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Comparative efficacy of different herbal anti-stressors in crossbred dairy cows during parturition stress

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ABSTRACT

The present study was conducted to evaluate the efficacy of different herbal anti-stressors on somatic and haemato-biochemical markers of stress during the peripartum period and their impact on milk yield in crossbred dairy cows. A total of 24 gravid crossbred cows approaching term were selected for the study and randomly divided into four equal groups. The control group (G_1) received no treatment, while the treatment groups (G_2, G_3, and G_4) were orally administered specific doses of different herbal anti-stressor preparations *viz*. Restobal, combination of Restobal, Exapar and Ketoroak, and Stenot Liquid, respectively. The results revealed that cows in the treatment groups exhibited significant improvements in various haemato-biochemical parameters, which were associated with an increase in milk yield compared to the control group. Specifically, the cows in treatment groups showed significantly higher levels of haemoglobin concentration, total erythrocyte counts, packed cell volume and neutrophil counts, along with elevated serum sodium and potassium levels. Additionally, there were reductions in serum cortisol, glutathione peroxidase, thiobarbituric acid reactive substances, catalase, creatine kinase and cholesterol levels compared to the control group. In conclusion, the herbal treatments used in this study were found to be efficacious in relieving stress earlier than the untreated control group, leading to enhanced milk production in crossbred dairy cows during the peripartum period.

Keywords: Anti-stressor, Dairy, Herbal, Milk yield, Parturition, Stress

Peripartum period is the most stressful condition as it induces several physiological changes for the onset of lactation (Konvicna *et al.* 2015; Ambily *et al.* 2019). Increased level of stress may cause different immune system and/or reproduction disorders which finally affects the productivity of dairy animals (Jerram *et al.* 2020). In bovines, majority of the production and reproduction associated diseases occur during the transition period of pregnancy which lasts from three weeks prior to calving and three weeks post-calving (LeBlanc *et al.* 2006; Reddy *et al.* 2018) and induce various haematological, blood biochemical, metabolic and endocrine changes during this phase in the life cycle of dairy animals (Tharwat *et al.* 2015; Vasantha *et al.* 2020). To overcome this parturition related stress, many anti-stressor medications have been developed including many herbal preparations with better efficacy (Bhamare *et al.* 2022a). In recent days, herbal anti-stressors are getting popularity for their better efficacy and safety. Keeping the above facts in view, an attempt was made to evaluate the efficacy of different herbal anti-stressor formulations on somatic and biochemical markers of stress during the peripartum period and their association with milk yield in crossbred dairy cows.

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MATERIALS AND METHODS

The study was conducted at North Lakhimpur, Assam, India which is located in the GPS coordinates of 27° 14' 10.7412'' N and 94° 5' 45.0240'' E from 29th January, 2020 to 31st January, 2022. A total of 24 gravid crossbred cows approaching term were selected for the study and randomly divided into four equal groups: G_1, G_2, G_3 and G_4. The cows in control (G_1) group received no treatment, while each treatment groups (G_2, G_3 and G_4) were subjected to different treatment regimens as outlined in Table 1. All the cows were provided *ad lib* access to feed and water throughout the experimental period.

Blood samples were collected from jugular vein of the cows on the 0th, 7th and 15th day of parturition and treatment supplementation. Each blood sample was divided into two parts: the first part (1 ml) was collected on disodium ethylene diamine tetracetic acid (EDTA) tubes for hemogram tests.

Table 1. Different treatment regimens applied in different groups

Group	No. of cows	Treatment	Dose and route
G_1	6	Control	No treatment
G_2	6	Restobal[1]	50 mL orally twice daily from about 5 days before to 5 days after parturition
G_3	6	Restobal[1] + Exapar[2] + Ketoroak[3]	Restobal @ 50 mL orally twice daily from about 5 days before to 5 days after parturition Exapar @ 100 mL orally after parturition twice on first day followed by 50 mL twice daily for next 5 days Ketoroak @ 200 mL orally once daily for 5 days after parturition
G_4	6	Stenot[4]	50 ml orally once daily for 10 days after parturition

[1]Marketed as Restobal® Liquid by *M/s* Ayurvet Limited, India. [2]Marketed as Exapar™ Liquid by *M/s* Ayurvet Limited, India. [3]Marketed as Ketoroak® Liquid by *M/s* Ayurvet Limited, India. [4]Marketed as Stenot Liquid by Natural Remedies Pvt. Ltd., Bengaluru.

The second part (5 ml) was placed in plain centrifuge tubes for separation of serum and the serum samples were stored at -20 ºC until used for subsequent biochemical analysis. Serum samples were evaluated for the estimation of various biochemical parameters *viz.* sodium (Na), potassium (K), chloride (Cl), catalase, glutathione peroxidase (GPx), thiobarbituric acid reactive substances (TBARS), aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), total protein, albumin and globulin, albumin to globulin ratio (A:G), blood urea nitrogen (BUN), creatinine, creatine kinase, glucose, cholesterol and cortisol with the help of commercially available test kits. Haematological parameters *viz.* total erythrocyte count (TEC), haemoglobin concentration (Hb), packed cell volume (PCV), total leukocytic count (TLC) and differential leukocytic counts (DLC) were also estimated. These parameters were evaluated according to the routine haematological procedures as described by Feldman *et al.* (2000). Rectal temperature (RT), heart rate (HR), respiration rate (RR) and milk yield of the individual animal was recorded day 0, 7 and 15 of treatment. Moreover, placental weight of the individual animal at parturition, and body weight of calf on day 0, 7 and 15 were also recorded during the study.

