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Injection doxorubicin chemotherapeutic management in TVT-Affected dogs

VVV Amruth Kumar and K Satish Kumar

Abstract
The objective of the present study was to evaluate the therapeutic efficacy of Inj. Doxorubicin in treatment of Transmissible venereal tumors. Dogs that were presented to the Veterinary Clinical Complex for a period of two years were screened for the presence of venereal tumors. They were subjected to various diagnostic tests to confirm TVT. The dogs that were confirmed for Transmissible Venereal Tumors were subjected to treatment with Inj. Doxorubicin. The clinical recovery, hematological and biochemical parameters and the therapeutic efficacy of treatment were enumerated.

Keywords: Transmissible venereal tumors, doxorubicin, chemotherapy, dogs

1. Introduction
The transmissible venereal tumour (TVT) in dogs, is a sexually transmitted benign reticuloendothelial tumour, that primarily affects the external genitalia of sexually developed animals (Gadmade *et al*., 2007) [6]. It is sometimes referred to as an infectious sarcoma, venereal granuloma or sticker tumour. There is no breed or sex preference for naturally occurring TVT in dogs (Rogers, 1997) [15]. Several treatments have been used in the treatment of TVT which include chemotherapy, radiotherapy, immunotherapy and surgery. Doxorubicin has long been shown to be efficacious as a single chemotherapeutic drug in the treatment of canine TVT.

2. Materials and Methods
The dogs presented to the clinic and those referred from various hospitals in and around Hyderabad to the Veterinary Clinical Complex, Bhoiguda over a period of 2 years formed the basis for the present study. Whole blood and serum samples of all the identified dogs formed the clinical material for laboratory examination. Two ml of whole blood was withdrawn from the cephalic or saphenous vein. Serum was separated immediately after clotting by centrifugation at 3000 rpm for 5 minutes and collected in Eppendorf tubes. Hematological parameters such as Hb, PCV, TEC, TLC and DLC were estimated as per the standard procedures. Biochemical parameters such as BUN, Creatinine, ALT, AST, ALT and TP were estimated by a semi-auto analyzer. Dogs selected for therapy had a BSA of 0.6 to 0.8 m^2. Fine needle aspiration samples are collected from tumor for cytology to know malignancy and biopsy samples are collected for histopathology to confirm tumour.

3. Results & Discussion
3.1 Diagnosis
Diagnosis of tumors was made based on clinical signs, FNAC and histopathology. Among the 458 dogs with neoplasia, dogs affected with Transmissible venereal tumors were the highest with 25.77%. Out of these diagnosed dogs, twenty dogs were included in this study.

3.1.1 Clinical signs
TVT was clinically diagnosed based on symptomatology which includes bleeding or discharges from the external genitalia (100%), nodules or cauliflower-like friable masses in the vulva (females) or in the prepuce / caudal penis (males) (100%), licking of the external genitalia (92.37%), ulceration of the tumor (44.07%), popliteal and/or inguinal lymph node

enlargement (28.81%), anorexia (24.58%), dysuria and/or tenesmus (16.10%) and lung metastasis (9.32%). These findings were in accordance with the findings of Boscos and Ververdis (2004) [4], Purohit (2008) [14], Tella *et al*. (2004) [17], Mellomartins *et al*. (2005) [11], Nak *et al*. (2005) [13] and Albanese *et al*. (2006) [2] who recorded location of tumour to be at vulvar lips, vaginal floor, perineal region and cervix. The results were depicted in the Table No.1 and Plate 1. Among the twenty dogs bleeding from the external genitalia and nodules or cauliflower-like friable masses in the vulva (females) or in the prepuce / caudal penis (males) were seen in all the twenty dogs (100%), other clinical signs like licking of the external genitalia, ulceration of the tumor, popliteal and/or inguinal lymph node enlargement, anorexia and/or inappetence, dysuria and/or tenesmus and lung metastasis were seen in 19, 9, 8, 8, 5 and 0 dogs respectively. In the present study, transmissible venereal tumors affected the external genitalia in dogs of both sexes. The tumors appeared as a solitary mass or as multiple tumors with pendular, nodular, papillary forms presenting a cauliflower-like appearance.

