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Effects of Splenectomy on Blood Constituents of Goats (*Capra hircus*)

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Abstract: The effects of splenectomy on haematological responses were investigated in adult goats. The splenectomized group maintained lower (PCV) and (Hb) concentration. Reticulocytosis was reported in splenectomized animals. After splenectomy, the lymphocyte and monocyte ratios were lower. The neutrophils and eosinophils ratios were higher compared to the sham operated control. Splenectomy increased the thrombocyte count significantly compared to the sham operated control. Splenectomy also resulted in non-significant decrease in serum total protein; the serum albumin and urea levels were not influenced by splenectomy. The sham operated control and splenectomized animals had higher plasma glucose level after surgical operations. Serum Na level was not influenced significantly by splenectomy.

Key words: Goats · Splenectomy · Erythrocytic indices · Leukocytes · Reticulocytes · Blood metabolites

INTRODUCTION

The spleen is a discrete organ located adjacent to the stomach and attached to the omentum in most higher vertebrates [1]. It is located on the left side of the abdominal cavity and is always covered by some ribs in most mammals [2]. In goats, the spleen is located in the left craniosacral abdomen between the diaphragm and the dorsal sac of the rumen [3]. The shape of the spleen is small triangular or quadrilateral in goats and sheep.

The spleen is the largest single secondary lymphoid organ and the most important organ of reticuloendothelial system; the anatomical structure of the spleen and its position in the route of the portal vein indicate its importance both in innate and in adaptive immunity [4]. The diverse regional anatomy based on a complex vascular system has the added feature of altering internal anatomy with changes in size and volume [5]. The main functions of the spleen are phagocytosis, haematopoiesis, lymphopoiesis or maturation, haemoglobin processing and storage and release of blood cells [1].

Splenectomy is a relatively safe and effective procedure for therapeutic and diagnostic purposes. The common diseases requiring splenectomy in humans include immune thrombocytopenic purpura (ITP), lymphoproliferative disorders, Hodgkin's disease and myeloproliferative disease [6]. Splenectomy was shown to prevent autotransfusion during experimental acute hypovolaemia in dogs [7]. It alters both humoral and cellular immunity [8]. On removal of the spleen, the incidence of severe infection increases [9, 10] and there are changes in many immunologic parameters [11]. One lasting change is a marked increase in number of B-lymophocytes in the blood, an effect that is constantly observed both in humans [12] and experimental animals [13, 14].

The effects of splenectomy have been previously studied in humans and some of the animal species, but there is paucity of information on the effects of splenectomy in goats. The objective of this study was to investigate the effects of splenectomy and associated surgical stress on the basic haematological parameters of goats.

MATERIALS AND METHODS

Animals and Diet: Twelve adult healthy non-gestating and non-lactating desert breed goats weighing an average

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Fig. 1: Extraction of the spleen

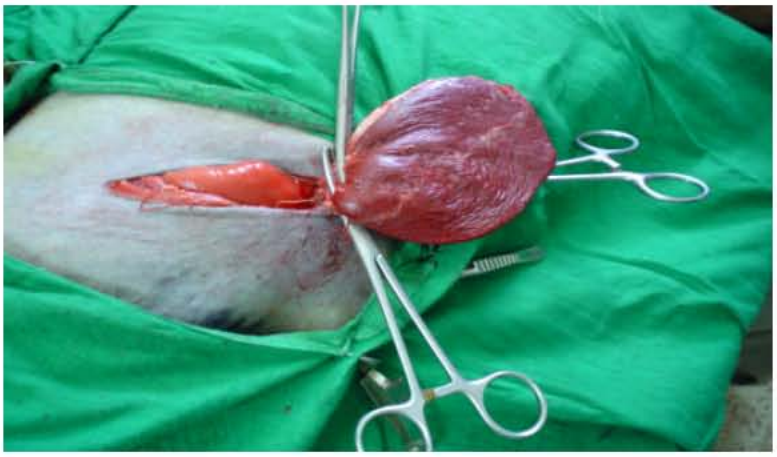

Fig. 2: Ligation of the blood vessels

of 16.0±0.5 kg were used in this experiment. The animals were kept in the pens for an adaptation period of 2 weeks before experimentation so that they were accustomed to handling, experimental conditions and collection of the blood samples. The animals were fed alfalfa hay (CP:18%; ME: 7.9 MJ kg^{-1}) and watered *ad libitum*. The study was conducted at the Department of physiology during February-March 2007.

