

Effect of Unilateral Adrenalectomy on the Blood Biochemistry of Black Bengal Goats (*Capra hircus*)

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Abstract: The present study was conducted to study the influence of unilateral adrenalectomy in the black Bengal goat and to observe its influence on stress alleviation capability in terms of changes in blood chemistry, and further emphasis was given to determine whether a single gland is sufficient for survival in these animals. Six female kids were unilaterally adrenalectomised and the other 6 were maintained as intact controls. Blood samples were drawn at regular intervals before and after surgery. Parameters included in the study were blood pH, blood volume, TEC, haemoglobin, PCV, TLC, differential leukocyte count, cortisol, glucose, total cholesterol, total protein, urea, and blood urea nitrogen. All the parameters were estimated by standard methods. All the blood biochemical parameters in the present study showed significant differences ($P < 0.01$) when compared to intact control animals. There was a significant increase in total plasma protein, total plasma cholesterol, blood urea, and blood urea nitrogen, and a significant decrease in plasma glucose and cortisol. The present study indicated that acute changes occurred in the blood biochemical parameters on unilateral adrenalectomy of the left adrenal gland that were later compensated for by the other intact right adrenal gland.

Key Words: Adrenalectomy, adrenal insufficiency, black Bengal goat, blood biochemistry, colorimetric method

Introduction

The adrenals are bilaterally symmetrical glands located anterior to the kidneys. Each gland has 2 anatomically and physiologically different parts, which are identified as the cortex and medulla. The cortex is derived from the mesoderm and produces cortisol, corticosterone, and aldosterone, whereas the medulla is ectodermal in origin and it produces epinephrine, norepinephrine, and dopamine. Adrenal cortical hormones are steroids and they primarily influence carbohydrate and electrolyte metabolism, and the hormones secreted by the medulla are amines and their effects are similar to those of sympathetic neurotransmitters. The adrenal glands play a role in the adaptation of animals to environmental adversities and stress.

The present study was undertaken to delineate the stress relieving capability of the adrenal gland in terms of

haemato-biochemical changes in black Bengal goats after unilateral adrenalectomy. Furthermore, emphasis was given to the compensatory mechanism of the intact adrenal gland to the lost gland. The parameters assessed in the study were blood volume, blood pH, TEC, PCV, Hb, TLC, DLC, plasma cortisol, total plasma protein, plasma glucose, blood urea, and blood urea nitrogen.

Materials and Methods

The study was conducted between March and June. It was generally hot and dry during the entire period of the study. The temperature was in the range of 23 to 34 °C and the humidity ranged between 62% and 94%. Twelve black Bengal female kids 4 to 6 months old were included in the study. Six female kids were unilaterally adrenalectomised and the other 6 were maintained as intact controls. The animals were in a clinically healthy state. To determine the normal values of the blood

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biochemical parameters of the animals, blood samples were drawn daily for a week from the jugular vein prior to surgery. Heparin was used as anticoagulant (20 IU per ml) and the plasma was separated by centrifugation at 4500 rpm for 30 min and stored at 4 °C until further analysis.

Surgical procedure

Single staged adrenalectomy was performed as described by Cowie and Stewart (1) in the experimental animals. The paracostal area was shaved and prepared aseptically. Pre-anaesthetic atropine sulphate (Morvel), 0.1 mg per kg BW, was administered subcutaneously 15 min prior to surgery followed by the sedative xylazine hydrochloride (Xylaxin, Indian Immunologicals), 0.1 mg per kg BW intramuscularly. The surgery was performed under local anaesthesia by infiltrating 2% lignocaine hydrochloride (Universal Medikit) at the site of incision. A paracostal incision from the last rib to the transverse process of first lumbar vertebrae was made. The approach was made through the abdominal muscles and by retracting the viscera to bring the left adrenal into view. It was carefully dissected free from surrounding tissues. The adrenal vein was ligated and the gland was excised. Muscles were closed by continuous suture using chromic catgut no. 1 and the skin incision was closed by interrupted sutures with monofilament of nylon.

Postoperative care

The adrenalectomised animals were administered dexamethasone (Rancortin, Ranbaxy), 0.5 mg per kg BW intramuscularly, to support them. They were also administered enrofloxacin (Vet India), 4 mg per kg BW intramuscularly, for 3 consecutive days to protect against infection (2). The skin sutures were removed 9-12 days after surgery.