The total dogs diagnosed (i.e., 118) with transmissible venereal tumors were classified based on WHO TNM Classification. Among these 20 dogs were selected from Stage I and Stage II. It was noticed that the tumor size was less than 3 cm in 12 dogs i.e., (T_1) and 8 dogs had tumors of 3-5 cm (T_2) size. Among these 20 dogs of the study, 12 dogs did not show any regional lymph node involvement (N_0) whereas 8 dogs showed unilateral lymph node involvement (N_1). Out of the 20 dogs, distant metastasis was not seen in any of the dogs (M_0). The results are depicted in Table No. 2.

3.1.2 Fine needle aspiration cytology (FNAC)

Examination of smears of fine needle aspiration samples obtained from TVT revealed round to oval-shaped cells with centrally located nuclei with coarsely aggregated chromatin, arranged in cord-like patterns. The nuclei had multiple and large basophilic prominent nucleoli (one / two). They also had lightly basophilic cytoplasm and multiple punctate vacuoles. The tumor cells showed criteria of malignancy like pleomorphism, anisocytosis and anisokaryosis. These findings were in agreement with the findings of Mellomartins *et al*. (2005) [11], Nak *et al*. (2005) [13], Albanese *et al*. (2006) [2], Purohit (2008) [14], Kabuusu *et al*. (2010) [9], Stockmann *et al*. (2011) [16], Behera *et al*. (2012) [3] and Ulcar *et al*. (2012) [18]. The results are depicted in plate 2.

3.1.3 Histopathology

Examination of biopsy samples in the present investigation in TVT-affected dogs revealed more cellular appearance with incompletely divided cells, multinucleated cells, round to oval-shaped cells containing mitotic figures and cells with clumping of chromatin as well as one or two prominent nucleoli. Similar findings were observed in histopathology of TVT biopsy samples by Menten (2002) [12], Meinkoth and Cowell (2002) [10] and Stockmann *et al*. (2011) [16]. The results are depicted in plate 2.

3.1.4 Electron microscopy

Electron microscopic studies in TVT affected dogs revealed numerous vesicles in the cytoplasm; dense round and shrunken mitochondria, dilatation of the cisterns of endoplasmic reticulum, anisokaryosis as well as heterochromatin with mild marginations. Nak *et al*. (2005) [13] and Stockmann *et al*. (2011) [16] described similar electron microscopic results. The results are depicted in plate 2.

3.1.5 X-ray

Thoracic radiographs were taken in all the TVT-affected dogs to detect the presence of pulmonary metastasis. Radiographic examination in the present study revealed multiple pulmonary masses of varying sizes which were nodular and radio-opaque in 11 dogs with stages III and IV TVT tumors. The results are depicted in Fig 1.

3.1.6 Ultrasound Scanning

In the present study ultrasonography examination of TVT-affected dogs did not reveal any intra-abdominal metastatic tumours. Ultrasonography examination of the TVT tumour mass revealed hyperechogenic texture. Ajadi *et al*. (2010) [1] stated that ultrasonography of the abdominal organs and regional lymph nodes may be done to assess the degree of spread or metastasis. The results are depicted in Fig 2.

3.1.7 Hemato-biochemical studies

Various hemato-biochemical parameters recorded among the dogs diagnosed for TVT tumors and taken up for therapy are presented as follows.

Hematological examination showed significant increase in neutrophils and decline in lymphocytes before treatment. The mean serum biochemistry parameters revealed significantly high mean ALT, AST, ALP and decreased serum protein when compared to the apparently healthy dogs (Table 3, 4 and Fig 4, 5).