The animals were randomly allocated to two groups. In the first group (8 goats), complete surgical procedure was performed with removal of the spleen according to the standard procedure [15]. In the second group (4 goats), sham-operation was performed without removal of the spleen.

Surgical Operation: The animals were fasted for 24 hr. The surgery was performed under local anaesthesia by subcutaneous injection of Lignocane with adrenaline. The skin of the abdomen was shaved and disinfected with absolute ethanol. Through a left side abdominal incision, the last rib was removed; then the spleen was exposed and the blood vessels were ligated (Figs. 1 and 2). The abdomen incision was sutured immediately after soaking with antibiotic (Oxytetracycline, 5%). After surgery, the animals were monitored until complete recovery. In the sham operated control animals only incision of abdomen and removal of the last rib were performed. All experimental animals received broad spectrum antibiotic (Oxytetracycline 5%), 5 ml /animal for 3 days after the operation. Both groups of animals were allowed a post-operative healing and recovery period of two weeks. The wound was dressed daily and the sutures were removed after healing. During the experimental period, the goats were kept in an animal house and offered alfalfa hay and water *ad libitum*. One of the splenectomized goat died 20 days post-splenectomy.

Blood Analysis: Blood samples were collected one day before operation, immediately before operation and on days 1, 3, 7, 10 and 13 following the operation. The haemoglobin concentration (Hb), packed cell volume (PCV), reticulocyte count, total leukocyte count (TLC) and differential leukocyte count (DLC) were determined according to the standard methods [16, 17].

Serum and Plasma Analysis: The concentration of serum total protein was determined using Biuret reagent [18]. Serum albumin concentration was determined by the colorimetric method [19]. Serum urea concentration was determined by the enzymatic method test (Berthlot) using a kit (Spinreact, S.A., Spain). The plasma glucose concentration was determined by enzymatic colorimetric method using a kit (Spinreact, S.A., Spain). The concentration of Na in serum was determined by flame photometer technique [20].

Statistical Analysis: The experiment was perfomed according to the complete randomized design. The SAS package (1988) was used to perform analysis of variance (ANOVA). The separation of means was done by Duncan Multiple Range Test [21].

RESULTS

Packed Cell Volume (PCV): Fig. 3 showed that the basal values for (PCV) were 25.0 – 27.5%. The pre-operation value of (PCV) was slightly higher in the goats that were splenectomized later. The splenectomized group showed increase in (PCV) immediately following the operation and at 24 hours post- operation. The result was significantly P<0.05) higher compared to (PCV) values obtained for the control group. The results indicated that the values returned to normal level after 10 days in splenectomized animals compared with 1 day in the control group.

Haemoglobin Concentration (Hb): Fig. 4 showed that the basal values for (Hb) concentration were 9.6–10.7 g/dL. The pre-operation value of (Hb) was slightly higher in subsequently splenectomized goats. The splenectomized group showed an increase in (Hb) concentration immediately after the operation and at 24 hrs post-operation. The value was significantly (P<0.05) higher compared to the respective values obtained for the control group. The (Hb) concentration returned to normal level after 10 days in splenectomized animals compared with 1 day in the control group.

Reticulocyte Count: The study indicated that the mean reticulocyte count was 3% in splenectomized animals, whereas in the control group, no reticulocytes were detected. Fig. 5 showed a blood film of a splenectomized goat with distinct moderate reticulocytosis.

Leukocytic Profile: The effect of splenectomy on total leukocyte count (TLC) is shown in Table 1. The pre-operation values of (TLC) were slightly higher in the splenectomized animals. In the post-operative period, the sham-operated control group maintained an almost steady value of TLC (~$7.5\times10^3/\mu L$) until the end of experimental period. The splenectomized group showed a sharp decrease in (TLC) immediately following the operation. It was significantly (P<0.05) lower compared to the respective values obtained for the control group.