Ethical approval: The clinical trial protocol was reviewed and approved by the Institutional Expert Committee chaired by the Director of Research (Veterinary), Assam Agricultural University, Khanapara, Guwahati; vide meeting held on 24-08-2019 with Proceedings Memo No. 99/774/2019-20/DRV/3345-54 Dated: 5-9-19.

Statistical analysis: The data were analyzed using R Software Version 4.2.2 (© The R Foundation for Statistical Computing Platform, 2022) and RStudio Software Version 1.1.463 (© RStudio, Inc., 2009-2018). Two-way analysis of variance test was conducted, followed by Post hoc difference test between means using Duncan's multiple comparison test to identify significant differences between groups and days of treatment and their interaction (Snedecor and Cochran, 1994; Maurya *et al.* 2014). Descriptive data are presented as Mean ± Standard Error. Statistically significant differences are indicated by different superscript letters and asterisks, where * denotes significant differences at $p<0.05$ and ** denotes highly significant differences at $p<0.01$. The value of $p>0.05$ were considered as statistically non-significant.

RESULTS AND DISCUSSION

The findings related to changes in blood biochemical parameters, haematological and physiological parameters, as well as calf weight and milk yield, across four different treatment groups (G_1, G_2, G_3 and G_4) at different days of treatment (day 0, day 7, and day 15) are presented in Table 2, Table 3, and Table 4, respectively.

Changes in blood biochemical parameters: The mean serum Na levels were increased significantly ($p<0.01$) on different days of treatment among the treatment groups (Table 2). Likewise, the mean serum K levels were increased significantly ($p<0.05$) on different days of treatment within the groups. On the contrary, the mean serum Cl levels among all groups were found to be stable without any significant difference. The present findings of serum Na, K and Cl levels are in agreement with the previous reports of Skrzypczak *et al.* (2014).

The mean serum catalase profile of G_1 group was significantly different ($p<0.01$) from the other treatment groups (G_2, G_3 and G_4). The activity was significantly reduced ($p<0.01$) on different days of treatment regimens, which might be due to the reduction of oxidative stress following different treatments. Maurya *et al.* (2014) had earlier reported that catalase activity increased in cows during the transition period.

Serum cortisol is a stress assessing hormone which is secreted in higher levels during the stress response to the body. In the present study, increased serum cortisol levels were noticed in all groups during the period of parturition, which indicates that the parturition process had severe stress in cows (Hudson *et al.* 1976). As the treatment days advanced, it showed a significantly decreasing trend in the treatment groups (G_2, G_3 and G_4) compared to control (G_1) group ($p<0.01$) indicating a reduction in the parturition stress following treatments. The present findings are in corroboration with the study of Abed *et al.* (2020), who reported that increased plasma cortisol levels at calving in Friesian cows.

The significant differences ($p<0.01$) in mean serum GPx

Table 2. Changes in various blood biochemical parameters in different treatment groups at different days of treatment