Assay of Haemato-Biochemical Parameters

About 5 ml of blood was collected 24 h after surgery from all the operated animals followed by 12 h intervals up to 5 days and 24 h intervals 6 to 7 days after surgery. Immediately after collection, the pH of the blood sample was determined using a pH meter (Systronic). Blood volume (3) was estimated using a plasma sample obtained by centrifugation of blood. The haematological parameters were estimated as per standard methods, i.e. total erythrocyte count (3), blood haemoglobin by the Drabkins method (cyanmethaemoglobin method) as described by Balasubramaniam and Malathi (4), packed

cell volume (3), total leukocyte count (3), and differential leukocyte count (3).

Cortisol was estimated spectrophotometrically with a Shimadzu spectrophotometer (Model RF 540) as per the method of Mattingly (5). The biochemical parameters were estimated colorimetrically as per standard methods, i.e. plasma glucose (6), total plasma protein (6), total plasma cholesterol (7), and blood urea and blood urea nitrogen (BUN) by the DAM method (8).

Statistical Analysis

The data were expressed in SI units and analysed statistically as per the standard method described by Snedecor and Cochran (9). All values were expressed as mean \pm standard error (SE) using a significant level of $P < 0.01$.

Results

The onset of the signs of adrenal insufficiency were observed as early as 24 h post-surgery and proceeded for approximately 72 h. Thereafter all the animals showed more or less normal values for all parameters studied. The most marked symptoms due to adrenal insufficiency in these animals were loss of appetite, slight weight loss, muscular weakness, and decreased body temperature.

The mean \pm standard error values of haemato-biochemical parameters of the black Bengal goats before and after adrenalectomy are presented in Tables 1, 2, and 3. The results revealed that, following unilateral adrenalectomy, the blood pH, TEC, Hb, PCV, TLC, DLC, plasma cortisol, plasma glucose, total plasma protein, total plasma cholesterol, blood urea, and blood urea nitrogen differed significantly from the control group ($P < 0.01$); however, there was no significant difference in the blood volume. Though non-significant, there was a gradual decrease in mean blood volume from 72.38 ± 0.84 to 65.14 ± 6.44 at 108 h following the adrenalectomy (Table 1).

Blood pH, TEC, PCV, and Hb levels were significantly decreased ($P < 0.01$) when compared to control values (Table 1). Unilateral adrenalectomy resulted in a significant ($P < 0.01$) reduction in mean lymphocyte and total leukocyte count (TLC) up to 48 h following surgery (Table 2). However, with neutrophil, monocyte, and eosinophil counts, a significant increase was observed for 48 h following adrenalectomy (Table 2).

Table 1. Mean and SE of blood volume, blood pH, TEC, Hb, and PCV at different hours of pre- and post-adrenalectomised black Bengal goats.

Parameters (Units)	Mean with standard error at different hours												
	Normal	24	36	48	60	72	84	96	108	120	144	168	192
Blood volume (ml/kg bwt)	72.38 ± 0.84	87.42 ± 7.15	76.67 ± 9.30	75.82 ± 5.28	73.39 ± 7.92	69.77 ± 5.60	66.68 ± 6.83	66.08 ± 0.92	65.14 ± 6.44	69.79 ± 7.56	72.55 ± 6.22	69.92 ± 3.50	69.79 ± 6.20
Blood pH	7.08 ^{ai} ± 0.03	7.00 ^{bi} ± 0.04	6.97 ^{bcd} ± 0.03	6.93 ^{cef} ± 0.04	6.78 ^{ad} ± 0.08	6.93 ^{cef} ± 0.04	6.90 ^{fg} ± 0.04	6.90 ^{cefg} ± 0.04	9.90 ^{fg} ± 0.04	6.92 ^{cefg} ± 0.03	6.86 ^d ± 0.02	6.94 ^{bcef} ± 0.04	6.93 ^{cef} ± 0.02
Total erythrocyte count (1 × 10 ⁶ /cumm)	8.68 ^a ± 0.41	6.75 ^{bc} ± 0.17	5.95 ^{bc} ± 0.33	6.13 ^{bc} ± 0.36	6.21 ^{bc} ± 0.60	5.73 ^{bc} ± 0.45	5.46 ^b ± 0.43	5.47 ^b ± 0.37	5.34 ^b ± 0.41	5.93 ^{bc} ± 0.43	5.15 ^b ± 0.23	5.72 ^b ± 0.75	7.70 ^{ac} ± 1.20
Haemoglobin (g%)	9.22 ^a ± 0.24	8.93 ^{ab} ± 0.07	9.16 ^{ad} ± 0.74	8.19 ^{abc} ± 0.14	7.87 ^{abc} ± 0.35	7.63 ^{bc} ± 0.24	8.06 ^{abc} ± 0.25	7.89 ^{abc} ± 0.16	7.51 ^c ± 0.27	7.82 ^{bcd} ± 0.16	7.73 ^{bc} ± 0.23	8.39 ^{abcd} ± 0.46	7.79 ^{bc} ± 0.14
Packed cell volume (%)	27.00 ^a ± 0.54	26.33 ^{ac} ± 0.56	25.17 ^{ab} ± 0.70	24.83 ^{abc} ± 0.75	23.00 ^{bcd} ± 0.45	22.66 ^{cd} ± 0.42	22.66 ^{cd} ± 0.42	22.50 ^{cd} ± 0.72	23.16 ^{bcdf} ± 0.79	22.83 ^{bcd} ± 1.08	22.83 ^d ± 0.49	22.83 ^{bcd} ± 0.75	23.17 ^{bcdf} ± 0.40