3.1.8 Therapy

Out of the 20 dogs diagnosed and taken up for the therapeutic trail 12 dogs were of stage I and 8 dogs of stage II and these were treated with Doxorubicin (Inj. Adriamycin) @ 30 mg/m^2 mixed with Normal saline and administered IV once in a week for a maximum of 5 weeks. Similar treatment was given by Gandimathi *et al*. (2011) [7] with Inj. Doxorubicin @ 30 mg/m^2 by slow i/v route with ten days interval for three times for TVT affected dogs and observed that five out of six cases showed complete response of tumour and one showed partial response with four doses of treatment and Nak *et al*. (2005) [13] conducted similar treatment for TVT with Inj. Doxorubicicn until complete remission was observed or a maximum of 3 weeks. The dogs which failed to recover completely were subjected to surgical excision.

3.1.9 Clinical improvement

Among 20 dogs treated 18 dogs (90%) showed a gradual reduction of tumor size by day 7 and complete response (CR) was observed by day 28 (week days after 4th dose), but the fifth dose was also administered in these dogs to completely eliminate the tumor cells. Two dogs (10%) showed only partial response (PR) of tumor even after 5 doses of Inj. Doxorubicin. In these dogs alleviation of clinical signs started slowly and 18 dogs showed a gradual improvement which started from day 7 with complete remission observed by day 28 (a week after 4th dose). In two dogs clinical signs like inappetence did not vane till the end of the therapy also. The inappetence aggravated from day 14 in these dogs which were anorectic from day 21 till day 35. Therefore these two dogs were given supportive treatment from day 21 till the completion of therapy. Anorexia was a result of anorexigenic peptides and other intermediary metabolites produced by

neoplasm. Hence they were given supportive treatment and were later subjected to surgical excision. Cizmeci *et al*. 2012 [5]; Gadmade *et al.* 2007 [6]; Gandhimathi *et al.* 2011 [7] and Huppes *et al.* 2014 [8].

3.1.10Laboratory findings

Hematological examination before during and after therapy revealed that Hb, PCV and TEC had significantly declined ($p<0.01$) from day 0 to day 35 which may be attributed to the tumoral bleeding and myeloid toxicity induced by the chemotherapeutic drug which was reported by Gadmade *et al.* (2007) [6] and Gandhimathi *et al.* (2011) [7]. Total leucocyte count was significantly high on day 0 before treatment ($p<0.01$) when compared to apparently healthy dogs. This leucocytosis before treatment showed a significant decline by day 14 during therapy ($p<0.01$) which continued until day 35. The mean neutrophil count which showed a significant increase on day 0 ($p<0.01$) showed a significant decline ($P<0.01$) by day 14 during therapy which continued until day 35. The mean lymphocyte count significantly increased ($P<0.01$) during the course of therapy. Similar findings were reported by Gadmade *et al.* (2007) [6], Cizmeci *et al.* (2012) [5] and Gandhimathi *et al.* (2011) [7]. Platelet count after therapy showed a significant decline ($p<0.01$) and was in agreement with the findings of Gadmade *et al.* (2007) [6] and Cizmeci *et al.* (2012) [5]. The thrombocytopenia may be attributed to the bone marrow suppression (Table 3, Fig 2).

The serum biochemistry parameter i.e., BUN of the treated dogs showed a non-significant increase on day 0 but increased significantly ($p<0.05$) during treatment when compared to before treatment. Cizmeci *et al.* (2012) [5] explained that BUN increase may be linked to decreased glomerular filtration rate. The serum creatinine revealed an insignificant increase was observed by day 35 during treatment. Increased Serum creatinine was because of increased catabolic activity. Similar results reported by Gadmade *et al.* (2007) [6], Cizmeci *et al.* (2012) [5] and Gandhimathi *et al.* (2011) [7]. The serum ALT, AST and ALP increased significantly ($P<0.01$) on day 0 when compared to the apparently healthy dogs. These parameters increased significantly ($P<0.01$) during therapy when compared to before therapy. AST increase may be because of muscle tissue damage, the findings were similar to those reported by Gadmade *et al.* (2007) [6], Cizmeci *et al.* (2012) [5] and Gandhimathi *et al.* (2011) [7]. The serum protein showed a significant decrease ($p<0.05$) on day 0 when compared to that of apparently healthy dogs, this further significantly decreased ($P<0.05$) during treatment by day 35. (Table 4, Fig 3)