Sl. No.	Parameter	Day 0				Day 7				Day 15				Significance level
		G_1	G_2	G_3	G_4	G_1	G_2	G_3	G_4	G_1	G_2	G_3	G_4	
1	Na (mmol/L)	131.793 ±0.534^{de}	131.810 ±0.569^{de}	131.470 ±0.402^{e}	131.480 ±0.569^{e}	132.950 ±0.676^{de}	138.578 ±1.062^{bc}	137.148 ±0.433^{c}	137.215 ±0.457^{c}	133.672 ±0.736^{d}	142.087 ±1.199^{a}	139.642 ±0.472^{b}	140.173 ±0.732^{ab}	**
2	K (mmol/L)	3.677 ±0.092^{c}	3.870 ±0.134^{abc}	3.703 ±0.147^{abc}	3.820 ±0.089^{abc}	3.897 ±0.101^{abc}	4.062 ±0.135^{abc}	3.802 ±0.143^{abc}	3.973 ±0.072^{abc}	3.998 ±0.102^{abc}	4.152 ±0.110^{a}	3.905 ±0.126^{abc}	4.165 ±0.069^{a}	*
3	Cl (mmol/L)	93.893 ±0.597	94.195 ±0.493	95.038 ±0.528	94.583 ±0.485	94.317 ±0.494	94.608 ±0.520	95.230 ±0.600	94.782 ±0.449	94.712 ±0.313	95.077 ±0.593	95.532 ±0.534	95.040 ±0.424	NS
4	Catalase (nmol/min/mL)	39.067 ±0.388^{a}	38.905 ±0.312^{ab}	39.000 ±0.594^{ab}	38.593 ±0.467^{ab}	37.785 ±0.324^{bc}	36.622 ±0.272^{cd}	36.492 ±0.416^{d}	36.210 ±0.507^{d}	36.892 ±0.234^{cd}	33.942 ±0.250^{e}	33.798 ±0.546^{e}	33.337 ±0.285^{e}	**
5	GPx (nmol/min/mL)	137.445 ±0.620^{a}	138.142 ±0.537^{a}	137.467 ±0.646^{a}	136.648 ±0.813^{a}	106.563 ±0.573^{b}	84.015 ±0.893^{de}	85.382 ±0.828^{cd}	80.717 ±1.752^{e}	88.348 ±2.656^{c}	62.553 ±1.136^{fg}	65.118 ±0.719^{f}	61.018 ±1.478^{g}	**
6	TBARS (μM)	2.423 ±0.005^{cd}	2.422 ±0.004^{cd}	2.413 ±0.002^{d}	2.433 ±0.005^{c}	2.470 ±0.010^{b}	2.340 ±0.006^{ef}	2.355 ±0.008^{e}	2.323 ±0.008^{f}	2.520 ±0.005^{a}	2.298 ±0.006^{g}	2.302 ±0.007^{g}	2.300 ±0.004^{g}	**
7	Cortisol (ng/mL)	26.717 ±1.443^{ab}	29.200 ±0.791^{a}	29.367 ±0.780^{a}	29.267 ±0.809^{a}	25.050 ±1.135^{bc}	23.917 ±0.769^{c}	22.800 ±1.049^{c}	22.367 ±0.861^{cd}	23.683 ±1.066^{c}	19.933 ±0.680^{de}	19.117 ±0.744^{e}	19.933 ±0.546^{de}	**
8	AST (U/L)	63.100 ±1.358^{d}	63.330 ±1.704^{d}	64.150 ±1.000^{d}	64.135 ±1.747^{d}	73.068 ±2.139^{c}	82.843 ±1.516^{b}	83.435 ±0.576^{b}	83.323 ±2.159^{b}	77.743 ±3.092^{c}	87.807 ±1.358^{ab}	91.545 ±1.049^{a}	92.560 ±2.182^{a}	**
9	ALT (U/L)	25.045 ±0.671^{d}	26.933 ±0.877^{abcd}	25.415 ±0.568^{bcd}	27.293 ±0.592^{abc}	25.332 ±0.630^{cd}	27.397 ±0.900^{abc}	25.820 ±0.733^{bcd}	27.578 ±0.423^{ab}	26.048 ±0.676^{bcd}	27.430 ±0.674^{abc}	26.698 ±0.601^{abcd}	28.265 ±0.426^{a}	*
10	ALP (U/L)	71.288 ±4.581	71.865 ±5.700	73.425 ±3.666	71.860 ±3.633	70.655 ±4.597	71.800 ±5.702	73.288 ±3.688	71.825 ±3.640	70.595 ±4.555	71.608 ±5.696	73.188 ±3.692	71.675 ±3.689	NS
11	TP (g/dL)	6.147 ±0.124	6.437 ±0.258	6.385 ±0.224	6.537 ±0.292	6.203 ±0.122	6.502 ±0.260	6.453 ±0.219	6.593 ±0.280	6.103 ±0.199	6.572 ±0.245	6.480 ±0.234	6.672 ±0.281	NS
12	Albumin (g/dL)	3.115 ±0.098^{ab}	2.873 ±0.114^{b}	3.012 ±0.143^{ab}	2.963 ±0.129^{ab}	3.198 ±0.114^{ab}	2.885 ±0.118^{b}	2.923 ±0.086^{b}	2.950 ±0.130^{ab}	3.318 ±0.126^{a}	2.943 ±0.119^{ab}	2.897 ±0.091^{b}	2.917 ±0.121^{b}	**
13	Globulin (g/dL)	3.160 ±0.077^{ab}	3.193 ±0.085^{ab}	3.258 ±0.081^{ab}	3.295 ±0.054^{a}	3.118 ±0.067^{ab}	3.097 ±0.084^{ab}	3.160 ±0.081^{ab}	3.212 ±0.053^{ab}	3.112 ±0.059^{ab}	3.015 ±0.095^{b}	3.093 ±0.077^{ab}	3.140 ±0.046^{ab}	*
14	A:G	0.987 ±0.030^{ab}	0.899 ±0.020^{b}	0.930 ±0.059^{b}	0.901 ±0.044^{b}	1.028 ±0.041^{ab}	0.931 ±0.023^{b}	0.930 ±0.043^{b}	0.920 ±0.044^{b}	1.069 ±0.048^{a}	0.976 ±0.025^{ab}	0.940 ±0.040^{b}	0.930 ±0.040^{b}	*

Table 2 contd...

