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Oncolytic effect of vincristrine and cisplatin on venereal granuloma in canine

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Abstract
The objective of the study was to evaluate a safe oncolytic drugs that could lead to fast and safe apoptic effect on canine transmissible venereal tumour (CTVT) and to determine various clinicophysiological and haematobiochemical parameters before and after treatment. Twelve dogs of either sex with canine transmissible venereal tumour (CTVT) used in the study were divided into two groups viz. A and B. The animals were administered with different drug viz. vincristine sulphate intravenously @ 0.025 mg/kg body weight, at weekly interval for four consecutive weeks and cisplatin @ 2.14 mg/kg intravenously once and repeat after 21 days in animals of group A and B respectively. The physical appearance in relation to onoclytic effect, histopathological changes, hematobiochemical parameters were studied at zero week, first, second, third and fourth week by using standard protocol. On the basis of parameter observed in this study, it was concluded that extent of apoptosis in CTVT cell at first and second week interval was more in the animals treated vincristine sulphate as compare to cisplatin as indicated by physical appearance and histopathological (H&E staining) examination. The early and best regression of the CTVT was observed in the animals treated with vincristine sulphate. Cisplatin regressed the CTVT masses upto some extent by apoptosis; Vincristine sulphate is effective drug for the treatment of CTVT even in metastatic conditions as compare to cisplatin.

Keywords: Vincristin sulphate, cisplatin, CTVT and apoptosis

Introduction
Cancer can be defined as a disease in which a group of abnormal cells grow uncontrollably by disregarding the normal rules of cell division. Canine transmissible venereal tumor (CTVT) is also known by various names such as stickers tumor, venereal granuloma, canine condyloma, transmissible sarcoma, transmissible lymphosarcoma. It is a tumor of the dog and other canids that mainly affects the external genitalia and is transmitted from animal to animal through sexual contacts but may also be passed on as the dog bites, sniffs or licks the tumor affected areas. As it is usually transmitted during coitus and mainly occurs in young, sexually mature animals (Kumar *et al.*, 2014 and 2015) [13, 14]. It is transplanted during coitus with intact viable cells across major histocompatibility complex (MHC) barriers within the same species and even to other members of the canine family. In India TVT is known to be the most frequently reported tumor in dogs ranging from 23-43 % of the total number of tumors in canine population. Uncontrolled sexual behavior and a large stray dog population appear to be one reason for such a high incidence of TVT. Tumors bleed easily and while becoming larger, normally ulcerate and become contaminated. Cytologically, TVT cells have a very distinct appearance. They are round to oval in shape and often contain mitotic figures, with chromatin clumping and one or two prominent nucleoli. Perhaps the most striking cytological finding, however, is the presence of multiple clear cytoplasmic vacuoles. Histological examination of TVTs usually reveals that the component cells grow in compact masses or confluent sheets. The management of TVT has not been very easy. Several treatments including surgery, radiotherapy, immunotherapy, biotherapy and chemotherapy have been applied for TVT. Surgery has been used extensively for the treatment of small, localized TVTs, although the recurrence rate can be as high as 50 - 68% in cases of large invasive tumors. Methods to prevent recurrence subsequent to surgery include excision along with cauterization, electrosurgical or cryosurgical excision or chemotherapy subsequent to surgical excision. Chemotherapy has become popular approach by using several chemotherapeutic agents like vincristine, doxorubicin and cisplatin. Vincristine sulphate has effective as a single chemotherapeutic agent in the management of canine CTVT but a part of this, it has considerable toxic effects (Verma *et al.*, 2014) [28].

Cisplatin has been especially interesting since it has shown anticancer activity in a variety of tumors including cancers of the ovaries, testes and solid tumors of the head and neck. It was discovered to have cytotoxic properties in the 1960s. It has a significant challenge with regard to their cure, because of drug resistance and considerable side effects. So effective drug delivering into the body is required to reduce their toxicity and side effects In view of the above facts, this study was undertaken with a primary objective to compare the chemotherapeutic effect of vincristine sulphate alone and cisplatin on transmissible venereal tumour in dogs.

Materials and Methods

All the twelve animals subjected to this study were divided in to two groups having six animals each. To obtain an effective dose for the regression of venereal tumours growth, different dose schedules of each drug was evaluated under pilot trial studies on separate dogs.