3.1.11 Side effects

The treatment side effects like vomiting, diarrhoea, anorexia and alopecia were seen during treatment with Inj. Doxorubicin. These signs started in 9 dogs from day 7 i.e., after 1st dose slowly and aggravated in 14 dogs by day 21. But the severities of the side effects seen during Inj. Doxorubicin treatment was severe. Such side effects were also observed by Gadmade *et al.* (2007) [6], Cizmeci *et al.* (2012) [5] and Gandhimathi *et al.* (2011) [7]. The side effects of chemotherapy were supportively treated successfully and therapy was continued uninterruptedly.

3.1.12 Recurrence

The cases were monitored for a period of six months for recurrence of any growths and the associated symptoms. The cases were also screened for metastatic spread to the internal organs by radiographs and ultrasonography. In two dogs that have undergone treatment, recurrence was seen after 4 months in one dog and 6 months in the other dog. These dogs were subjected to surgical excision. These were in accordance with the findings of Gadmade *et al.* (2007) [6] and Cizmeci *et al.* (2012) [5].

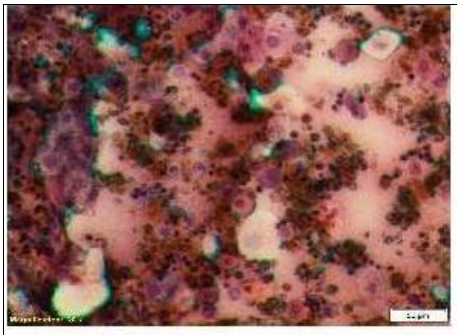
4. Conclusion

In the present study, dogs affected with TVT were successfully treated with Inj. Doxorubicin@30mg/m^2 and the dogs showed side effects were managed with supportive therapy. The dogs that failed to recover completely were subjected to surgical excision.

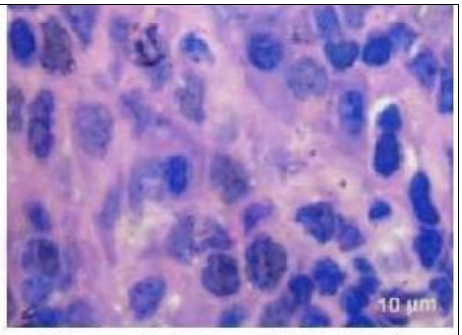
Table 1: Clinical signs of Transmissible venereal tumours in dogs (n=118)

S. No	Clinical Sign	Number	Percent
1	Bleeding or discharges from external genitalia	118	100
2	Nodules/Cauliflower like friable masses in the prepuce and /caudal penis /vulva	118	100
3	Licking of external genitalia	109	92.37
4	Ulceration	52	44.07
5	Lymph node enlargement (inguinal/popliteal)	34	28.81
6	Anorexia /In appetence	28	24.58
7	Dysuria / Tenesmus	19	16.10
8	Metastasis	11	9.32

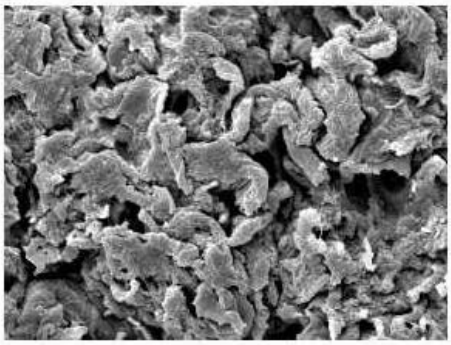
Plate 1: Clinical signs in dogs affected with transmissible venereal tumour