Table 2 concluded

Sl. No.	Parameter	Day 0				Day 7				Day 15				Significance level
		G_1	G_2	G_3	G_4	G_1	G_2	G_3	G_4	G_1	G_2	G_3	G_4	
15	BUN (mg/dL)	14.745 ±1.203	14.997 ±1.591	14.457 ±1.290	14.832 ±1.182	14.143 ±1.157	14.730 ±1.580	14.215 ±1.324	14.667 ±1.200	13.672 ±1.227	14.168 ±1.562	13.965 ±1.259	14.310 ±1.180	NS
16	Creatinine (mg/dL)	0.615 ±0.037 ^{b}	0.723 ±0.040 ^{ab}	0.710 ±0.062 ^{ab}	0.817 ±0.102 ^{a}	0.642 ±0.030 ^{b}	0.733 ±0.045 ^{ab}	0.765 ±0.080 ^{ab}	0.832 ±0.105 ^{a}	0.655 ±0.033 ^{b}	0.757 ±0.047 ^{ab}	0.800 ±0.089 ^{ab}	0.853 ±0.108 ^{a}	*
17	Creatine kinase (ng/mL)	137.883 ±0.229 ^{a}	138.538 ±0.461 ^{a}	138.250 ±0.313 ^{a}	137.882 ±0.226 ^{a}	134.172 ±0.218 ^{b}	130.555 ±0.370 ^{cd}	131.375 ±0.399 ^{c}	130.443 ±0.472 ^{cd}	130.260 ±0.285 ^{d}	125.488 ±0.268 ^{e}	125.370 ±0.313 ^{e}	124.802 ±0.363 ^{e}	**
18	Glucose (mg/dL)	45.195 ±1.445	45.790 ±1.447	47.512 ±1.338	46.192 ±2.056	45.868 ±1.436	46.240 ±1.430	48.095 ±1.406	46.748 ±2.113	46.753 ±1.396	46.792 ±1.428	48.618 ±1.409	47.315 ±2.097	NS
19	Cholesterol (mg/dL)	139.048 ±0.401 ^{a}	138.535 ±0.399 ^{a}	138.523 ±0.360 ^{a}	139.385 ±0.207 ^{a}	134.727 ±0.276 ^{b}	133.070 ±0.120 ^{c}	132.448 ±0.172 ^{c}	134.105 ±0.426 ^{b}	129.903 ±0.493 ^{d}	124.733 ±0.343 ^{e}	125.110 ±0.390 ^{e}	125.683 ±0.365 ^{e}	**

Means with different superscript letters differ significantly ($p<0.05$) from one another in a row; NS: Statistically non-significant at $p>0.05$; *: Statistically significant at $p<0.05$; **: Statistically highly significant at $p<0.01$; Na=Sodium; K=Potassium; Cl= Chloride; GPx= Glutathione Peroxidase; TBARS=Thiobarbituric Acid Reactive Substances; AST= Aspartate Aminotransferase; ALT= Alanine Aminotransferase; ALP= Alkaline Phosphatase; TP= Total protein; A:G= Albumin to Globulin Ratio; BUN= Blood Urea Nitrogen.

levels among all groups was in agreement with the previous reports of Maurya *et al.* (2014). Likewise, the mean blood TBARS levels were significantly reduced ($p<0.01$) among the treatment groups. Being lowest on day 15 as compared to day 7 and day 0, suggesting that the antioxidant effect of treatment regimens.

Chandra *et al.* (2013) had reported an increase in blood TBARS levels during calving in Sahiwal cows.

The serum transaminases (ALT and AST) are the most sensitive indicator of hepatocellular injury (Shrimali *et al.* 2016). Moreover, elevation in alkaline phosphatase (ALP), lactate dehydrogenase (LDH), gamma-glutamyl transferase (GGT), serum proteins, and bilirubin have significant importance in the degree of cholestasis and synthetic capacity of the liver (Hauptman *et al.* 2001; Limdi and Hyde 2003). In the present study, the mean serum AST levels were found to be significantly different ($p<0.01$) between control and treatment groups on different days of treatment. However, there were no significant differences among the G_2, G_3 and G_4. These findings are in agreement with the previous reports of Yadav *et al.* (2019), who observed enzyme activities were within the normal range, indicating that the integrity and functionality of liver tissues were obviously maintained during these periods. It was also opined that the lowest plasma AST level was recorded on the day of parturition, and it increased thereafter significantly in crossbred cows (Yadav *et al.* 2019).

During the study, the mean serum ALT levels of group G_1 and G_3 were found to be significantly different ($p<0.05$) from group G_2 and G_4. The present findings are in agreement with the previous reports (Kaneko *et al.* 1997). On the contrary, Yadav *et al.* (2019) reported lowest value of ALT was recorded on the day of parturition, followed by a significant increase from day 3 onwards, reaching the highest value on day 45 of the peri-partum period. However, in the present study, a non-significant increase in the mean serum ALT levels on different days of treatment, but these levels were within the normal reference range, indicating a healthy liver. The mean serum ALP levels did not exhibit significant differences across the groups and on different days of treatment, which indicates stable liver function without significant differences in all treated groups. In contrast to the present study, Yadav *et al.* (2019) recorded ALP significantly increased ($p<0.05$) on the day of parturition, which decreased significantly ($p<0.05$) after parturition from day 3 onwards reaching the lowest value on day 45 in crossbred cows.

Similarly, the mean serum TP levels in group G_2, G_3 and G_4 showed a non-significant increasing trend from day 0 to day 15. This variation in TP levels on different days of treatment might be attributed to metabolic stress during parturition. The findings of the present study corroborate with the latest findings of Mohammed *et al.* (2021).