Table 1: Effect of splenectomy on leukocytic indices in goats

		Time							
Parameter	Treatment	Pre-1day	Pre-operation	Post-operation	1day	3days	7days	10days	13days
TLC ($x10^3/\mu l$)	Control	7.5±1.1	8.6±1.7	7.1±2.3	7.1±2.3	7.8±1.0	7.3±0.8	8.1±1.7	7.9±0.9
	Splenectomized	8.6±1.3^{a}	9.5±2.0^{a}	6.4±2.1^{b}	10.4±2.0^{a}	9.4±1.4^{a}	9.1±1.5^{a}	9.3±1.5^{a}	9.3±1.7^{a}
Lymphocyte (%)	Control	59.0±2.6	62.7±2.5	60.7±5.9	57.3±2.5	57.3±4.0	57.7±0.6	59.0±2.6	60.7±3.5
	Splenectomized	60.0±3.4^{a,b}	62.5±2.8^{a}	30.0±8.2^{e,f}	31.7±7.1^{e}	40.1±4.6^{d}	53.5±3.6^{c}	57.0±4.6^{b,c}	61.4±2.1^{a,b}
Neutrophil (%)	Control	33.3±3.2	31.0±1.7	38.0±1.0	36.3±3.2	35.0±5.0	36.3±1.2	34.0±1.7	31.7±2.9
	Splenect-omized	32.5±3.5^{d}	33.3±3.7^{d}	60.6±8.4^{a}	61.5±7.1^{a}	53.4±5.1^{b}	42.8±3.8^{c}	38.6+3.7^{c}	33.5+2.6^{d}
Monocyte (%)	Control	4.7±0.6	2.7±0.6	2.7±0.6	2.7±1.0	1.7±0.6	2.3±0.6	3.3±0.6	4.3±0.6
	Splenectomized	3.4±0.7^{a}	2.0±0.8^{b,c}	2.4±0.9^{b,c}	2.3±1.2^{b,c}	2.1±0.8^{c}	1.6±0.7^{c}	1.9±0.8^{b,c}	2.3±0.7^{c}
Eosinophil (%)	Control	2.3±0.6	2.0±0.0	4.0±1.0	3.7±1.2	6.0±1.7	3.7±0.6	3.3±1.5	2.7±0.6
	Splenectomized	2.5±0.9^{c}	3.0±2.3^{c}	5.3±2.7^{a,b}	5.3±1.6^{a,b}	5.4±0.9^{a}	3.6±1.3^{b,c}	3.8±1.2^{b,c}	3.3±1.0^{c}
Basophil (%)	Control	1.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.3±0.6	0.3±0.6	0.7±0.6
	Splenectomized	0.6±0.5^{a}	0.0±0.0^{c}	0.3±0.5^{b,c}	0.1±0.4^{b,c}	0.4±0.5^{b,c}	0.0±0.0^{b,c}	0.3±0.5^{b,c}	0.3±0.5^{b}

