotransferases have been reported with pyrethroids [19]. Administration of either nitrate or fluoride or their combination also elevated phosphatase activity in rats [20]. Studies relating to the effects of fenvalerate and nitrate in combination have not been documented. The liver plays an important role in metabolism to maintain energy level and structural stability of body. It is also the site of detoxification where toxic compounds get biotransformed into less harmful products to reduce toxicity [21]. However, these toxic compounds damage the liver cells and produce hepatotoxicity. High plasma concentrations of ALT and AST are considered as an index of liver damage [22]. An increase of aminotransferase activity in the extracellular fluid or plasma is a sensitive indicator of cellular damage [23]. Alkaline phosphate is a zinc-containing enzyme [24] which is secreted in liver, biliary tracts, small intestines, bones, lungs and kidney. Elevated levels in blood may indicate damage to the liver and other organs due to non-specific irritation [8]. Although the exact cause of increased GGTP level in the present study could not be ascertained, cholestatic disorders of all species are associated with increased GGTP activity [25].

The results for the effect of fenvalerate and nitrate combination on LDH are shown in Figure-1. Fenvalerate and sodium nitrate co-administration produced significant increase in plasma LDH levels from 14th day onwards. Maximum elevation of 62.5% was observed on 21st day of treatment which was comparatively higher than that in fenvalerate (32.5%) and sodium nitrate (40.8%) exposed group.

LDH is a cytosolic enzyme and any cellular damage to liver, lung, muscle, kidney and heart releases this enzyme into systemic circulation [26]. LDH is elevated in natural organophosphates or carbamate poisoning [27]. The increase in plasma LDH activity in present investigation reflects damage to a range of tissues including skeletal or cardiac muscles, kidney and liver.

The effects produced by fenvalerate and sodium nitrate co-administration on glucose, total protein and albumin levels are shown in Table-2. Combined exposure to fenvalerate and sodium nitrate produced significant increase in plasma glucose levels from 14th day onwards. Maximum increase of 60.6% was observed on 21st day of treatment which was comparatively higher than that observed in fenvalerate (39.9%) and sodium nitrate (53.1%) exposed groups. Fenvalerate and sodium nitrate co-exposure in buffalo calves produced significant decrease in plasma protein levels on 7th day and then 14th day onwards. Maximum decline of 29.2% was observed on 21st day of treatment which was comparatively higher than fenvalerate (20.5%) and sodium nitrate (7.69%) exposed groups. No signi-

Table-2. Effect of sub-acute oral administration of fenvalerate (1 mg/kg/day) and sodium nitrate (20 mg/kg/day) combination on plasma glucose, total proteins and albumin in buffalo calves.