The mean serum albumin level of G_1 was recorded to be significantly different ($p<0.01$) from G_2, G_3 and G_4. On the contrary, non-significant differences in mean serum globulin levels were recorded and are in agreement with

Table 3. Changes in various haematological and physiological parameters in different treatment groups at different days of treatment

Sl. No.	Parameter	Day 0				Day 7				Day 15				Significance level
		G_1	G_2	G_3	G_4	G_1	G_2	G_3	G_4	G_1	G_2	G_3	G_4	
1	Hb (g/dL)	9.523 ±0.011 ^{c}	9.498 ±0.015 ^{cd}	9.460 ±0.030 ^{d}	9.515 ±0.021 ^{cd}	9.623 ±0.004 ^{b}	9.643 ±0.013 ^{b}	9.638 ±0.026 ^{b}	9.615 ±0.025 ^{b}	9.715 ±0.004 ^{a}	9.737 ±0.012 ^{a}	9.763 ±0.028 ^{a}	9.755 ±0.016 ^{a}	**
2	TEC (10^6/µl)	5.332 ±0.024 ^{d}	5.342 ±0.010 ^{d}	5.327 ±0.018 ^{d}	5.322 ±0.009 ^{d}	5.402 ±0.022 ^{c}	5.482 ±0.010 ^{b}	5.452 ±0.016 ^{b}	5.447 ±0.016 ^{bc}	5.478 ±0.027 ^{b}	5.603 ±0.008 ^{a}	5.588 ±0.009 ^{a}	5.592 ±0.010 ^{a}	**
3	PCV (%)	32.382 ±0.086 ^{d}	32.473 ±0.073 ^{d}	32.428 ±0.152 ^{d}	32.605 ±0.103 ^{d}	33.197 ±0.091 ^{c}	34.273 ±0.061 ^{b}	34.270 ±0.187 ^{b}	34.560 ±0.189 ^{b}	33.562 ±0.156 ^{c}	35.272 ±0.088 ^{a}	35.218 ±0.190 ^{a}	35.525 ±0.138 ^{a}	**
4	TLC (/cumm)	6871.333 ±32.781	6821.833 ±47.638	6818.667 ±55.671	6847.833 ±40.745	6887.167 ±36.656	6838.333 ±44.303	6826.333 ±56.751	6899.500 ±47.579	6907.500 ±45.940	6851.167 ±43.262	6841.167 ±56.953	6912.167 ±48.836	NS
5	Neutrophils (/cumm)	2345.333 ±19.174 ^{c}	2352.167 ±20.213 ^{c}	2359.167 ±38.679 ^{c}	2360.500 ±14.771 ^{c}	2489.667 ±34.248 ^{ab}	2496.667 ±31.994 ^{a}	2519.833 ±42.212 ^{a}	2561.333 ±26.920 ^{a}	2389.333 ±40.514 ^{bc}	2489.000 ±23.111 ^{ab}	2483.000 ±53.559 ^{ab}	2469.667 ±21.556 ^{ab}	**
6	Lymphocytes (/cumm)	5268.833 ±61.803 ^{bc}	5401.500 ±31.607 ^{ab}	5410.667 ±53.486 ^{ab}	5526.333 ±29.299 ^{a}	5135.667 ±74.693 ^{cde}	5153.000 ±41.796 ^{cde}	5225.500 ±73.413 ^{cd}	5378.833 ±23.334 ^{ab}	5021.500 ±54.444 ^{ef}	4942.500 ±35.258 ^{f}	5064.167 ±70.865 ^{def}	5199.000 ±27.133 ^{cd}	**
7	Monocytes (/cumm)	255.500 ±6.541 ^{a}	265.000 ±4.926 ^{a}	267.333 ±6.195 ^{a}	255.833 ±8.396 ^{a}	211.500 ±2.766 ^{bc}	209.000 ±1.633 ^{bcd}	217.000 ±4.017 ^{bc}	221.333 ±4.702 ^{b}	193.000 ±3.347 ^{ef}	184.000 ±3.246 ^{f}	196.167 ±5.250 ^{def}	203.333 ±4.499 ^{cde}	**
8	Eosinophils (/cumm)	256.500 ±6.212 ^{ab}	247.167 ±4.183 ^{b}	266.500 ±2.363 ^{a}	257.000 ±7.076 ^{ab}	231.333 ±2.777 ^{c}	208.833 ±2.822 ^{de}	220.500 ±3.181 ^{cd}	227.333 ±5.731 ^{c}	213.667 ±2.140 ^{d}	191.167 ±1.956 ^{f}	193.667 ±5.631 ^{f}	200.333 ±5.071 ^{ef}	*
9	Basophils (/cumm)	47.833 ±3.736 ^{a}	49.667 ±4.514 ^{a}	49.500 ±1.746 ^{a}	56.167 ±4.175 ^{a}	36.000 ±2.191 ^{b}	31.333 ±2.728 ^{bc}	28.000 ±2.708 ^{bcd}	28.167 ±3.219 ^{bcd}	24.833 ±2.664 ^{cde}	18.500 ±0.957 ^{e}	18.000 ±1.693 ^{e}	19.667 ±1.085 ^{de}	**
10	RT (°F)	102.130 ±0.531	101.721 ±0.752	101.539 ±0.338	101.661 ±0.772	100.746 ±0.295	101.632 ±0.234	100.661 ±0.472	101.361 ±0.872	100.361 ±0.772	100.311 ±0.742	101.734 ±0.537	101.362 ±0.771	NS
11	HR (beats/min)	77.457 ±1.031^{a}	75.671 ±0.674^{ab}	75.643 ±0.554^{ab}	74.343 ±0.851^{b}	74.643 ±0.854^{c}	73.343 ±0.851^{de}	74.131 ±0.612^{cd}	74.243 ±0.853^{c}	71.354 ±0.451^{f}	71.341 ±0.551^{f}	74.112 ±0.614^{d}	72.113 ±0.653^{ef}	*
12	RR (breaths/min)	23.546 ±1.027	24.556 ±1.024	24.146 ±1.026	23.156 ±1.031	22.645 ±0.637	23.542 ±1.022	22.146 ±1.025	22.146 ±1.021	22.912 ±1.016	23.126 ±1.025	23.556 ±1.020	23.146 ±1.021	NS

