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Impact of Silymarin and nano-zinc oxides in liver functions of Murrah buffalo calves

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Abstract
This study aimed to explore the impact of silymarin and nano-zinc oxides on liver functions of Murrah buffalo calves. Twenty-eight Murrah buffalo calves were used in this experiment and randomly assigned into four groups (n = 7) according to body weight (136±5.16 kg) and age (10.52±0.50 months). Group 1st acted as a control offered basal diet without any supplementation, however, group 2nd, 3rd, and 4th given basal diet with silymarin (600mg/kg DM/calves/day, T_1), nano-zinc (50mg/kg DM/calves/day, T_2), and both silymarin + nano-zinc (600mg/kg DM/calves/day + 50mg/kg DM/calves/day, T_3), respectively, for 120 days of the experimental period. The jugular blood was collected at 7.00 am in EDTA containing test-tube before feeding and watering for the analysis of alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP). A statistical (P< 0.05) reduction in ALT activity of the treatments was observed with respect to control, but reduction was greater in T3 groups, however, no statistical difference was noticed between T_1 and T_2 groups. The AST activity was varied statistically (P< 0.05) in the groups, and reported greater significant (P< 0.05) reduction in the activity of AST in buffalo calves who received both silymarin and nano-zinc compared to all other groups. A statistical (P< 0.05) reduction was observed in the activity of mean ALP in groups supplemented either with silymarin and nano-zinc or both than control but the reduction was highest in the T_3 group of Murrah buffalo calves. In conclusion, we find that the supplementation of silymarin and nano-zinc improved the function of liver of Murrah buffalo calves by reducing ALT, AST, and ALP.

Keywords: Silymarin, Nano-zinc, Murrah buffalo calves, liver functions

Introduction
The Murrah buffalo is mainly kept for the production of milk, also known as milch breed of buffalo. The genetic improvement of growth of Murrah buffaloes is of great importance in the large ruminant industry in India (Kul *et al.*, 2018) [11]. Murrah buffalo is the world's best dairy type buffalo and capable for producing more than 35 kg milk/day. Murrah has been used as to improver breed in India and other countries (Kumar *et al.*, 2017) [12]. Murrah buffaloes are jet black in colour, black eyes, active and prominent in females, but slightly shrunken in males and should not be walled, i.e., the cornea should not have whiteness. Their ears are short, thin, and alert. They have short and tightly curved horns. Bulls weigh around 545 kg and cows around 445 kg. Average production of milk is 2250 kg in a lactation period of 310 days (Pramod *et al.*, 2018) [17]. *Silybum marianum*, commonly called as milk thistle (Family: Compositae/Asteraceae) is one of the most important herbal medicinal plant which are used in traditional treatment for liver failures and many diseases, drug, and food poising as well as treatment for foetal diseases such as viral hepatitis (Banae *et al.* 2011) [3]. From the recent years, several research have been indicated that silymarin has antioxidant, antiatherosclerotic, antihypertensive, anti-obesity, anti-diabetic, anti-inflammatory, and anti-carcinogenic effects. The anticancer activity of silymarin, as well as of silibinin was demonstrated against various cancer cells, such as breast, skin, colon, cervix, ovary, prostate, lung and hepatocellular cancers (Denev *et al.*, 2020) [6]. The main active ingredients of its seeds are silybin, silycristin, silydianin, and isosilybin. Some features of *S. marianum* include anti-oxidation and immune modulators (Behboodi *et al.*, 2017) [4]. There are many possible mechanisms by which silymarin can improve the oxidative status and antioxidant defence mechanisms and its beneficial effect in protecting against systemic oxidative stress (Farmer *et al.*, 2016) [7]. Nano zinc oxide (nZnO) is a new substance that has been produced and marketed using nanotechnologies. This substance has found many applications in the pigments, food and electronics industries as well as in medicine (Mohamed *et al.*, 2017) [14].

Nano-sized nutrients and supplements have been claimed to have an improved bioavailability or functionality and thereby minimize the concentrations needed in the food product (Nasser *et al.,* 2016). Nano form of zinc supplementation raises the surface area that would augmentation of mineral absorption and thereby its utilization leading to reduction in the quantity of its supplementation and ultimately decline in feed cost. Feeding minerals with higher bioavailability not only minimize its cost of supplementation it also the excretion of excess minerals and thereby reduced environmental pollution (Belewu and Adewumi, 2021). Therefore, the present study was conducted to investigate Impact of silymarin and nano-Zinc oxides in liver lunctions of Murrah buffalo calves on serum parameter like Alanine aminotransferase (ALT/SGPT), Aspartate aminotransferase (AST/GOT) and Alanine aminotransferase (ALT/GPT).