Means in a row having different superscripts differ significantly (P < 0.01).

Table 2. Mean and SE of total leukocyte count and differential leukocyte count at different hours of pre- and post-adrenalectomised black Bengal goats.

Parameters (Units)	Mean with standard error at different hours												
	Normal	24	36	48	60	72	84	96	108	120	144	168	192
Total leukocyte count (×10 ³ cumm)	7.01 ^a ± 0.58	6.92 ^a ± 0.49	4.83 ^{bc} ± 0.26	4.08 ^b ± 0.18	5.32 ^{bc} ± 0.23	6.16 ^{ac} ± 0.71	5.10 ^{bc} ± 0.35	4.24 ^{bd} ± 0.31	4.29 ^{bd} ± 0.34	4.33 ^{bd} ± 0.26	5.03 ^{bc} ± 0.17	4.48 ^{bd} ± 0.28	5.11 ^{bc} ± 0.30
Neutrophil (%)	33.33 ^{ab} ± 0.80	42.83 ^{ab} ± 3.19	33.83 ^{ab} ± 3.35	39.50 ^{ab} ± 1.98	31.40 ^{ab} ± 2.28	29.67 ^a ± 5.38	33.33 ^{ab} ± 1.93	44.50 ^{bc} ± 4.61	29.83 ^a ± 2.75	45.83 ^b ± 5.83	38.00 ^{ab} ± 2.65	29.83 ^a ± 3.65	31.60 ^{ac} ± 6.01
Basophil (%)	0.17 ± 0.17	0.17 ± 0.17	0.17 ± 0.17	0.67 ± 0.21	0.50 ± 0.22	0.17 ± 0.17	0.17 ± 0.17	0.50 ± 0.22	0.67 ± 0.21	0.67 ± 0.21	0.17 ± 0.17	0.50 ± 0.22	0.17 ± 0.17
Eosinophil (%)	1.50 ^{ab} ± 0.22	3.50 ^{abc} ± 0.85	3.83 ^{abc} ± 1.19	3.67 ^{abc} ± 1.02	2.17 ^{abc} ± 0.75	1.33 ^{ab} ± 0.80	1.17 ^a ± 0.50	2.50 ^{abc} ± 0.89	2.50 ^{abc} ± 0.56	2.17 ^{abc} ± 0.83	1.17 ^a ± 0.67	1.33 ^{ab} ± 0.47	4.17 ^{bc} ± 1.01
Lymphocyte (%)	59.33 ^{acd} ± 1.28	46.00 ^{abc} ± 2.82	54.50 ^{abcd} ± 5.04	46.66 ^{abce} ± 2.46	55.16 ^{abcd} ± 3.81	59.00 ^{acd} ± 4.44	58.17 ^{abcd} ± 2.55	45.66 ^{ab} ± 3.40	60.83 ^{acd} ± 3.59	43.40 ^b ± 7.31	54.50 ^{abcd} ± 2.57	61.17 ^{cd} ± 3.00	56.83 ^{abcd} ± 5.83
Monocyte (%)	5.67 ± 0.84	6.67 ± 0.92	7.50 ± 1.38	8.00 ± 1.80	8.33 ± 1.82	9.83 ± 2.06	7.16 ± 0.83	6.83 ± 1.85	6.33 ± 1.23	6.50 ± 0.99	5.67 ± 1.17	7.17 ± 1.30	7.33 ± 0.61

Means in a row having different superscripts differ significantly (P < 0.01).