	Treatment							Post-treatment
Days	**0**	**3**	**7**	**10**	**14**	**17**	**21**	**7**
Glucose (mg/dl)								
Group I	59.8 ± 2.25^{a}	60.1 ± 1.95^{a}	58.8 ± 1.96^{a}	60.0 ± 0.98^{a}	57.9 ± 1.50^{a}	60.2 ± 1.87^{a}	60.6 ± 2.36^{a}	58.0 ± 0.80^{a}
Group II	56.7 ± 1.72^{a}	60.3 ± 1.65^{a}	60.8 ± 1.42^{ab}	63.2 ± 1.87^{ab}	67.6 ± 2.07^{b}	77.6 ± 2.14^{b}	79.3 ± 2.95^{b}	63.3 ± 2.28^{a}
Group III	53.9 ± 2.24^{a}	61.5 ± 2.81^{a}	69.5 ± 4.04^{b}	70.2 ± 2.77^{b}	71.2 ± 2.32^{b}	76.8 ± 3.64^{b}	82.5 ± 2.98^{b}	61.1 ± 2.75^{a}
Group IV	56.1 ± 4.39^{a}	60.1 ± 3.11^{a}	64.1 ± 3.61^{ab}	67.4 ± 3.69^{ab}	70.8 ± 4.33^{b}	80.4 ± 3.70^{b}	90.1 ± 5.43^{b}	65.2 ± 3.46^{a}
Total plasma proteins (g/dl)								
Group I	6.88 ± 0.24^{a}	6.71 ± 0.19^{a}	7.03 ± 0.24^{a}	6.69 ± 0.18^{ab}	6.94 ± 0.24^{a}	6.80 ± 0.25^{a}	7.09 ± 0.30^{a}	6.6 1± 0.24^{a}
Group II	7.16 ± 0.15^{a}	6.83 ± 0.25^{a}	7.10 ± 0.12^{a}	6.88 ± 0.18^{b}	6.46 ± 0.27^{ab}	5.96 ± 0.17^{b}	5.69 ± 0.22^{b}	6.31 ± 0.15^{ab}
Group III	6.37 ± 0.18^{a}	6.56 ± 0.18^{a}	6.71 ± 0.15^{ab}	6.43 ± 0.14^{ab}	6.14 ± 0.14^{b}	5.99 ± 0.06^{b}	5.88 ± 0.10^{b}	6.09 ± 0.05^{b}
Group IV	7.12 ± 0.10^{a}	6.69 ± 0.19^{a}	6.46 ± 0.13^{b}	6.28 ± 0.14^{a}	6.10 ± 0.16^{b}	5.47 ± 0.14^{b}	5.04 ± 0.13^{c}	5.87 ± 0.13^{b}
Albumin (g/dl)								
Group I	2.95 ± 0.10^{a}	3.01 ± 0.14^{a}	2.91 ± 0.16^{a}	2.96 ± 0.15^{a}	3.05 ± 0.15^{a}	3.18 ± 0.17^{a}	3.15 ± 0.25^{a}	2.83 ± 0.14^{a}
Group II	2.81 ± 0.17^{a}	2.87 ± 0.22^{a}	2.94 ± 0.30^{a}	3.04 ± 0.30^{a}	3.23 ± 0.27^{a}	2.95 ± 0.19^{a}	2.79 ± 0.15^{a}	2.86 ± 0.14^{a}
Group III	3.04 ± 0.08^{a}	3.01 ± 0.14^{a}	2.99 ± 0.21^{a}	3.08 ± 0.26^{a}	2.84 ± 0.14^{a}	2.80 ± 0.13^{a}	2.92 ± 0.22^{a}	2.95 ± 0.22^{a}
Group IV	3.18 ± 0.17^{a}	3.07 ± 0.14^{a}	3.09 ± 0.23^{a}	3.02 ± 0.14^{a}	3.08 ± 0.26^{a}	2.87 ± 0.22^{a}	2.69 ± 0.15^{a}	2.94 ± 0.30^{a}

Values given are mean ± SE, n= 4*, *Means with at least one common superscript do not differ significantly ($p<0.05$). Group I - Control, II - Fenvalerate, III - Sodium nitrate, IV - Fenvalerate and Sodium nitrate combination.

Table-3. Effect of sub-acute oral administration of fenvalerate (1 mg/kg/day) and sodium nitrate (20 mg/kg/day) combination on plasma cholesterol, blood urea nitrogen and creatinine in buffalo calves.

