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Research Article

THERAPEUTIC EFFECTS OF FEEDING GILOY (*TINOSPORA CORDIFOLIA*) AND FENUGREEK (*TRIGONELLA FOENUM-GRAECUM*) ON BLOOD BIOCHEMICAL PROFILE IN JERSEY CROSSBRED COWS

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ABSTRACT: The study was conducted to evaluate the effect of Giloy (*Tinospora cordifolia*) and Fenugreek (*Trigonella foenum-graecum*) supplementation on blood biochemical parameters. The lactating cows were randomly divided into four groups, each group having six animals. T_1, T_2 and T_3 group cows received Giloy stem powder (150 g), Fenugreek seed powder (150 g), and a combination of both the herbs (75 g of each) respectively, mixed with the concentrate feed for 60 days while the control group (T_0) received only the concentrate. Blood sampling was done fortnightly, from Day 0 to Day 75. Blood plasma samples were analyzed for biochemical parameters viz. glucose, protein profile (Total protein, albumin, globulin and A: G ratio) and renal profile (urea nitrogen and creatinine).). It was found that the feeding of Giloy stem powder lead to significantly lower levels ($p \leq 0.05$) of blood glucose in the blood of lactating dairy cows. Most of the other blood biochemical parameters remained unchanged in control and treated animals.

Key words: Giloy (*Tinospora cordifolia*), Fenugreek (*Trigonella foenum-graecum*), Blood biochemical profile, Jersey crossbred.

INTRODUCTION

The Himalayas are a rich repository of medicinal herbs. However, the areas with rich biodiversity remain potentially unexplored. The traditional knowledge about wild and cultivated veterinary medicinal plants used by indigenous people has not been much documented in the state. Discovery and development of newer drugs to replace ineffective or less effective drugs is a continuous phenomenon in modern medicine (Pattanayak *et al.* 2016). Giloy (*Tinospora cordifolia*), also known as *guduchi*, occupies the top spot in "*Ayurvedic Materia Medica*" and it has been designated as "*Rasayana*" (Bhattacharyya and Bhattacharya 2013). This plant finds mention in ancient Sanskrit literature like *Charak Samhita* and *Sushruta Samhita* as a potential healer of many diseases. The root extract of *T. cordifolia* is shown to exert hypolipidemic activity (Stanley *et al*. 1999), hypoglycaemic activity and to reduce serum and tissue cholesterol, phospholipids and free fatty acids (Stanley *et al.* 2000). The alcoholic extract of *T. cordifolia* (500mg/kg body weight, orally) decreased the increased levels of serum creatinine, blood urea nitrogen and alkaline phosphatase in cisplastin induced nephrotoxicity in rats (Khanam *et al.* 2011). *T. cordifolia* supplementation in lactating Murrah buffaloes caused a higher plasma catalase activity and growth hormone concentration (Mir *et al.* 2013). *T. cordifolia* supplemented group in Murrah buffaloes showed that plasma glucose concentration was higher ($p>0.05$) than the control (Mir *et al.* 2014).

Fenugreek (*Trigonella foenum-graecum*) is a leguminous herb cultivated in India. The endosperm of the seed is rich in galactomannan and the young seeds mainly contain carbohydrates and sugar. Mature seeds contain the amino acid, fatty acid, vitamins and saponins. The main chemical constituents of fenugreek are fibres, flavonoids, polysaccharides, saponins and polysaccharides and some identified alkaloids such as trigonelline and choline (Toppo *et al.* 2009). Lower blood glucose ($p<0.05$) and urea ($p<0.01$) levels were reported in goats when fed on with fenugreek seeds (Alamer and

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Basiouni 2005, Al-Janabi 2012). Fenugreek seeds have also been reported to lower the urea and creatinine content in plasma (Hamden *et al.* 2010). Blood plasma total protein, albumin, globulin, cholesterol, glucose and total lipids were found to have no significant difference on fenugreek supplementation in a study conducted on dairy goats (Al-Shaikh *et al.* 1999). Fenugreek seeds treatments showed significantly ($p<0.05$) increased blood glucose, total protein, albumin and creatinine in buffaloes (Abo El-Nor *et al.* 2007). Contrarily, fenugreek seeds have been evaluated for significantly decreased serum glucose, creatinine levels in streptozotocin-induced diabetic rats (Eidi *et al.* 2007). Treatment with fenugreek seed powder restored the levels of serum urea and creatinine levels as well as alkaline phosphatase, aspartate aminotransferase and alanine amonitransferase activities in gamma radiation irradiated rats which showed an increase in these biochemical parameters post exposure (El-Tawil 2009). The scientific literature lacks reports of effects of the herbs in dairy cattle and thus study was designed to evaluate their therapeutic effects in lactating dairy animals.