The mean plasma cortisol and glucose levels decreased significantly (P < 0.01) until 72 h (Table 3). However, the total plasma protein, total plasma cholesterol, and blood urea levels increased significantly (P < 0.01) up to 72 h (Table 3). Almost all parameters had gradually reached near normal values by the end of the study period.

Discussion

The initial delay in the onset of symptoms after adrenalectomy up to 24 h might be due to exogenous administration of glucocorticoid following surgery (10,11). One of the major symptoms evident in adrenalectomised animal is severe dehydration (12). This dehydration is primarily due to failure to retain body

Table 3. Mean and SE of plasma cortisol, plasma glucose, total plasma cholesterol, total plasma protein, blood urea, and blood urea nitrogen at different hours of pre- and post-adrenalectomised black Bengal goats.

Parameters (Units)	Mean with standard error at different hours												
	Normal	24	36	48	60	72	84	96	108	120	144	168	192
Plasma cortisol (µg/100 ml)	8.54 ^{ac} ± 0.47	10.39 ^b ± 0.68	8.98 ^a ± 0.60	8.89 ^{ac} ± 0.56	7.88 ^{ac} ± 0.58	7.41 ^c ± 0.35	7.92 ^{ac} ± 0.60	7.95 ^{ac} ± 0.59	7.89 ^{ac} ± 0.31	7.56 ^{ac} ± 0.41	7.93 ^{ac} ± 0.36	7.98 ^{ac} ± 0.47	8.35 ^{ac} ± 0.61
Plasma glucose (mg/dl)	49.65 ^{abc} ± 2.49	55.36 ^{abc} ± 3.90	47.20 ^{ac} ± 4.10	45.23 ^{ac} ± 4.11	41.98 ^a ± 3.79	43.62 ^a ± 3.68	52.06 ^{abc} ± 1.93	53.03 ^{abc} ± 2.95	61.10 ^{bc} ± 2.25	52.09 ^{abc} ± 4.96	43.93 ^a ± 4.98	45.75 ^a ± 2.14	44.40 ^a ± 3.36
Total plasma cholesterol (mg/dl)	136.14 ^{ac} ± 7.32	176.07 ^{abde} ± 5.72	181.55 ^{bde} ± 5.85	212.06 ^{bcg} ± 3.09	135.11 ^{ae} ± 6.79	170.20 ^{ab} ± 15.42	210.09 ^{bcg} ± 13.37	243.40 ^{cf} ± 17.16	220.14 ^{cd} ± 14.97	172.71 ^{ab} ± 4.20	160.12 ^{eh} ± 9.00	173.08 ^{abeg} ± 15.36	207.63 ^{bcdg} ± 13.20
Total plasma protein (g/dl)	6.36 ^{ad} ± 0.29	6.18 ^{abcd} ± 0.29	5.60 ^{ad} ± 0.23	4.80 ^b ± 0.20	5.76 ^{abcd} ± 0.42	5.58 ^{abd} ± 0.47	5.33 ^{ab} ± 0.20	6.33 ^{acd} ± 0.39	5.73 ^{abcd} ± 0.20	5.95 ^{abcd} ± 0.21	5.32 ^{abc} ± 0.11	5.81 ^{abcd} ± 0.18	6.87 ^{cd} ± 0.47
Blood urea (mg/dl)	31.82 ^{ab} ± 3.47	27.59 ^a ± 4.50	43.91 ^{bc} ± 3.43	45.58 ^{cd} ± 3.28	46.47 ^{cd} ± 5.59	50.97 ^c ± 5.10	39.77 ^{abc} ± 3.65	37.15 ^{abd} ± 4.16	35.18 ^{abd} ± 3.70	31.18 ^{ab} ± 2.51	41.42 ^{bcd} ± 4.29	37.50 ^{abcd} ± 3.52	34.33 ^{abd} ± 3.27
BUN (mg/dl)	14.86 ^{ab} ± 1.62	12.87 ^a ± 2.11	20.51 ^{bcd} ± 1.60	21.23 ^{bcd} ± 1.52	21.70 ^{bc} ± 2.61	23.80 ^b ± 2.38	18.61 ^{ab} ± 1.70	17.35 ^{ab} ± 1.94	16.43 ^{ac} ± 1.73	14.56 ^{ad} ± 1.17	19.34 ^{abc} ± 2.00	17.51 ^{abc} ± 1.64	16.04 ^{ac} ± 1.52