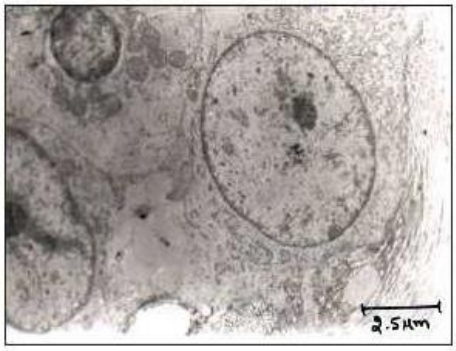
FNAC of TVT showing large round cells with anisocytosis and anisokaryosis



Histopathology multiple mitotic figures with anisocytosis and multiple nuclei large sized



Scanning Electron Microscopic pictures of TVT showing loosely packed cells depicting friable nature of the tumour



7720x 1.9 bar: Cells showing different histological features with altered loss of cytoplasm cell organelles but prominent altered shape and size of mitochondria discontinued and distorted Rough Endoplasmic reticulum with mopderarate dilatation of cisternae;periphery of cells surrounded by proliferating connective tissue all 3 cells showing altered cytoplasm and nucleus ratio with dense chromatin material it appears to be prominent malignant features

Plate 2: Microscopic pictures of TVT

Table 2: W.H.O. (TNM) classification of Transmissible venereal tumours (n=118).

S No	TNM Classification	Dogs selected for chemotherapy	Staging of Tumours				
			Stage I	Stage II	Stage III	Stage IV	Total
1	T1aN0M0	5	9	-	-	-	9
2	T1bN0M0	3	7	-	-	-	7
3	T1CN1aM0	4	7	6	-	-	13
4	T2aN0M0	-	-	12	-	-	12
5	T2bN0M0	4	-	10	-	-	10
6	T2bN1aM0	4	-	12	-	-	12
7	T2bN1bM0	-	-	3	-	-	3
8	T3aN1bM1	-	-	-	6	3	9
9	T3bN1bM0	-	-	-	7	7	14
10	T3bN2bM2	-	-	-	4	6	10
11	T4aN1aM1	-	-	-	4	5	9
12	T4aN1bM0	-	-	-	4	6	10
	Total	20	23	43	25	27	118