MATERIALS AND METHODS

The study trial was conducted on Jersey crossbred, healthy cows in various stages of lactation, maintained at the Instructional Livestock Farm, College of Veterinary and Animal Sciences, CSKHPKV, Palampur (Himachal Pradesh), India after approval of study from Directorate of Research, CSKHPKV. Necessary permission for conducting the trial was obtained from In charge, Instructional Livestock Farm. The experimental animals were maintained in loose housing system, under standard feeding and management conditions being followed at Livestock farm. The animals were fed twice daily and watered *ad libitum*. The major fodder provided to the cows during entire study consisted of Setaria, Maize, Sorghum, local grass. In addition, the animals were also offered concentrate during milking time.

The Jersey crossbred lactating cows randomly divided into four groups, each group having six animals. T_1, T_2 and T_3 group cows received Giloy stem powder (150 g), or Fenugreek seed powder (150 g), and a combination of both the herbs (75 g of each) respectively, mixed with the concentrate feed for 60 days while the control group (T_0) received only the concentrate. The herbal treatment was administered at a fixed time daily *i.e.* afternoon milking hours, to all the animals till day 60 of the experimental trial. Blood samples from experimental dairy cows were collected at regular intervals of 15 days till Day 75 of the experiment. First sampling was done a day before the start (Day 0) of feeding trial. Sampling on Day 75 was done to evaluate residual effect of treatment, if any. The harvested blood plasma was then stored at -20°C till further analysis. The blood samples were taken by asceptic venipuncture technique (jugular vein) and were collected in heparinised tubes. Blood plasma biochemical parameters viz. Glucose, Protein profile (Total protein, Albumin, Globulin) and Renal profile (Urea Nitrogen, Creatinine) were determined using biochemical estimation kits (Manufactured by Agappe Diagnostics Ltd., Agappe Hills, Distt. Ernakulam Kerala, India) on automatic blood biochemistry analyzer (Mispa Nano, Agappe) on automatic blood biochemistry analyzer (Mispa Nano, Agappe). The results so obtained in the study were analyzed using computer software 'SAS Enterprise Guide'. The data was analyzed by using ANOVA at 5 % level of significance.

RESULTS AND DISCUSSION

Plasma glucose (mg/dL)

Glucose (mg/dL) in the blood plasma of lactating cows treated with herbal supplements and control group are depicted below in Fig. 1 and tabulated in Table 2.

Blood glucose concentration remained in normal physiological range (Kaneko *et al.* 2008). The control (T_0) group did not show much variation in blood glucose during first 60 days. A significantly elevated blood

Table 1. Composition of concentrate feed supplied to dairy cows (per 100kg).

Ingredient	Quantity (kg)	Ingredient	Quantity (kg)
Maize	30	Mineral Mixture	3
Wheat Bran	10	Urea	1
Deoiled Rice Bran	15	Cottonseed Cake	8
Ground Nut Cake	10	Soya flakes	5
Lime Powder	1	Mustard Cake	9
Molasses	6	Bypass fat	1
Salt	1		
Total			**100**

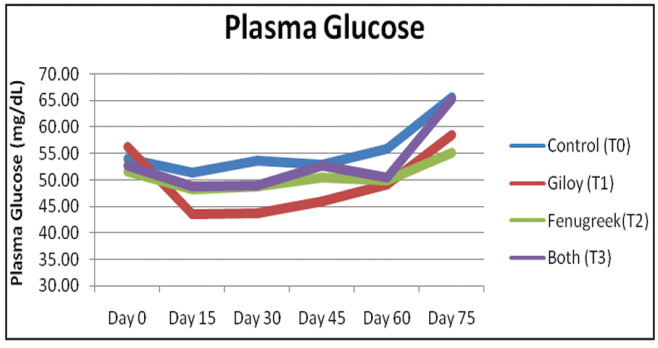

Fig. 1. Glucose (mg/dL) in the blood plasma of lactating cows treated with herbal supplements and control group.

glucose level was observed on day 75. The blood plasma glucose concentration in giloy fed cows (T_1 group) was significantly ($p<0.05$) lower as compared to control group from day 15 onwards. T_2 and T_3 groups did not exhibit any significant variation in blood glucose. An interesting observation was the significant increase in blood glucose values on day 75. As this elevation occurred in control as well treatment groups, it cannot be linked to herbal supplementation.