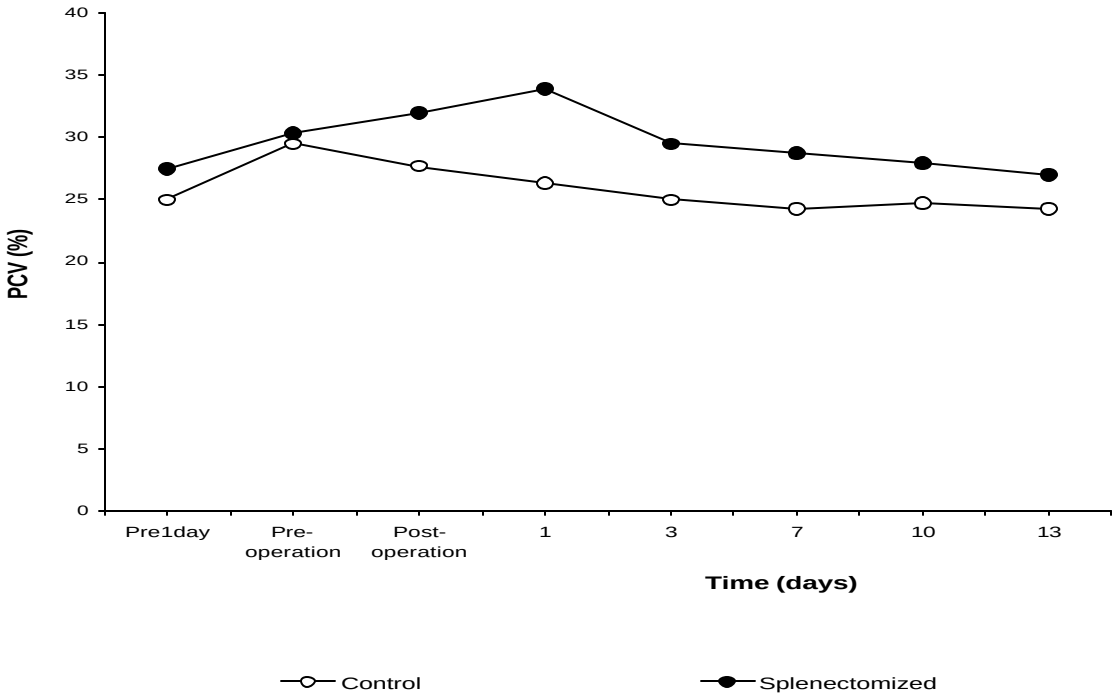

Fig. 3: Effect of splenectomy on packed cell volume (PCV) in goats

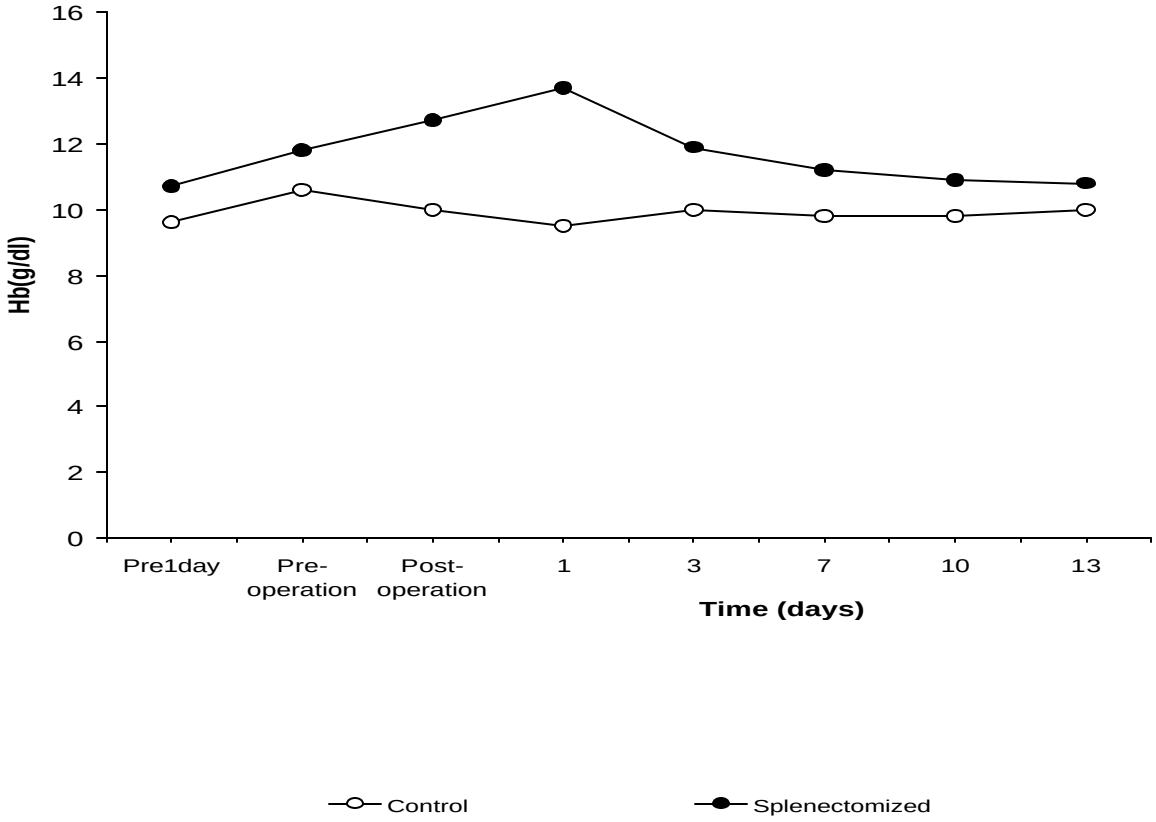

Fig. 4: Effect of splenectomy on haemoglobin concentration (Hb) in goats