The following schedule of experimentation was followed in the animals of different groups.

Groups Drug schedule

a) Vincristine Sulphate @ 0.025 mg/kg intravenously once in a week for four consecutive weeks
b) Cisplatin @ 2.14mg/kg intravenously once and repeat after 21 days

[1]**Vincristin Sulphate:** Cipla Ltd.
[2]**Cisplatin:** Neon Laboratories Ltd.

Observations

Clinical Parameters

Physical and clinical symptoms were noticed before and after treatment i.e at 0, 1st, 2nd, 3rd and 4th week of the treatment. The subjected animals were observed for up to 2 months after the end of experiments for untoward symptoms as well as recurrence of tumour.

Response of Onoclytic Drugs

The extent of apoptotic effect of different drugs were determined by observing and assessing the clinical size of tumour mass and comparison the regression of tumour mass with their initial base size before treatment.

Histopathological Examination

For the identification of the nature of tumor, the histo-pathological studies were conducted by routine paraffin embedding method. The tissue biopsy samples from different groups were collected in 10% buffer formalin solution at 0, 1st, 2nd, 3rd and 4th week of experimentation.

Haematoxylin and Eosin Staining

Normal and cytological changes of venereal tumour cell at different time interval of treatment were observed. Serial sections were cut at 6-8 microns thickness from each specimen by automatic section cutting machine and stained with haematoxylin and eosin. The slides were observed under microscope and lesions were recorded.

Haematobiochemical parameters

Haematobiochemical parameters were studied on 0, 1st, 2nd, 3rd and 4th week of treatment. Nearly 5 ml venous blood were collected from recurrent tarsal vein or sephaneous vein by the sterilized disposable syringe and then transferred into sterilize glass vials (2 ml) provided with heparin as an anticoagulant and in other test tubes (3 ml) for the serum collection.

Different haematological parameters viz. haemoglobin (Hb), packed cell volume (PCV), total erythrocyte count (TEC), total leucocyte count (TLC) and differential leucocyte count (DLC) were analysed. The serum was analyzed for different biochemical parameters included serum total protein, serum albumin, serum glucose, serum urea nitrogen, serum creatinine, serum aspartate amino transferase (AST), serum alanine amino transferase (ALT) and serum gamma glutamyl transpeptidase (GGT) by fully automatic blood analyzer.

Results and Discussion

The present study was carried out to evaluate the onoclytic effect of vincristine sulphate and cisplatin on transmissible venereal granuloma in twelve dogs of either sex divided into two groups *viz.* A and B of six animals each. The onoclytic effects of the different oncolytic drugs were made on the basis of physical appearance, regression activities and histopathological studies and apoptotic studies.

Gross appearance of canine venereal tumour

In this study, cases presented had a characteristic appearance of TVTs: cauliflower shaped nodules of various sizes (0.5–5 cm in diameter) with ulcerated surfaces. In males the tumours masses were appeared generally as single, multiple, small to large, sessile or pedunculated and soft nodular masses on the base of penis. In females, the neoplasms were usually solitary cauliflower like on vulvar region in the external genitalia. The consistencies of the neoplastic masses were soft and relatively firm. All the tumour masses were irregular, soft and fragile in consistency. In few of the cases the tumours were fulminating in nature and animal became cachexic. Primary genital tumour in the male dog was usually found on the caudal part of the penis, from the crura to bulbus glans or the area of the glans penis and occasionally on the prepuce. These findings collaborate the findings of Rezae *et al.*, 2016 [20]. In some cases the tumours were so large that it extending to scrotum and there was difficulty in exposing penis. The neoplasms in both male and female dogs were pink to bright red in colour because of the rich blood supply and had a tendency to bleed associated with tumour fragility. Persistent or intermittent serosanguineous or bloody discharge with often foul smelling were evident with CTVT affected patients and most of the animals showed a constant licking behaviour to the genitalia. In all animals physical parameters (rectal temperature, urination and defecation) were normal except in one case where there was severe retention of urine due to pressure of growth on the urinary tract. The present findings associated with the physical appearance and location of venereal granuloma were also supported by Das and Das, (2000) [4]; Goldschmidt and Hendrick (2002) [8]; Thangathurai *et al*. (2008) [25]; Kisani and Adamu (2009) [10].