An increase, as well as a decline in blood glucose levels after herbal supplementation, has been reported by various research workers. Wadood *et al.* (1991) and Stanley *et al.* (2003) documented a decline in blood glucose levels on *T. cordifolia* extract administration in lab animal studies. However, Mir *et al.* (2014) reported higher blood glucose levels in Murrah buffaloes in *T. cordifolia* treated group. Alamer and Basiouni (2005) found lower blood glucose in fenugreek fed goats. Contrarily, Abo El-Nor *et al.* (2007) reported an increase in blood glucose levels in fenugreek fed buffaloes. Vijayakumar *et al.* (2005) and Hamden *et al.* (2010) documented a decline in blood glucose levels on fenugreek extract administration in lab animal studies. Giloy (*Tinospora cordifolia*) is a documented herbal galactogogue (Mallick and Prakash 2011, Mir *et al.* 2014). Various studies reported a negative correlation between blood glucose and milk yield (Mouffok *et al.* 2013, Chládek and Máchal 2004, Djokoviæ *et al.* 2017). Thus the decline in the blood glucose level can be attributed to the enhanced glucose demand by mammary gland due to higher milk yield in the crossbred cows.

Plasma protein profile

Total Protein, Albumin, Globulin and Albumin: Globulin ratio in the blood plasma of lactating cows treated with herbal supplements and control are tabulated in Table 3, 4, 5 and 6.

The values for total protein were slightly higher than standard reference (6.74 to 7.46 g/dL) for cattle (Kaneko

Table 2. Glucose (mg/dL) in the blood plasma of lactating cows treated with herbal supplements and control group (Mean±S.E.).

Group	Day 0	Day 15	Day 30	Day 45	Day 60	Day 75
T_0	54.03^{bc} ±0.62	51.38^{cx}±1.79	53.55^{bcx}±0.65	52.90^{bcx}±1.02	55.95^{bx}±1.97	65.57^{ax}±0.69
T_1	56.22^{a} ±1.82	43.60^{cy}±2.07	43.63^{cy}±1.20	45.87^{bcy}±0.78	49.10^{by}±0.76	58.53^{ay}±2.17
T_2	51.57^{a} ±2.17	48.22^{axy}±1.96	48.75^{axy}±2.85	50.48^{axy}±2.76	49.93^{ay}±2.65	55.15^{ay}±2.42
T_3	52.77^{b} ±1.39	48.75^{bxy}±2.04	48.92^{bxy}±2.31	52.77^{bx}±1.58	50.40^{bxy}±1.69	65.30^{ax}±1.61

Figures with different superscripts (a, b, c) differ significantly ($p<0.05$) between rows.
Figures with different superscripts (x, y, z) differ significantly ($p<0.05$) between columns.
T_0-Control, T_1- Giloy, T_2-Fenugreek, T_3- Both (Fenugreek+Giloy).

Table 3. Total Protein (g/dL) in the blood plasma of lactating cows treated with herbal supplements and control group (Mean±S.E.).

Group	Day 0	Day 15	Day 30	Day 45	Day 60	Day 75
T_0	7.66^{a}±0.19	7.40^{a}±0.11	7.60^{a}±0.09	7.55^{a}±0.07	7.60^{a}±0.09	7.60^{a}±0.09
T_1	7.58^{a}±0.17	7.17^{a}±0.25	7.45^{a}±0.16	7.50^{a}±0.15	7.43^{a}±0.19	7.45^{a}±0.24
T_2	7.45^{a}±0.11	7.10^{a}±0.29	7.65^{a}±0.19	7.35^{a}±0.18	7.30^{a}±0.13	7.33^{a}±0.25
T_3	7.42^{ab}±0.19	7.17^{b}±0.18	7.60^{a}±0.24	7.47^{ab}±0.23	7.30^{ab}±0.21	7.30^{ab}±0.40

Figures with different superscripts (a, b, c) differ significantly ($p<0.05$) between rows.
Figures with different superscripts (x, y, z) differ significantly ($p<0.05$) between columns.
T_0-Control, T_1- Giloy, T_2-Fenugreek, T_3- Both (Fenugreek+Giloy).

Table 4. Albumin (g/dL) in the blood plasma of lactating cows treated with herbal supplements and control group (Mean±S.E.).