Means with different superscript letters differ significantly ($p<0.05$) from one another in a row; NS: Statistically non-significant at $p>0.05$; *: Statistically significant at $p<0.05$; **: Statistically highly significant at $p<0.01$; TEC= Total Erythrocyte Count; Hb= Haemoglobin; PCV= Packed Cell Volume; TLC=Total Leukocyte Count; RT= Rectal temperature; HR= Heart rate; RR= Respiration rate.

Table 4. Changes in calf weight and milk yields in different treatment groups at different days of treatment

Sl. No.	Trait	Day 0				Day 7				Day 15				Significance level
		G_1	G_2	G_3	G_4	G_1	G_2	G_3	G_4	G_1	G_2	G_3	G_4	
1	CW (kg)	24.353 ±0.882 e	26.192 ±1.055 bcde	24.752 ±0.834 de	24.785 ±0.650 de	25.023 ±0.894 cde	27.253 ±1.081 abcd	25.858 ±0.843 bcde	25.803 ±0.675 bcde	26.267 ±0.895 abcde	28.988 ±0.980 a	27.947 ±0.828 ab	27.748 ±0.691 abc	**
2	MY (kg)	3.950 ±0.304 e	5.167 ±0.441 de	5.400 ±0.473 d	4.817 ±0.375 de	5.333 ±0.459 d	7.283 ±0.277 c	7.950 ±0.528 bc	7.150 ±0.208 c	7.133 ±0.406 c	9.050 ±0.496 ab	9.917 ±0.651 a	9.350 ±0.316 a	**

Means with different superscript letters differ significantly ($p<0.05$) from one another in a row; NS: Statistically non-significant at $p>0.05$; *: Statistically significant at $p<0.05$; **: Statistically highly significant at $p<0.01$; CW= Calf weight; MY= Milk Yield.

the reports of Jhambh *et al.* (2016) in dairy cows and fall within the normal reference range. The globulin fraction was lower than the albumin fraction in contrast to Magnus and Lali (2009). Benjamin (1978) opined that a higher level of globulin in serum is an indicator of bacterial infection. The mean albumin to globulin ratio (A:G) of cows in G_1 (control) was significantly different ($p<0.01$) from G_2, G_3 and G_4 but non-significantly different on different days of treatments. These findings are in collaboration with the reports of Jhambh *et al.* (2016) in dairy cows. Magnus and Lali (2009) stated that the A:G ratio should be equal in normal animals. The altered A:G ratio in this study might be due to parturition related stress and activities.

In this study, the mean serum BUN profiles did not exhibit significant differences among the groups and on different days of treatment indicate no significant renal function impairment due to the treatments. Many researchers have reported serum urea to be influenced by diet and as an indicator of total protein intake in Egyptian buffaloes (Ashmawy 2015). However, the serum BUN levels recorded in the present study were within the normal reference range and are in accordance with the reports by Radostitis *et al.* (2007). However, the differences in mean serum creatinine levels were non-significant on different days of treatment but significant among groups. The present findings are in agreement with the results of Ashmawy (2015). The mean serum creatine kinase levels reduced significantly ($p<0.01$) on different days of treatment among the groups and across G_2, G_3 and G_4 from day 0 to day 7 and further from day 7 to day 15 (Table 2). These findings are in accordance with the reports of Al-Watar *et al.* (2021), who reported increased creatine kinase levels due to cellular damage and inflammatory processes. The higher levels of creatine kinase on pre-treatment days might be due to the separation of placenta and associated tissue damage and inflammatory changes during parturition. However, the reduction in mean creatine kinase levels in all treatment groups might be attributed to the reduction in stress associated with parturition following treatments.

The differences in mean blood glucose levels were non-significant between the groups and days of treatments. These findings are similar to the findings observed by Ashmawy (2015) in Egyptian buffaloes. The mean blood cholesterol values of cows in G_2 and G_3 varied significantly ($p<0.01$) with G_1 and G_4 and days of treatment perhaps due to adjustments in physiological processes. These findings are as per with the study of Jambh *et al.* (2016), which reported higher cholesterol values in lactating cows and buffaloes.

Changes in haematological and physiological parameters: The changes in various haematological and physiological parameters at different time intervals in different treatment groups are given in Table 3. The non-significant differences in the mean blood Hb, TLC, neutrophils, monocytes and basophils levels but significantly different ($p<0.01$) PCV, TEC, lymphocytes and eosinophils profiles were found among the different groups. The blood Hb, PCV, TEC, neutrophils, lymphocytes, monocytes, eosinophils and basophils profiles were significantly different ($p<0.01$) on different days of treatments. These findings are in agreement with the observations of Reddy *et al.* (2018) in parturition-stressed buffaloes.