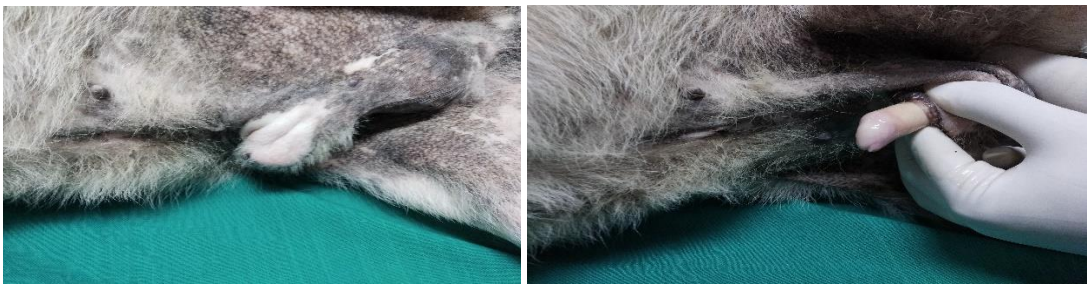

Fig a-b: Group A- before and after treatment with vincristine sulphate

Fig c-d: Group A- before and after treatment with vincistine

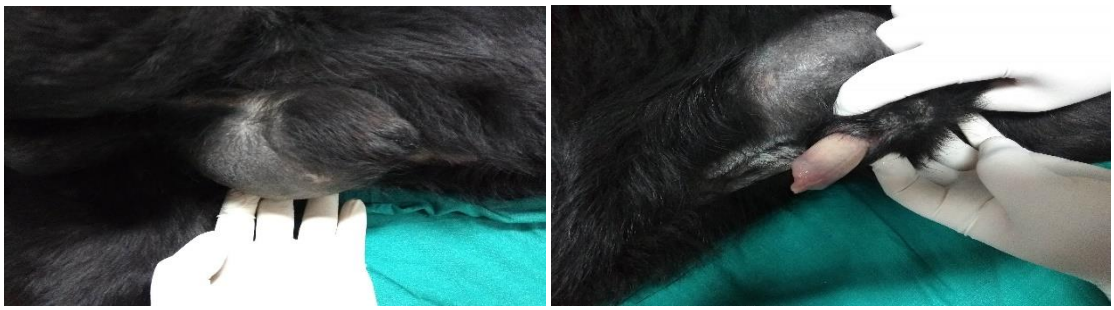

Fig e-f: Group B- before and after treatment with cisplatin

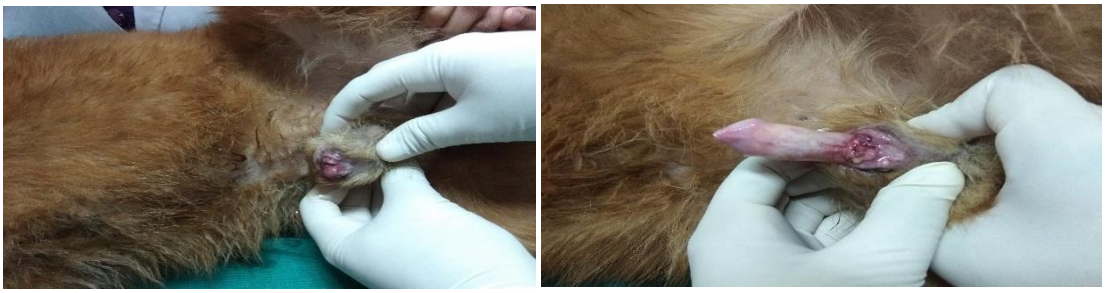

Fig g-h: Group B- before and after treatment with cisplatin

Plate 1: Photograph showing gross appearance of the venereal tumours before and after the treatments

Regression of tumour

In the group A complete regression of tumours mass was observed at the end of third week (fig. a, b and c, d). In group B two animal did not respond to the cisplatin therapy and there was no change in the physical appearance (Plate-1; Fig. e & f). The tumours masses were more or less same size throughout the period of study. In the group B moderate regression of tumours mass was recorded at the end of fourth weeks and regression of tumours mass was seen only in the peripheral area of the site of injection of nodular mass while, the rest of areas were unaffected (Plate-1; Fig. g to h). The venereal tumours regressed after two week of treatment changed to pea shaped structure of pink to red colour in the male dogs of group A. After 4 weeks the complete regression of venereal tumour was observed as 100% and 40% in the animals of group A, and B, respectively. Reduction in venereal tumour was started rapidly after first injection of vincristine sulphate in the animals of group A. After one week of treatment in group A remarkable regression of the growth was noticed along with markable reduction in the oozing of bloody and fowl discharge. On physical examination animals of group B showed 40% reduction in tumours growth after fourth week and there was no cessation of discharge.