Group	Day 0	Day 15	Day 30	Day 45	Day 60	Day 75
T_0	3.23^{a}±0.05	3.00^{bxy}±0.06	3.10^{bx}±0.03	3.13^{abx}±0.02	3.08^{b}±0.05	3.08^{b}±0.03
T_1	3.12^{a}±0.05	2.87^{by}±0.04	2.97^{aby}±0.03	2.98^{aby}±0.02	2.97^{ab}±0.07	2.97^{ab}±0.11
T_2	3.17^{a}±0.07	2.95^{abxy}±0.03	3.10^{abx}±0.06	3.13^{abx}±0.05	2.87^{b}±0.16	2.97^{ab}±0.10
T_3	3.27^{a}±0.05	3.05^{abx}±0.04	3.18^{ax}±0.02	3.22^{ax}±0.03	2.88^{b}±0.10	3.10^{a}±0.11

Table 5. Globulin (g/dL) in the blood plasma of lactating cows treated with herbal supplements and control group (Mean±S.E.).

Group	Day 0	Day 15	Day 30	Day 45	Day 60	Day 75
T_0	4.43^{a}±0.16	4.40^{a}±0.11	4.50^{a}±0.11	4.43^{a}±0.10	4.53^{a}±0.14	4.53^{a}±0.14
T_1	4.47^{a}±0.16	4.30^{a}±0.22	4.48^{a}±0.16	4.52^{a}±0.14	4.46^{a}±0.16	4.48^{a}±0.15
T_2	4.28^{a}±0.14	4.15^{a}±0.27	4.55^{a}±0.21	4.22^{a}±0.20	4.43^{a}±0.27	4.37^{a}±0.20
T_3	4.15^{a}±0.21	4.12^{a}±0.21	4.42^{a}±0.22	4.25^{a}±0.21	4.42^{a}±0.22	4.20^{a}±0.32

Table 6. Albumin: Globulin ratio in the blood plasma of lactating cows treated with herbal supplements and control group (Mean±S.E.).

Group	Day 0	Day 15	Day 30	Day 45	Day 60	Day 75
T_0	0.73^{a}±0.03	0.68^{a}±0.02	0.69^{a}±0.02	0.71^{axy}±0.03	0.68^{a}±0.02	0.68^{a}±0.02
T_1	0.70^{a}±0.03	0.67^{a}±0.03	0.67^{a}±0.03	0.66^{ay}±0.02	0.67^{a}±0.01	0.66^{a}±0.02
T_2	0.75^{a}±0.04	0.73^{a}±0.05	0.69^{a}±0.04	0.75^{axy}±0.04	0.65^{a}±0.07	0.68^{a}±0.03
T_3	0.80^{a}±0.05	0.75^{a}±0.04	0.73^{a}±0.04	0.77^{ax}±0.04	0.65^{a}±0.06	0.75^{a}±0.05

Table 7. Urea Nitrogen (mg/dL) in the blood plasma of lactating cows treated with herbal supplements and control group (Mean±S.E.).

Group	Day 0	Day 15	Day 30	Day 45	Day 60	Day 75
T_0	23.98^{by}±2.14	27.28^{by}±1.02	29.65^{ab}±2.41	34.23^{a}±1.72	28.48^{abx}±0.74	29.78^{ab}±2.47
T_1	29.83^{ax}±1.00	32.83^{ax}±2.16	32.02^{a}±2.07	32.03^{a}±3.56	26.03^{axy}±1.44	30.52^{a}±3.23
T_2	25.95^{bxy}±1.20	27.57^{by}±0.85	28.45^{b}±0.78	34.10^{a}±1.99	24.33^{by}±0.83	27.60^{b}±2.76
T_3	26.18^{bcxy}±1.50	29.25^{abcxy}±1.29	32.25^{a}±2.16	34.05^{a}±1.64	24.58^{cy}±1.49	31.80^{ab}±2.93

Table 8. Creatinine (mg/dL) in the blood plasma of lactating cows treated with herbal supplements and control group (Mean±S.E.).

Group	Day 0	Day 15	Day 30	Day 45	Day 60	Day 75
T_0	0.75^{a}±0.05	0.78^{a}±0.05	0.75^{a}±0.04	0.78^{a}±0.03	0.83^{a}±0.06	0.83^{a}±0.05
T_1	0.72^{a}±0.07	0.67^{a}±0.04	0.72^{a}±0.03	0.73^{a}±0.02	0.77^{a}±0.04	0.82^{a}±0.07
T_2	0.75^{a}±0.04	0.67^{a}±0.03	0.73^{a}±0.02	0.72^{a}±0.02	0.78^{a}±0.05	0.77^{a}±0.12
T_3	0.79^{ab}±0.03	0.73^{b}±0.02	0.83^{ab}±0.06	0.77^{ab}±0.02	0.78^{ab}±0.05	0.92^{a}±0.07

In Table No. 4, 5, 6, 7, 8, figures with different superscripts (a, b, c) differ significantly ($p<0.05$) between rows.
Figures with different superscripts (x, y, z) differ significantly ($p<0.05$) between columns.
T_0-Control, T_1- Giloy, T_2-Fenugreek, T_3-Both (Fenugreek+Giloy).

et al. 2008). The plasma total protein values in control (T_0) and treatment groups (T_1, T_2 and T_3) were found to be in normal range and did not show much variation during the trial.