Haematology revealed the elevated levels of Hb, TEC, PCV and neutrophil counts in treated groups (G_2, G_3 and G_4) than control group (G_1) in day 15 of treatment. This increase in mean TEC might be due to increase in erythropoiesis in the treated groups. The present findings are in accordance with the previous report of Sivajothi *et al.* (2018), who reported an elevation in total erythrocyte count in the treated group of buffaloes, which had a role in the development of immunity. Non-significant differences in physiological parameters *viz*. rectal temperature, heart rate and respiration rate were observed (Table 3). However, there was significant difference ($p<0.05$) in terms of heart rate on different days of treatment. These findings are in accordance with the reports of Reddy *et al.* (2018).

Changes in placental weight at parturition: The average weights of placenta among all groups at parturition showed no significant difference, which aligns with the findings of Vijayakumar *et al.* (2019) in crossbred Jersey cows. Cows in treatment group, G_4 exhibited highest average placental weight (2.570±0.032 kg) among all groups at parturition, followed by G_3 (2.568±0.063 kg), G_2 (2.527±0.071 kg). In contrast, cows in G_1 exhibited the average placental weight

of 2.507±0.078 kg at parturition.

Changes in calf weight and milk yield: Though, non-significant differences were recorded in mean calf weights among all groups at parturition. G_2 had slightly higher average calf weight as compared to the other groups (Table 4). This finding is in agreement with the observation of Vijayakumar *et al.* (2019) in crossbred Jersey cows. At day 15 of treatment, the mean differences in calf weight among groups ($p<0.01$) became more pronounced in the present study as compared to day 0 and day 7 indicating positive impact of herbal treatment on calf weight during the study period.

The average milk yield varied significantly among different animal groups and on days of treatment ($p<0.01$). However, the interaction effect between the groups and days of treatment did not exhibit significant differences. Interestingly, cows in G_3 consistently had the highest milk yield across all days (Fig. 1). Most groups, except G_1 (untreated control group), exhibited higher average milk yield than the observed grand mean of milk yields, particularly on day 7 and day 15 of treatment, indicating an overall improvement in milk yield in the groups treated with polyherbal anti-stressors.

As the treatment days advanced the biochemical parameters and/or stress markers estimated in serum such as cortisol, GPx, TBARS, creatine kinase, cholesterol levels were found to be significantly decreased while, haemato-biochemical parameters *viz*. TEC, Hb, PCV, serum sodium and potassium levels and neutrophil counts were found to be significantly increased in treatment groups (G_2, G_3 and G_4) as compared to control (G_1) group (Table 2 and Table 3). Interestingly, the treatment groups showed significantly higher milk yields as compared to the control group (Fig. 1), indicating the efficacy of these treatments in improving dairy cow productivity during the peripartum period.

The polyherbal product Restobal® (M/s Ayurvet Limited, India) used in the present study contains several herbal ingredients viz., *Ocimum sanctum*, *Phyllanthus emblica*, *Mangifera indica* and *Withania somnifera*. Whereas, the polyherbal product Exapar™ Liquid (M/s Ayurvet Limited, India) used in this study contains *Aloe barbadensis*, *Aristolochia indica*, *Citrullus colocynthis*, *Cyperus rotundus*, *Caesalpinia bonducella*, *Desmodium gangeticum*, *Gardenia gummifera*, *Gloriosa superba*, *Gossypium herbaceum*, *Inula racemose*, *Leptadenia reticulata*, *Lepidium sativum*, *Plumbago zeylanica*, *Peganum harmala*, *Piper longum*, *Rubia cordifolia*, *Saraca indica*, *Tribulus terrestris* and *Uraria picta.* Additionally, the polyherbal product Ketoroak® Liquid (M/s Ayurvet Limited, India) contains ingredients like *P. niruri* (Bhuiamlaki), *Tephrosia purpurea* (Sharpunkha), *Asparagus racemosus* (Shatavari), *Glycyrrhyza glabra* (Mulethi). In contrast, the polyherbal product Stenot Liquid (Natural Remedies Pvt. Ltd., Bengaluru) used in this study possess key ingredients such as tulsi, ashwagandha, guduchi, amalaki, vidari, amra, and silajatu.

O. sanctum has been used as traditional remedy against stress since many decades in India (Ravindran *et al.* 2005; Bathala *et al.* 2012; Saxena *et al.* 2012). *P. emblica* contains polyphenols that protect cell constituents from oxidative damage through potent free radical scavenging. It has defensive antioxidant mechanisms and increases the levels of glutathione, antioxidant capacity, and the activities of super oxide dismutase, catalase, glutathione peroxidase, glutathione reductase, and glutathione-S-transferase (Saha and Verma 2015; Barbosa *et al.* 2020). *M. indica* possesses antioxidant, immunomodulatory, anti-allergic, anti-inflammatory, antitumor, antidiabetic, lipolytic, anti-bone resorption, monoamine oxidase-inhibiting, antimicrobial and antiparasitic properties (Shah *et al.* 2010). *W. somnifera* have antistress, anti-inflammatory, antioxidant, antitumor, antipyretic, immunomodulatory and hemopoetic properties (Anju 2011; Kalra and Kaushik 2017). Restobal® liquid (M/s Ayurvet Limited, India) has been used as supportive therapy against the vaccination stress in buffaloes (Sivajothi *et al.* 2018), parturition stress in buffaloes (Reddy *et al.* 2018) and heat stress in Gir cows (Bhamare *et al.* 2022b).