	Treatment							Post-treatment
Days	**0**	**3**	**7**	**10**	**14**	**17**	**21**	**7**
Cholesterol (mg/dl)								
Group I	65.6± 2.88^{a}	67.3 ± 1.82^{a}	64.8 ± 2.46^{a}	66.3 ± 2.43^{a}	65.1 ±2.50^{a}	63.4 ± 1.89^{a}	64.1 ± 3.08^{a}	62.9 ± 2.15^{a}
Group II	64.4 ± 2.97^{a}	65.9 ± 2.67^{a}	66.2 ± 1.91^{ab}	64.9 ± 1.73^{a}	63.9 ± 1.98^{a}	64.5 ± 2.18^{a}	67.5 ± 1.60^{a}	63.0 ± 1.67^{a}
Group III	66.3 ± 4.27^{a}	69.5 ± 2.96^{a}	71.5 ± 1.32^{b}	74.8 ± 1.80^{b}	75.0 ± 1.47^{b}	79.3 ± 2.17^{b}	83.3 ± 3.84^{b}	73.0 ± 2.58^{b}
Group IV	69.0 ± 1.29^{a}	70.8 ± 1.38^{a}	72.0 ± 2.04^{b}	75.5 ± 2.10^{b}	77.5 ± 2.90^{b}	83.6 ± 1.75^{b}	89.8 ± 2.32^{b}	76.3 ± 1.31^{b}
Blood urea nitrogen (mg/dl)								
Group I	9.25 ± 0.85^{a}	10.5 ± 0.65^{a}	10.3 ± 0.85^{a}	9.50 ± 0.87^{a}	10.5 ± 0.65^{a}	11.0 ± 1.08^{a}	11.5 ± 1.32^{a}	10.5 ± 0.65^{a}
Group II	10.5 ± 0.87^{a}	9.8 ± 0.85^{a}	10.0 ± 1.47^{a}	12.5 ± 1.19^{ab}	13.0 ± 0.91^{ab}	15.8 ± 0.48^{b}	17.3 ± 0.63^{b}	12.8 ± 1.11^{a}
Group III	11.3 ± 0.85^{a}	12.5 ± 0.65^{a}	11.8 ± 0.85^{a}	14.0 ± 1.08^{b}	15.5 ± 1.44^{bc}	16.3 ± 0.85^{b}	17.0 ± 0.91^{b}	12.5 ± 1.04^{a}
Group IV	10.0 ± 0.91^{a}	11.5 ± 0.65^{a}	11.0 ± 0.91^{a}	14.3 ± 1.49^{b}	17.8 ± 2.02^{c}	17.5 ± 1.04^{b}	19.0 ± 0.91^{b}	11.8 ± 1.11^{a}
Creatinine (mg/dl)								
Group I	1.56 ± 0.09^{a}	1.60 ± 0.08^{a}	1.48 ± 0.11^{a}	1.54 ± 0.08^{a}	1.50 ± 0.08^{a}	1.60 ± 0.07^{a}	1.43 ± 0.06^{a}	1.61 ± 0.05^{a}
Group II	1.59 ± 0.06^{a}	1.50 ± 0.06^{a}	1.69 ± 0.05^{a}	1.80 ± 0.05^{a}	1.91 ± 0.11^{b}	2.12 ± 0.11^{b}	2.19 ± 0.13^{b}	1.82 ± 0.07^{b}
Group III	1.39 ± 0.04^{a}	1.44 ± 0.04^{a}	1.50 ± 0.03^{a}	1.56 ± 0.03^{a}	1.63 ± 0.03^{a}	1.74 ± 0.10^{a}	1.84 ± 0.20^{ab}	1.60 ± 0.05^{a}
Group IV	1.40 ± 0.04^{a}	1.44 ± 0.03^{a}	1.49 ± 0.05^{a}	1.72 ± 0.17^{a}	1.94 ± 0.10^{b}	2.09 ± 0.15^{b}	2.23 ± 0.19^{b}	1.65 ± 0.07^{a}

Values given are mean ± SE, n= 4*, *Means with at least one common superscript do not differ significantly ($p<0.05$). Group I - Control, II - Fenvalerate, III - Sodium nitrate, IV - Fenvalerate and Sodium nitrate combination.

ficant alteration was observed in albumin levels in any of the treated groups.