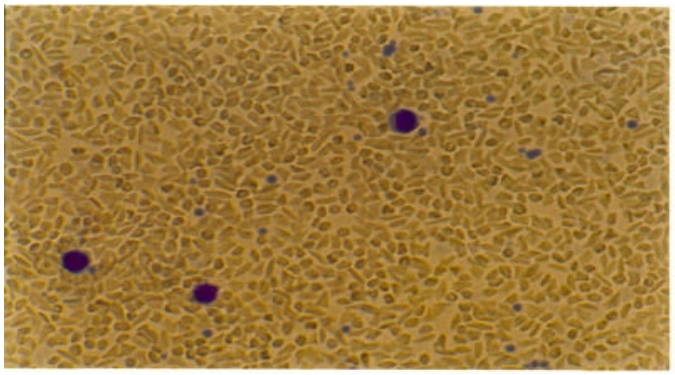

Fig. 5: Reticulocytosis after splenectomy

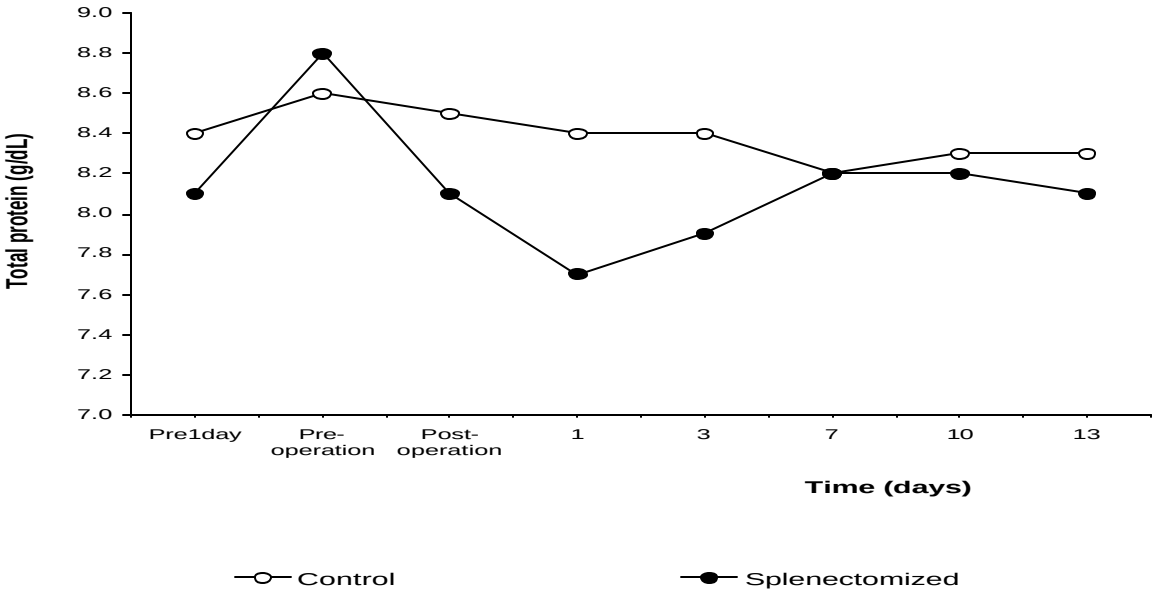

Fig. 6: Effect of splenectomy on serum total protein concentration in goats