The albumin values are in agreement with the standard reference range for cattle (3.03 to 3.55 g/dL) given by Kaneko *et al.* (2008). The plasma albumin values in the case of control (T_0) group and treatment groups (T_1, T_2 and T_3) the albumin values declined on day 15 to a lower value and remained low within the group for entire study period with no particular trend observed. The albumin values from day 15 to day 45 in the T_1 group were found to be lower as compared to control group. However, in T_2 and T_3 albumin values were similar to the corresponding values of the control group. The albumin levels did not vary much after thereafter.

The plasma globulin (g/dL) in the blood plasma is higher than the standard reference values (3.00 to 3.48 g/dL) for cattle (Kaneko *et al.* 2008). Frequent vaccination and exposure to various microbes (in comparison to the animals for which the reference values are quoted) might be the reason for higher globulin values in Jersey crossbred cows used in the present study. The variations observed were statistically non-significant.

In general the A: G ratio declined slightly from day 15 onwards in all the groups. However, the differences were non-significant. The A: G value is indicative of the immune status of the animal. The decline in A: G values in attributable to increase in plasma globulins which could be due to the fact that animals were vaccinated early at the beginning of the trial (day 5).

Maher and NMB (2013) and Al-Shaikh *et al.* (1999) reported no significant effect of fenugreek supplementation on plasma total protein levels in dairy cows and goats respectively. However, Nasser (2013) and Abo El-Nor *et al.* (2007) reported an increase in total protein levels on fenugreek supplementation in cows. Aher and Wahi (2010) observed that the immunomodulatory action of *T. cordifolia* lead to an increase in the immunoglobulin levels in rats. Similarly, it has been documented that fenugreek feeding in cows lead to elevated globulin levels (Nasser 2013). However, Maher and NMB (2013) found no significant difference in plasma globulin levels in Friesian cows after fenugreek supplementation.

Plasma renal profile

The plasma urea nitrogen and creatinine levels (mg/dL) in the blood plasma of lactating cows treated with herbal supplements and control are tabulated in Table 7 and Table 8 respectively.

The standard reference range for plasma urea nitrogen is 20-30 mg/dL (Kaneko *et al.* 2008). Slightly higher values observed in the present study may be due to variation in dietary protein intake. No specific trend in the variation of plasma urea nitrogen and creatinine values could be seen in control (T_0) as well as treatment (T_1, T_2 and T_3) groups. The plasma urea nitrogen concentration in the cows receiving fenugreek seed powder or a combination of fenugreek and giloy (T_2 and T_3 groups) was found to be significantly lower on day 60. A decline in blood urea levels after herbal supplementation has been reported by various research workers. Nasser (2013) reported a decline in blood urea levels on fenugreek supplementation in the dairy cows. Alamer and Basiouni (2005) and Al-Janabi (2012) reported a decline in blood urea levels in goats fed with fenugreek seeds.

Giloy (*Tinospora cordifolia*) is a documented herbal galactogogue (Mallick and Prakash 2011, Mir *et al.* 2014). Also there is an evidence that there is a negative correlation between blood glucose and milk yield (Mouffok *et al.* 2013, Chládek and Máchal 2004, Djokoviæ *et al.* 2017). Thus the decline in the blood glucose level can be attributed to the enhanced glucose demand by mammary gland due to higher milk yield in the crossbred cows.

CONCLUSION

The study can be concluded that the blood biochemical parameters showed some variation in some groups. In the giloy fed group (T_1), the plasma glucose levels were significantly lower than the control group animals. The lactating dairy cows supplemented with fenugreek (T_2) or both giloy and fenugreek (T_3) did not exhibit any significant variation in plasma glucose levels during the supplementation period. Protein profile (total protein, albumin, globulin and A: G ratio) was not affected much during the course of giloy supplementation. However, lower levels of plasma albumin and higher levels of plasma globulins were seen from day 15 to day 45. A: G ratio was significantly lower in giloy fed animals in comparison to control on day 45. No significant variations were observed in the protein profile values of group T_2 and T_3 animals. No significant effect on the renal profile (urea nitrogen and creatinine) could be attributed to the giloy supplementation. Similarly, in animals receiving fenugreek or both the herbs (T_2 and T_3) urea nitrogen levels exhibited nonspecific variation which appears to be not linked to herbal supplementation.

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