Plate 3: Therapy of male dogs with TVT

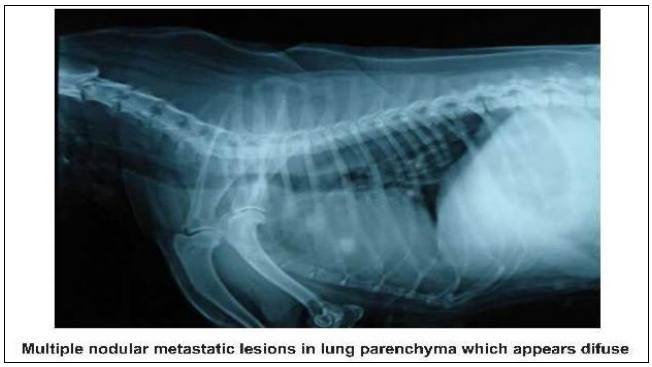


Fig 1: X ray of TVT affected dogs

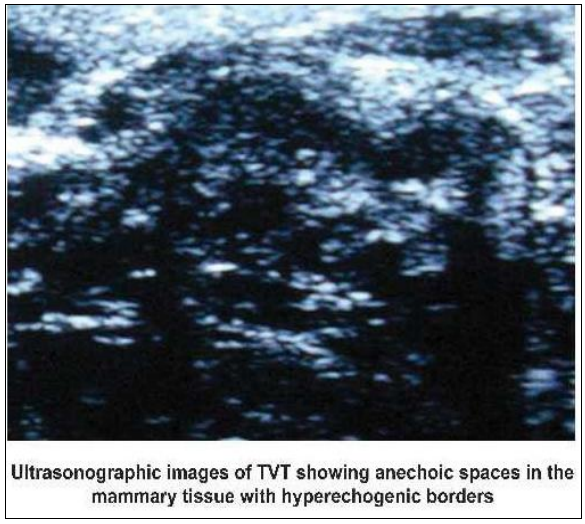
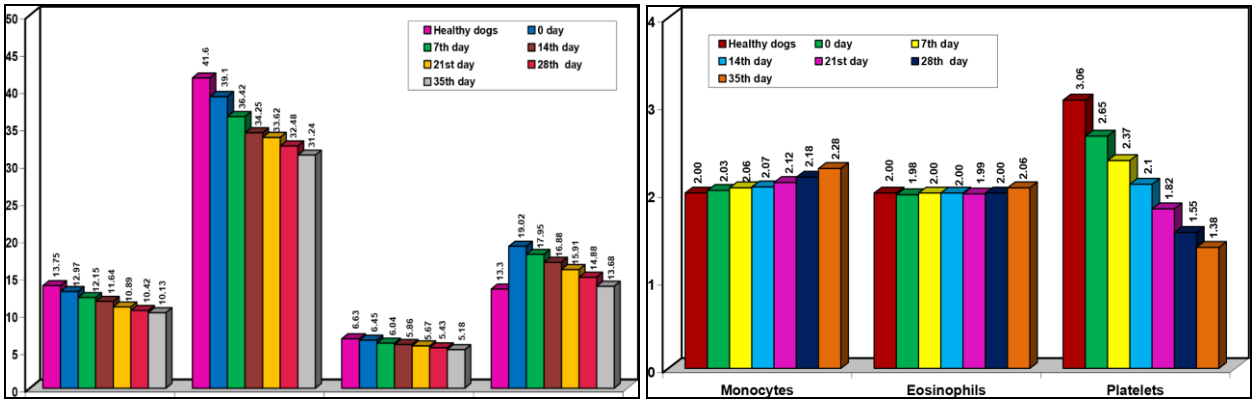


Fig 2: USG of TVT affected dogs

Table 3: Mean hematological values of dogs in Apparently Healthy and Treatment dogs (TVT) before, during and after therapy

S. No.	Parameter	Apparently Healthy dogs (n=10)	Treatment dogs Before therapy (n=20)	Treatment Dogs During and After therapy (n=20)				
			0 day	7th day	14th day	21st day	28th day	35th day
1	Haemoglobin (g/dl)	13.75±0.51	12.97±1.02	12.15±1.80	11.64±1.04	10.89±1.72	10.42±1.68	10.13±2.04##
2	PCV %	41.60±0.91	39.10±1.68	36.42±2.24	34.25±1.66	33.62 ±2.04	32.48±1.90	31.24±2.22##
3	TEC (x 10^6/ µl)	6.63±0.3	6.45±1.22	6.04±0.88	5.86±1.24	5.67±1.76	5.43±2.06	5.18±1.84##
4	TLC (x 10^3/ µl)	13.30±0.86	19.02±2.38**	17.95± 2.26	16.88±1.88	15.91± 1.48##	14.88 ±1.45	13.68 ±0.26##
5	DLC (%)							
	Neutrophils (%)	72.0±0.32	78.98± 1.22**	74.48± 1.44	69.32± 1.52##	64.48± 1.80	60.61± 1.14	54.35±2.62##
	Lymphocytes (%)	24.0±0.14	17.01 ±0.38**	21.46± 0.74	26.61± 0.52##	31.41 ±0.14	35.21± 0.14	41.31±1.30##
	Monocytes (%)	2.00±0.36	2.03±0.72	02.06 ±0.32	02.07± 0.32	02.12±0.30	2.18±0.44	02.28±0.52
	Eosinophils (%)	2.00±0.80	1.98±0.36	02.00± 0.22	02.00± 0.22	1.99±0.38	2.00± 0.22	2.06±0.08
6	Platelets (x 10^5/dl)	3.06±0.24	2.65±0.62*	2.37±0.22	2.10±0.68	1.82±0.22	1.55±0.42	1.38±0.24##