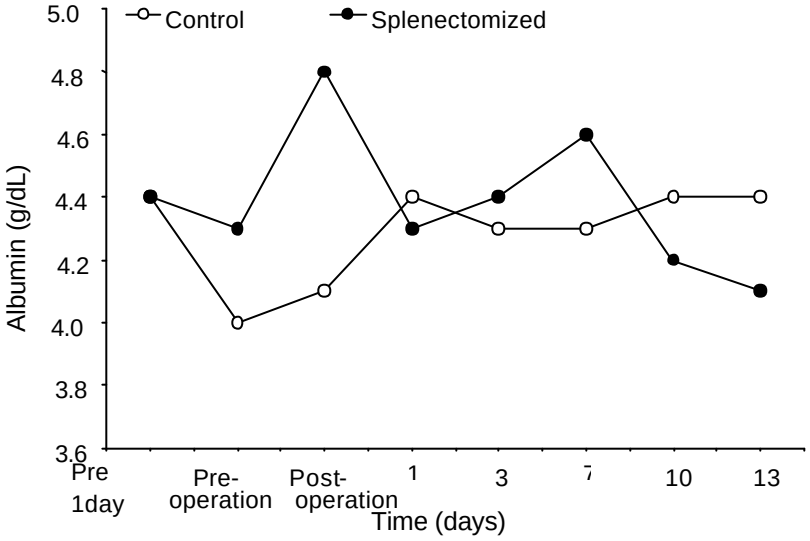

Fig. 7: Effect of splenectomy on serum albumin concentration in goats

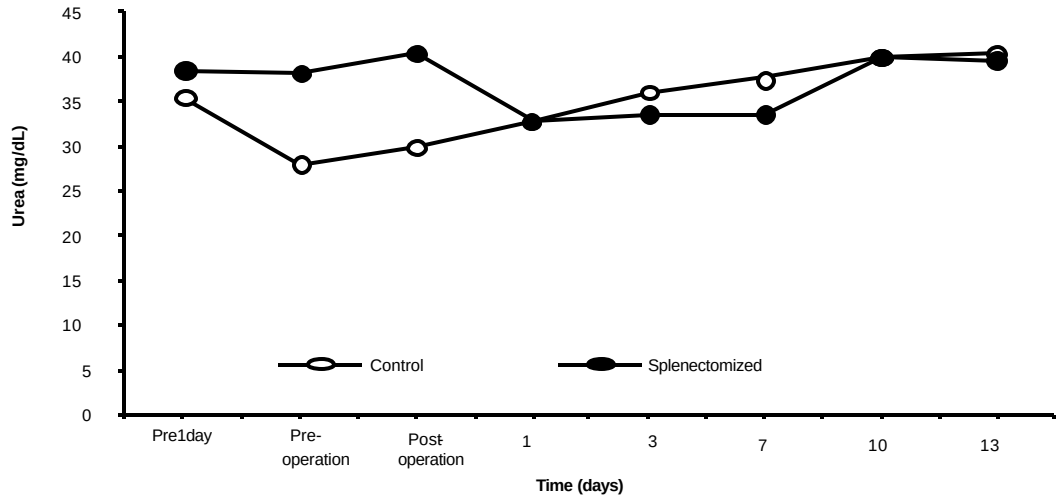

Fig.8 Effect of splenectomy on serum urea concentration in goats.