Regression effects of vincristine and cispatin in canine transmissible venereal tumour have also been reported by Murchison (2008) [18] who suggested that vincristine induces apoptosis in CTVT in canine. The induction of apoptosis by vincristine sulphate was associated with the caspase-3 and 9. The activation of caspase family protines play a key role in apoptosis. The present findings of apoptosis or regression of CTVT are in accordance with observation of Decker *et al.* (2015) [5] who have reported the regression of tumour mass in

the present findings with vincristine treatment may be due to its binding to tubulin, a microtubular protein within the cells, acts as a spindle poison. The spindles then cannot act in mitosis, arresting the cell cycle in metaphase (Barton, 2001; Garden, 2010; Ganguly *et al.*, 2016 and Antonov, 2017) [2, 7, 6, 1]. Nak *et al.* (2005) [19] have also reported that 31 dogs out of 38 dogs suffering with CTVT cured by using vincristine. Even in the case of metastasis, the cure rate for CTVTs is over 90%. Sharma *et al.* (2011) [22] and Antonov, (2017) [1] have reported that vincristine sulphate at the rate of 0.025 mg/kg body weight intravenously at weekly intervals on 3 to 4 occasions was the most effective, safe and convenient chemotherapeutic agent, which gave a better survival time in CTVT patients with extra genital metastasis.

Histopathological Studies

The histopathological staining at different time interval in various group of animals before and after administration of drugs are given photograph depicted in plate 2.