**: Significant at P < 0.01 when compared to apparently healthy dogs;
*: Significant at P < 0.05 when compared to apparently healthy dogs
##: Significant at P < 0.01 when compared to before therapy;
#: Significant at P < 0.05 when compared to before therapy



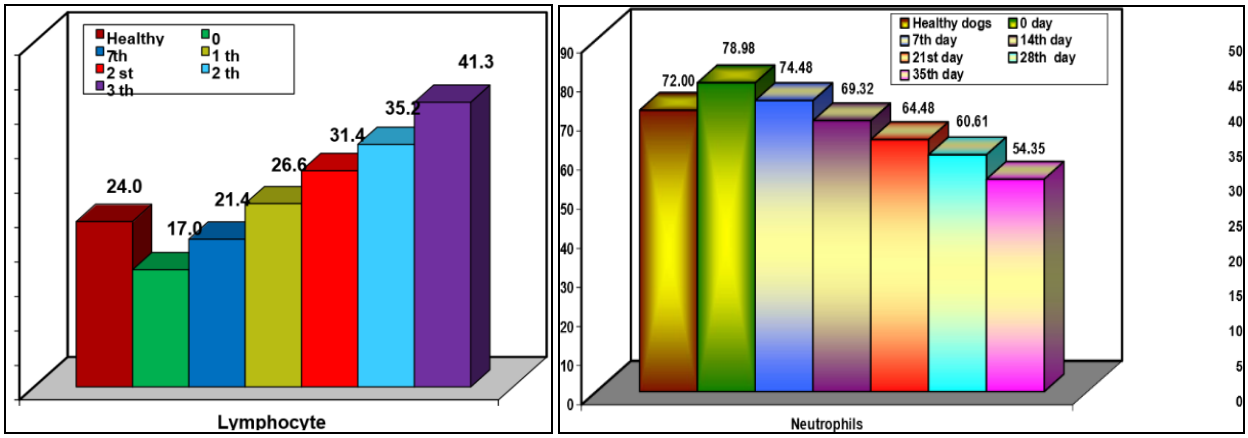


Fig 4: Depicting the mean hematological parameters of Group-I (Apparently healthy dogs) and Group-III TVT affected dogs before, during and after therapy