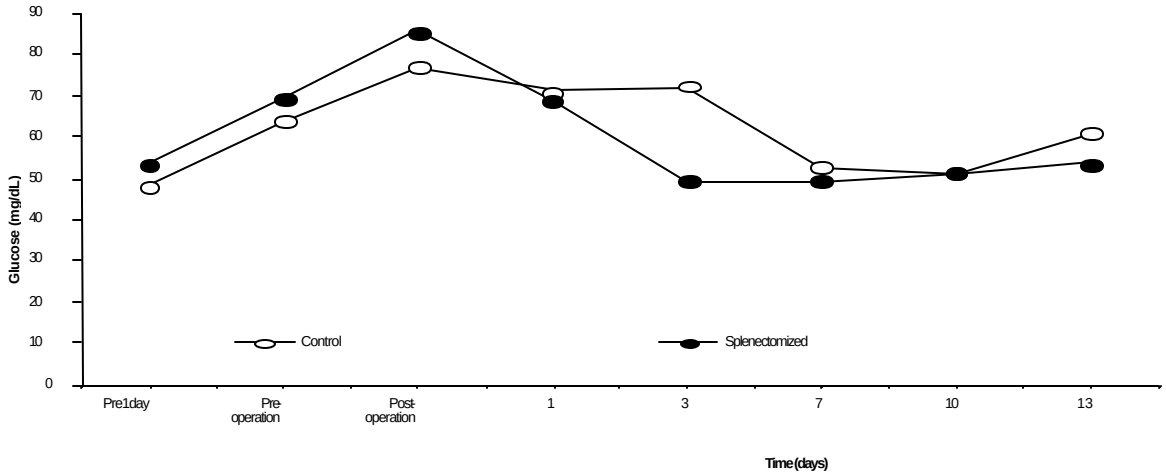

Fig. 9: Effect of splenectomy on plasma glucose level in goats

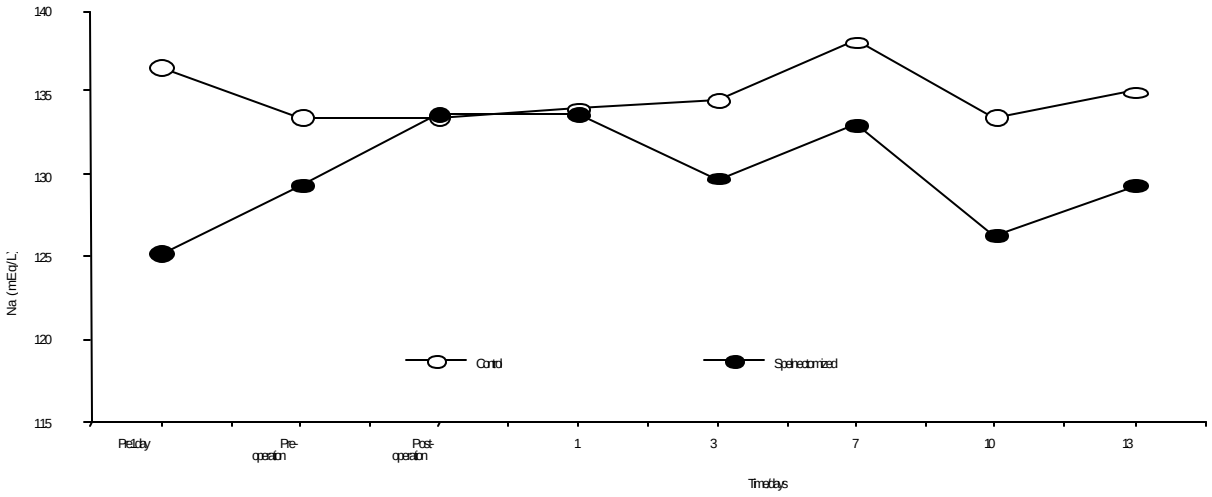

Fig. 10: Effect of splenectomy on serum Na concentration in goats

The splenectomized group showed a sharp increase in (TLC) to ˜10×10^3 /μL and slightly lower values were maintained until day 13.

The results of differential leukocyte count (DLC) (Table 1) showed a significant (P<0.001) decrease in lymphocyte ratio of splenectomized group (Table 1) compared to the sham-operated group. A significant (P>0.001) increase in neutrophil ratio Table 1) was obtained compared to the control. These ratios returned to normal values at day 13. Also the splenectomized animals had higher ratio of eosinophils compared to decreased ratio of monocytes; there was no marked change in the ratio of basophils.

Thrombocyte Count: The mean value of thrombocyte count in splenectomized animals (401,600/μL) was higher than in normal control animals (221,600/μL).

Serum Total Protein: Fig. 6 showed that the pre-operative values of serum total protein in both groups were close to each other (8.1 – 8.4 mg/dL). Following the operation, the splenectomized group showed a decrease in concentration of total protein in day 1 and it returned to the normal value after 7 days.

Serum Albumin: Fig. 7 showed the level of serum albumin concentration due to splenectomy. The results indicated that there was a small increase in albumin level after splenectomy.

Serum Urea: Fig.8 showed the value of serum urea concentration due to splenectomy. The pre-operative values of both groups were close to each other (36.5-39.0 mg/dL). Following the operation, the splenectomized group showed a decrease in urea level in day 1 and it returned to the normal values after 10 days.

Plasma Glucose: Fig. 9 showed that the initial pre-operation plasma glucose levels of the control and spelenectomized group were close to each other (˜48-54mg/dL). Following the operation, paradoxically both groups showed an increase in glucose level; the increase was more pronounced in spelenectomized animals. In both groups, the glucose level returned to normal after 7 days. Thereafter, both groups maintained low values until day 13.

Serum Na Concentration: Fig. 10 showed the level of Serum Na concnetration in response to splenectomy. The basal value range for Na concentration was 125-137mEq/L. Following the operation, the splenectomized group showed an increase in Na level and it returned to the normal value after 10 days.