The blood sugar level is an important indicator of on-going body homeostatic mechanisms. The blood sugar levels are tightly regulated by the body, but certain metabolic and toxic conditions cause the levels to increase or decrease abnormally. Several animal studies have also shown altered glucose homeostasis following acute or chronic exposures to insecticides due to stress-induced release of catecholamines [28-29]. Increase in enzymatic activity of liver specific enzymes in the present study is indicative of liver damage which can also be the contributing factor for hyperglycemia since the liver is an important site for metabolism of glucose. Decrease in plasma protein levels are usually seen in chronic liver diseases due to impaired synthesis and in renal diseases due to loss of proteins. The declining protein levels of plasma in the present study could either be due to impairment of protein synthesis or due to increased protein catabolism following combined exposure to toxicants.

The effect produced by combined exposure of fenvalerate and sodium nitrate on cholesterol, BUN and creatinine are presented in Table-3. Elevation in plasma cholesterol levels was observed following fenvalerate and sodium nitrate co-administration from 7th day onwards. Maximum increase of 30.1% was observed on 21st day of treatment which was comparatively greater than that in nitrate-exposed group. No significant alteration was produced in fenvalerate- treated group. An increasing trend in BUN levels from 10th day onwards was observed following combined exposure to fenvalerate and sodium nitrate. Maximum increase of 90% was observed on 21st day of combined treatment of toxicants which was comparatively higher than that of fenvalerate (64.8%) and sodium nitrate (50.4%)

alone treated groups. Co-administration of fenvalerate and sodium nitrate caused significant increase in plasma creatinine levels from 14th day onwards in buffalo calves. Maximum increase of 59.3% was observed on 21st day of treatment which was higher than that of fenvalerate (37.7%) treated group indicating greater effect of toxicants on renal system following co-exposure of toxicants.

Cholesterol is esterified in liver and is used for the synthesis of bile acids and steroid hormones. Esterification is less in liver diseases and hypocholesterolemia as seen in animals with bile duct occlusion, whereas hypocholesterolemia is seen in severe liver dysfunctions [30]. Liver plays an active role in the metabolism of cholesterol so disruption of liver function in present study might be responsible for the increased cholesterol levels. BUN levels increase in blood/serum during renal insufficiency, high protein diet or other factors which increase catabolism of proteins. Blood urea nitrogen indicates the glomerular filtration but is not a sensitive marker as 75% of the kidney should be non functional for BUN elevation [31]. Plasma creatinine levels help in evaluating renal functions especially the glomeruli because creatinine is excreted in urine after filtration [32]. Increase in plasma BUN and creatinine levels in present study are indicative of renal damage, which may be attributed to urinary obstruction that potentiates decreased secretion of urea from the body. Greater elevation of BUN and creatinine levels on combined exposure might have occurred due to additive effect of toxicants on renal system.

The present findings, therefore, suggest that both test chemicals, to varying degrees, have the potential of causing damage to various organs of body. However, co-exposure of calves to both fenvalerate and nitrate produced more severe damage, as indicated by the greater disturbance in the homeostasis of biochemical parameters. Consistent to our findings, Dubey et al. [8] reported exacerbated oxidative stress and biochemical alterations in rats co-exposed to deltamethrin and fluoride. In another study, Sodium fluoride and deltamethrin have been reported to aggravate the oxidative injury of each another in rat erythrocytes [33]. Similarly Gill et al. [34] reported that combined exposure to fipronil and sodium fluoride aggravate their effects on toxic signs, cholesterol and creatinine levels in buffalo calves.

Conclusion

Co-exposure to fenvalerate and sodium nitrate cause damage to liver, muscle and kidney thereby causing a leakage of liver and muscle specific enzymes into blood and also an incline in plasma levels of glucose, cholesterol, BUN and creatinine. The study clearly indicates that a cocktail of fenvalerate and sodium nitrate induce a cascade of reaction in the exposed animals, thereby augmenting the toxicological damage. Therefore, this is an indication that the interaction of these compounds in nature could be responsible for aggravating their toxic potential in livestock.

Authors' contributions

The present research investigation was carried out by KKG as a part of her doctoral research programme under the guidance of HSS (Major advisor) and RK (Co-advisor). All authors read and approved the final manuscript.

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Competing interests

The authors declare that they have no competing interests.

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