Means in a row having different superscripts differ significantly (P < 0.01).

water as a result of sodium diuresis and partly due to intracellular movements of water during aldosterone deficiency (13). This dehydration could be a reason for the gradual decrease in blood volume noted in the study (14). After 144 h the blood volume tends to increase towards the normal range (15). The probable reason for this is the restoration of aldosterone level by the other gland.

The decrease in blood pH observed in the present study might be due to acidosis as a result of sodium diuresis and hypoglycaemia in adrenalectomised animals. The gradual reduction in circulating level of TEC until 108 h after surgery might be correlated with the findings reported by Estergreen and Van Demark (16,17). It is probably due to increased erythrophagocytosis as a result of deficiency of glucocorticoids following adrenalectomy (18). Due to haemoconcentration the level of haemoglobin suddenly increased 36 h after the operation. Then the level of haemoglobin gradually decreased and this finding correlated with findings reported by Estergreen and Van Demark (17). Deficiency of glucocorticoid after adrenalectomy leads to a decrease in TLC. The decrease in TLC that is evident from the results obtained can be correlated with the results reported by

McDonald and Pineda (18). After adrenalectomy, there was a reduction in both TEC and TLC. This reduction in total cell content of the blood could be the reason for the reduction in PCV values (19). Deficiency of glucocorticoids further led to neutropenia, lymphocytophilia, monocytophilia, and eosinophilia.

All the biochemical parameters in the present study showed significant differences (P < 0.01) when compared to the intact control animals. There was a significant increase in total plasma protein, total plasma cholesterol, and blood urea, and a significant decrease in plasma glucose and plasma cortisol as reported earlier by Cowie and Stewart (1). The reduced cortisol level at 72 h could be due to corticosteroid deficiency following adrenalectomy (20). One of the important effects of glucocorticoid is the stimulation of hepatic gluconeogenesis, which involves conversion of non-carbohydrate sources into glucose. The net result is an increase in hepatic glycogen and a tendency to increase blood glucose level (21). Due to a deficiency of glucocorticoid after unilateral adrenalectomy, these events fail to take place, leading to a decrease in plasma glucose level (22). Cholesterol is the precursor for steroid synthesis (18). In the absence of steroid synthesis due to

adrenalectomy, the cholesterol remain unutilised and further, due to the absence of steroid synthesising adrenal cortical cells, the cholesterol remains in the blood, leading to an increase in its circulating level (23).

Glucocorticoids inhibit the synthesis and stimulate the breakdown of proteins in muscle, skin, adipose, lymphoid tissue, and connective tissue (18,24). Enhancement of protein catabolism is accompanied by the release of free amino acids. This process supports hepatic gluconeogenesis. Due to a deficiency of glucocorticoids after adrenalectomy the above event does not take place and the protein remains unutilised, leading to an increase in plasma protein level (25).

The rise in blood urea is due to impairment of renal function (1). Continued sodium and fluid loss due to adrenal insufficiency lead to dehydration, hypotension, reduced renal blood flow, and increased blood levels of non-protein nitrogenous substances. This reduced renal blood flow is the cause of renal function impairment and as a result more urea is retained in the blood instead of its elimination through the urine.

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In conclusion, the present study revealed the importance of the adrenal gland in combating stress conditions in goats. With a detailed study, particularly on secretion of adrenal cortex and its effects on various metabolic processes, it is appropriate to conclude that the adrenal cortex is essential for maintenance of homeostasis and for survival in these animals. The effect of unilateral adrenalectomy of the left adrenal gland persisted in these animals for 72 h post-surgery and then the animals showed normal behaviour as the right adrenal gland took up the function of the lost gland through a compensatory mechanism. The study indicates the existence of a compensatory mechanism between the 2 adrenal gland so that when one loses its function the other gland takes up the function of the lost gland to maintain the physiological functions at near normal level.

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