Material and Methods

The study design was approved by the Institutional Animal Ethics Committee (IAEC), which works under the control of the CPCSEA rules laid down by the Government of India.

The research was performed in LRC at Sardar Vallabhbhai Patel University of agriculture and technology Meerut (U.P) India. In this experiment, 28 Murrah buffalo calves were chosen with average body weight (136±0.50 kg) and age (9-12 months) and categorized randomly into four groups, 7 animals in each group. The feed and fodders were fed to the experimental calves in the form of total mixed ration (TMR), which had concentrate, green fodder (berseem and oat) and straw (wheat) in the ratio of 50:30:20 to meet their nutrient requirement as per the direction of NRC (2001). TMR basal diet offered to all four groups was the same except groups 2nd, 3rd, and 4th additionally supplied silymarin (600 mg/kg DM/day/calf, T_1), nano-zinc (50 mg/kg DM/day/calf, T_2), and both silymarin and nano-zinc (600 mg/kg DM + 50 mg/kg DM, T_3), respectively. The Accurate weighed dose of silymarin, nano-zinc or both was mixed in the little amount of concentrate and fed to each animal individually for 120 days trial period. Fresh and clean tap water was given *ad libitum*. The TMR was prepared daily in the early morning and fed two times a day at 8:00 a.m. and 6.00 p.m.

Jugular vein blood was congregated in the EDTA-containing glass test tube at 7.00 am during 0, 30, 60, 90 and 120 days of the experimental period before watering and feeding. Blood samples were centrifuged at 3000 rpm for 30 minutes and plasma was kept in the Eppendorf tube and stored at -20ºC for the analysis of ALT, AST and ALP. These variables were analyzed by commercial ERBA kits (ERBA diagnostics Mannheim Germany).

The generated data of ALT, AST, and ALP were statistically analyzed by the MIXED model of SPSS software V 19.0. The means were compared by applying 'Duncan's Multiple Range Test'.

Results and Discussions

The activity of ALT of all four groups over a period of 120 days is given in table 1. The activity of ALT was similar statistically among the groups before starting supplementation of silymarin and nano-zinc. During the 1st, 2nd, and 4th months of the study, a statistical (P< 0.05) reduction was observed in ALT activity of the treatments fed either with silymarin, nano-zinc or both with respect to control. The mean value of ALT activity significantly declined (P< 0.05) in T_3 (28.14 IU/L) than in T_2 (30.71 IU/L), T_1 (31.85 IU/L), and control (36.32 IU/L), respectively. In agreement with our research, Banaee *et al.,* (2010) reported that the activity of alanine aminotransferase in serum of fishes supplemented with 100 mg/kg silymarin was showed significantly declined in comparison to the control group. The result of the current research was in contrast with Fazil *et al.*, (2013) [9] who find that nanoparticles of zinc oxide (25–200 mg) significantly increased serum ALT levels in male rats. Jung *et al.* (2010) [10] also observed an increasing trend in the activity of ALT in mice fed with zinc oxide nanoparticles compared with the control. In the dispute with our research, Belewu and Adewumi, (2021) found no effect of zinc nanoparticle supplementation on the activity of ALT.

The impact of silymarin and nano-zinc supplementation on the AST activity of Murrah buffalo calves is presented in Table 1. The AST activity statistically showed no difference in all four groups before starting the feeding of silymarin and nano-zinc. Whereas, AST activity was significantly (P<0.05) reduced in T_3 as compared to control, T_1 and T_2 during 30, 60, and 120 days of the experimental period. The mean AST and AST activities on day 90 of the study showed a significant (P< 0.05) difference among the groups and was reported highest reduction in the group that received both silymarin and nano-zinc. Similar to the present findings, Shahad and Ahmed, (2020) also observed a decrease in AST activity in quails who were fed silymarin in their diet with dose rates of 0.5 and 1.0 g/kg.

Table 1: Effect of silymarin and nano-zinc supplementation on ALT, AST and ALP of Murrah buffalo calves.