The herbal ingredients of Exapar Liquid *viz*. *Aloe*

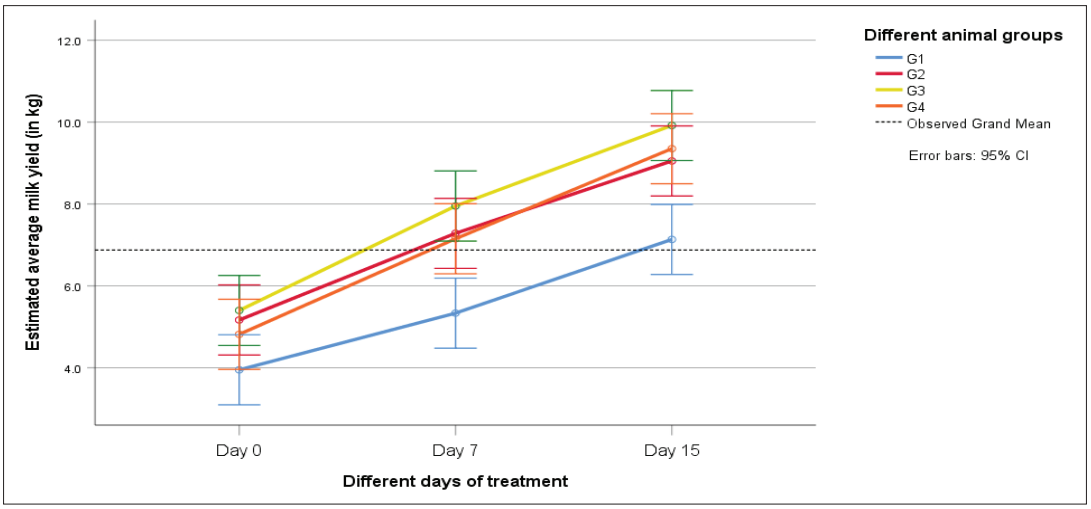

Fig. 1. Average milk yield (in kg) of different animal groups estimated at different days of treatment

barbadensis and *Gloriosa superba* among others possess antibacterial, antifungal, anti-inflammatory, immunomodulatory, antioxidant, and healing properties (Jadhav *et al.* 2020). *Piper longum*, *Tribulus terrestris* and *Cyperus rotundus* are well known for their potent anti-inflammatory activity (Hussain *et al.* 1992). *Uraria picta*, *Tribulus terrestris*, *Plumbago zeylanica* and *Leptidium sativum* are scientifically proven to have adaptogenic and immunomodulatory properties (Satyavati *et al.* 1987). Koppad *et al.* (2009) reported that Exapar Liquid significantly improved the local immune response in animals, as evidenced by a significant increase in total immunoglobulins, phagocytic response and polymorphonuclear cells. Khanna *et al.* (1997) observed that Exapar Liquid is effective in improving the reproductive health of buffaloes by promoting the early expulsion of the placenta, facilitating uterine involution, reducing the period of postpartum anestrus, and improving conception rates. *Phyllanthus niruri* has an anti-oxidant property (Harish and Shivanandappa 2006) and has been used in the treatment of ketosis.Thakur and Bigoniya (2014) had reported that increase in milk yield may be attributed to the anti-oxidant and free radical scavenging properties of *Phyllanthus niruri*. Manjekar *et al.* (2008) also reported increased milk yield following recuperation from ketosis attributable to the hepatoprotective effect of *Phyllanthus niruri*. In the Ayurvedic system of medicine, *Asparagus racemosus* has been extensively used for the treatment of stress-related immune disorders and to improve general health. Bakshi *et al.* (2004) reported galactagogue properties of *Asparagus racemosus* while *Glycyrrhyza glabra* has antidepressant and anti-stress properties (Trivedi and Sharma 2011).

The anti-stressor preparations used in the present study had herbal ingredients with many beneficial effects such as anti-stress, antidepressant, anti-inflammatory, antioxidant, immunomodulatory, anti-allergic, antimicrobial, antiparasitic, hepatoprotective and galactagogue properties, etc. Moreover, the results of the study indicated that indigenous polyherbal preparation Restobal® (M/s Ayurvet Limited, India) or Stenot (Natural Remedies Pvt. Ltd., Bengaluru) can be used as supportive therapy in the management of parturition stress in crossbred dairy cows during peripartum period.

In conclusion, the findings of the present study provided an idea that the animals during transition period of parturition suffer from severe stress altering various haematological, blood biochemicals and physiological parameters, which can be ameliorated using ayurvedic supplements. Earlier recovery might enhance the milk production and hasten the subsequent attainment of postpartum ovarian rebound in crossbred dairy cows.

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AUTHORSHIP CONTRIBUTION STATEMENT

SNY, NA, and PKB designed the work, performed the research work and drafted the manuscript. DP performed the statistical analysis of data and drafted the manuscript. All authors read and approved the final manuscript.

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