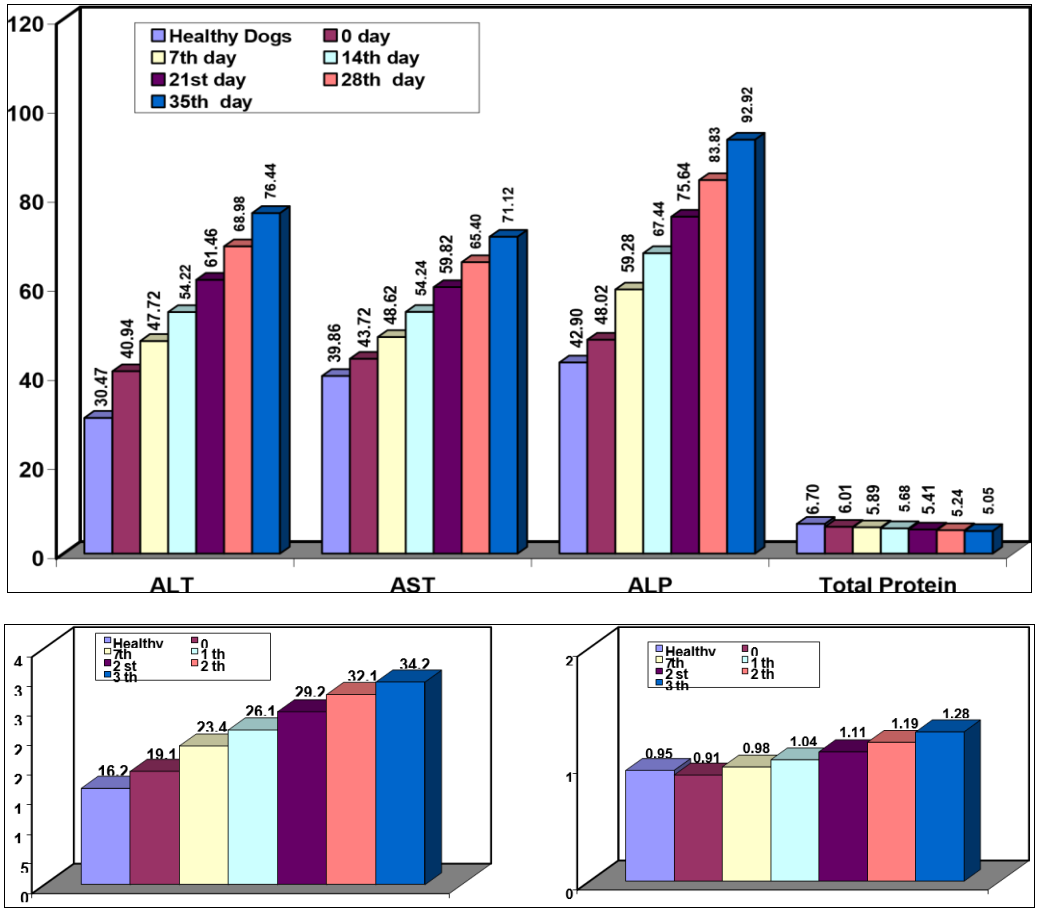


Fig 5: Depicting the mean Biochemical parameters of Group I (Apparently healthy dogs) and Group III TVT-affected dogs before, during and after therapy

Table 4: Mean serum biochemistry of dogs in Apparently Healthy sand Treatment dogs (TVT) before, during and after therapy

S. No.	Parameter	Apparently Healthy dogs (n=10)	Treatment dogs Before therapy (n=20)	Treatment Dogs During and After therapy (n=20)				
			0 day	7th day	14th day	21st day	28th day	35th day
1	BUN (mg/dl)	16.29± 0.44	19.18 ±1.32	23.46± 2.82	26.16±2.36	29.23± 2.14	32.18±2.02	34.26±1.54#
2	Creatinine (mg/dl)	0.95± 0.62	0.91 ±0.28	0.98± 0.18	1.04±0.36	1.11±0.42	1.19 ±1.38	1.28±1.76
3	ALT (IU/L)	30.47 ± 0.98	40.94 ±1.32**	47.72± 2.22	54.22±2.50	61.46±1.12	68.98±1.36	76.44±1.24##
4	AST (IU/L)	39.86±0.36	43.72 ±0.22**	48.62± 2.12	54.24± 0.90	59.82±0.82	65.40±1.14	71.12±0.64##
5	ALP (IU/L)	42.90±0.80	48.02±1.36**	59.28±2.20	67.44±2.52	75.64± 2.16	83.83±1.92	92.92±1.64##
6	Total Protein (g/dl)	6.70±0.14	6.01±0.12*	5.89±0.30	5.68±0.14	5.41 ±0.22	5.24 ±0.18	5.05±1.22#

**** :** Significant at P < 0.01 when compared to apparently healthy dogs;
*** :** Significant at P < 0.05 when compared to apparently healthy dogs
: Significant at P < 0.01 when compared to before therapy;
: Significant at P < 0.05 when compared to before thera

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