Variable	Days	Treatment				SEM	P- Value		
		Control	T_1	T_2	T_3		T	D	T×D
ALT (IU/L)	0	34.10	32.90	31.57	29.05	1.13			
	30	37.38^c	32.46^b	32.83^b	28.54^a	1.01			
	60	36.62^b	30.57^a	30.56^a	28.29^a	1.24			
	90	35.61^b	30.93^{ab}	30.81^{ab}	27.53^a	1.37			
	120	37.89^c	32.37^b	27.78^a	27.28^a	0.79			
	Mean	36.32^c	31.85^b	30.71^b	28.14^a	1.11	.000	.309	.179
AST (IU/L)	0	97.49	82.73	78.60	73.25	1.98			
	30	96.99^c	82.53^b	78.81^b	70.21^a	1.67			
	60	95.72^c	81.27^b	79.88^b	70.97^a	1.59			
	90	98.76^d	83.65^c	77.83^b	68.19^a	1.79			
	120	95.22^c	83.03^b	79.03^b	68.95^a	2.20			
	Mean	96.84^d	82.64^c	78.83^b	70.32^a	1.85	.000	.878	.891
ALP (IU/L)	0	104.98	101.65	98.98	92.10	3.81			
	30	107.32	99.89	98.81	97.53	3.35			
	60	103.65	94.46	97.90	95.00	4.20			

	90	101.62	101.57	99.00	94.27	3.12			
	120	104.94	98.05	97.85	94.28	3.41			
	Mean	104.50^{b}	99.13^{a}	98.51^{a}	94.64^{a}	3.58	.001	.822	.988

T_1, silymarin (600 mg/kg DM/d) supplemented group; T_2, nano-zinc (50 mg/kg DM/d) supplemented group; T_3, silymarin (600 mg/kg DM/d) and nano-zinc (50 mg/kg DM/d) supplemented group; SEM, Standard error mean; T, effect of treatment, D, effect of day; T × D, interaction between treatment and day

$^{a, b, c}$Mean bearing different superscripts in a row showed a statistical difference at $P < 0.05$

A significant lower activity of AST was found in treatments in Zn-fed West African dwarf goats (Belewu and Adewumi, 2021). In disaccord with the present findings, Anil *et al.,* (2020) [2] reported that there was no significant difference in AST activity of cross-bred calves supplemented with nano-zinc oxide (zinc sulphate) @ 25ppm in the basal diet. Fathi (2016) [8] and Sahoo *et al.,* (2014) [18] also reported no significant effect on the AST activity of broiler chicks treated with nano-zinc oxide. In long-term exposure of mice, the doses of nano-zinc oxides did not affect the serum levels of aspartate aminotransferase (Wang *et al.*, 2016) [19].

The plasma concentration of ALP in all four groups over a period of 120 days trial period has been depicted in Table 1. The activity of ALP was reduced statistically ($P < 0.05$) in supplemented groups either with silymarin, nano-zinc or their combination than control but the reduction was highest in the T_3 group of Murrah buffalo calves. However, no statistical difference was observed among the T_1, T_2, and T_3 groups. The mean values of ALP were 104.50, 99.13, 98.51, and 94.64 IU/L in control, T_1, T_2, and T_3, respectively. During 0, 30, 60, 90, and 120 days of the study period, ALP activity did not differ statistically among the groups. In agreement with the present finding Najafzadeh *et al*., (2013) [15] reported a decline in ALP activity with supplementation of zinc nanoparticles @ 20mg/kg BW in lamb. Similarly, Armanini *et al.,* (2021) reported silymarin reduces the secretion of hepatic enzymes such as alanine aminotransferase, aspartate aminotransferase and alanine aminotransferase. Banaee *et al.* (2010) also found the use of silymarin (800 mg/kg) as a food supplement in the diet of fishes caused the decline in ALP activity when compared with the control group. In contrast to our finding, Fathi *et al.,* (2016) [8] reported higher activity of ALP in broiler chickens that received 20 mg/kg nano-zinc oxides in their diets.

Conclusion

The present research suggested that supplementation of both silymarin and nano-zinc have positive impact in liver functions also improved the functions of ALP, AST and ALT in Murrah buffalo calves. So supplementation of silymarin and nano-zinc or combination of both (Silymarin + Nano-zinc) can improve the growth performance and antioxidant status in dairy animals.

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