DISCUSSION

This study showed that splenectomy in goats resulted in significantly higher (PCV) level (Fig. 3) and an increase in (Hb) concentration (Fig. 4) immediately after surgical operation. The closely related changes in these erythrocytic indices indicated that the erythrocytes were not stored in the spleen causing an increase in (PCV) and (Hb) concentration in systemic circulation. The maintenance of lower (PCV) and (Hb) values in sham-operated animals suggests that the cellular elements of blood including erythrocytes were trapped in the spleen.

The results indicated that reticulocytosis was evident after splenectomy (Fig. 5). The maturation of reticulocytes occurs in the circulation and spleen [22]. In the absence of spleen, they become mature and accumulate in the circulation. It has been reported [3] that the goat demonstrates only a mild to moderate reticulocytosis response to induction of haemorrhagic anaemia. Reticulocytosis in peripheral blood can be considered as an indicator of effective erythropoiesis that was previously reported in humans [23] and in animals [17].

The current results also indicated that the leukocytic profile of goats was influenced by splenectomy (Table 1). Splenectomy resulted in decreases in total leukocyte count, (TLC) (Fig. 6) and in the ratios of lymphocytes and monocytes. The operation also resulted in an increase in the ratios of neutrophils and eosinophils. The post-operative decrease in (TLC) is presumably associated with acute response to removal of spleen which constitutes a source of leukocytes. Also it may be partly related to the effect of the antibiotic that was administered to the animals during and after surgical operation. The increase in (TLC) of splenectomized goats after 24 hrs could be a physiological response to removal of the spleen. Although the mechanisim involved has not been clarified, presumably it could be associated with elimination of the spleen that maintains destruction of the leukocytes. However, the concept of a humoral regulation of leukocytes production by the spleen cannot be ruled out. Splenectomy usually results in a significant decrease in blood residency time of recirculating lymphocytes and in an enhanced accumulation of recirculating lymphocytes in lymph nodes in humans [14].

The increase in platelets count in splenectomized goats reported in the present study could be associated with the removal of the normal site of platelet destruction. The thrombocytosis could also be related to the injuries caused by surgical operation. Thrombocytosis after splenectomy was reported in dogs [24] and in humans [6].

The results indicate that the serum concentrations of total protein and urea decreased (Figs. 6, 8), whereas albumin concentration increased after splenectomy (Fig. 7). The decrease in total protein may be attributed to reduction in the level of immunoglobulins. The decrease in serum concentrations of total protein and urea could be related to hormones secreted in response to surgical trauma. Following surgical operations, protein catabolism stimulated by increased cortisol concentration results in muscle wasting and is usually associated with increased nitrogen excretion in urine [25]. Protein degradation leads to release of amino acids and transport to the liver for gluconeogenesis; deamination in the liver leads to characteristic increase in urea production and excretion [26]. The decline in serum total protein and urea levels could also be attributed to secretion of arginine vasopressin (AVP), which promotes water retention, haemodilution and production of concentrated urine; AVP secretion may continue for 3-5 days depending on the severity of the surgical injury [25]. Similarly reduction in serum total protein level post-splenectomy has been noted in humans [27, 28].

The plasma glucose level was increased in normal and splenectomized goats (Fig. 9). The rise in glucose level in both experimental groups is presumably related to endocrine responses associated with surgical trauma. Cortisol and catecholamines secreted in response to surgical trauma, facilitated glucose production due to increased hepatic glycogenolysis and gluconeogensis [27]. In response to surgical trauma, the usual mechanisms which regulate glucose production and homeostasis are ineffective because of initial failure of insulin secretion followed by insulin resistance [29].

The slight increase in serum Na level following splenectomy is attributable to renin secretion from the juxtaglomerular cells which stimulates release of aldosterone from the adrenal cortex, promoting Na and water reabsorption from the distal tubules in the kidney [30].

CONCLUSION

Splenectomy can be performed successfully in goats under local anaesthesia. The post-operative changes in erythrocytic and leukocytic indices are related mainly to removal of spleen. The changes in blood metabolites were presumably influenced mainly by hormones secreted in response to surgical trauma.

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