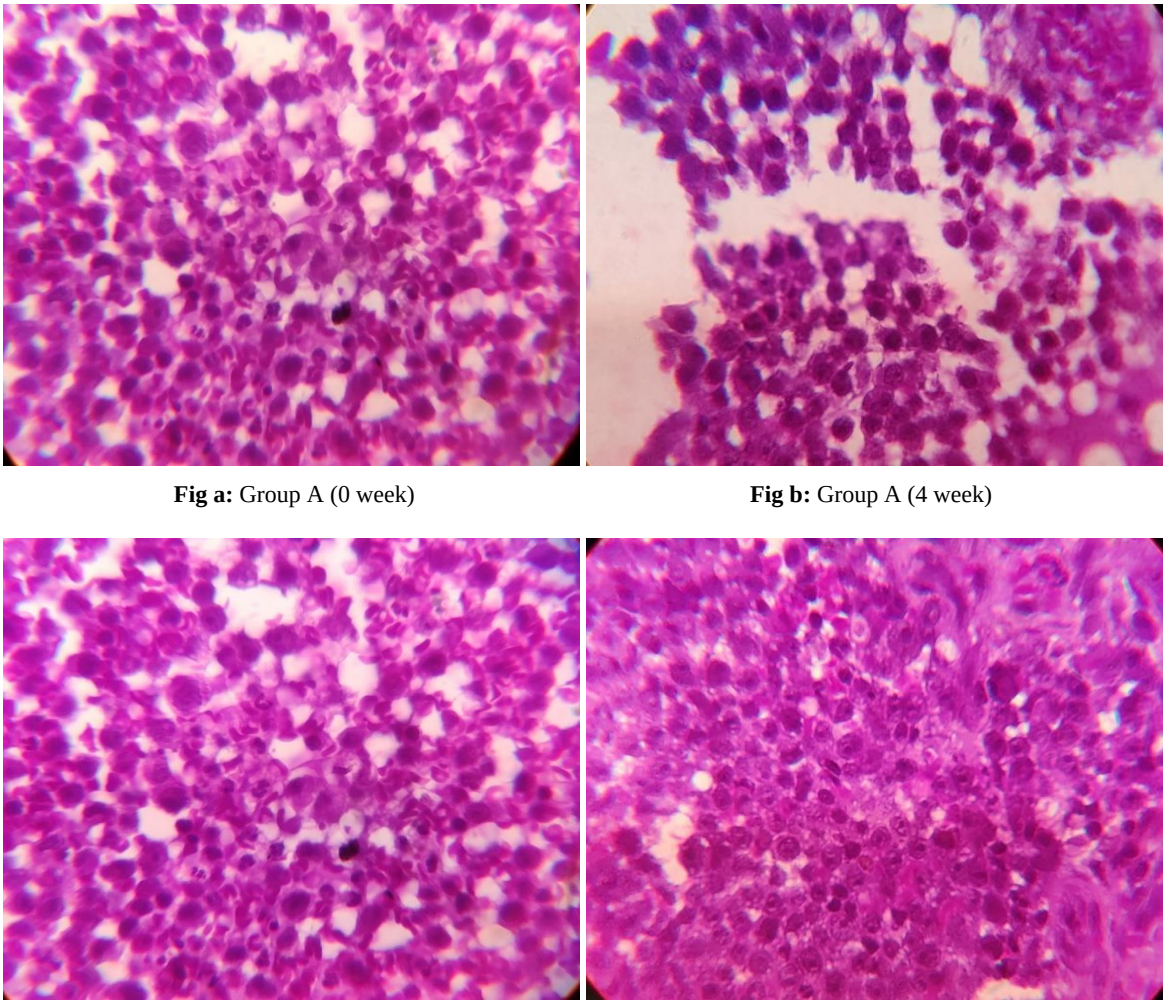

Fig a: Group A (0 week)

Fig b: Group A (4 week)

Fig c: Group B (0 week)

Fig d: Group B (4 week)

Plate 2: Photomicrograph showing histopathological section at different time intervals.

There were variable regressive changes by histopathological staining were seen in animal of the groups A and B at zero and fourth week. The histopathological section from the animals of group B at fourth week showed few neoplastic cells-initiated regression phase with coarse and granular nuclei having scanty vacuolation of cytoplasm (Plate 2. Fig.d). Histopathological examination of venereal tumour of group A at second week time interval revealed a large area of necrosis of neoplastic tissue. Neoplastic cells were highly vacuolated with oval or round, large or small nucleus with coarse and granular chromatin and the interlaced intercellular collagen fibers.

Histopathological examination of group A at third week interval showed a large area of necrosis of neoplastic tissue. Neoplastic cells were highly vacuolated with oval or round, large or small nucleus with coarse and granular chromatin and the interlaced intercellular collagen fibers. The nucleus was absent in most of the degenerative places of necrosed area. In high power magnification, the cytoplasm was abundant with vacuolation and had low mitotic figures. At multiple places, there was necrosis of individual or group of neoplastic cells which appeared as irregular empty spaces seen throughout the section of tumour (Fig. b.). The number of apoptotic cells were more in comparison to biopsy sample of 0 week intervals. Presence of shrunk isolated apoptotic cells, with a condensed chromatin and fragmented nucleus and apoptotic bodies were also observed. Abundant inter-cellular stromal collagen accumulation was revealed in the progressive regression of tumour mass in the animals of group A at third week and fourth week interval of the study (Fig. b.). Mature connective tissue was seen in histopathological section of the animals of group A at fourth week time intervals.

Histopathological studies revealed a sheet of round cells containing round vesicular nuclei, the borders of which could

not be easily differentiated. Trompieri (2009) [26] has reported that venereal tumour of dogs contained uniform populations of round or oval shaped large cells with scant but well-defined cytoplasm, arranged in compact groups. The nuclear chromatin was fine diffusely distributed, with large and oval shaped nuclei containing prominent and centrally placed nucleoli.

There was prominent infiltration of lymphocytes, plasma cells and few macrophages. Mitotic figures were also seen. The presence of round individual cells in an arborizing fibrovascular network could help in diagnosing the tumour histologically. These observations are in accordance with the findings of Maclachlan and Kennedy, 2002 [15] and Krithiga *et al.,* 2005 [11]. However, the cells were baso-eosinophilic with haematoxylin and eosin (H&E) staining. A great variation in the cellular (anisocytosis) and nuclear morphology (anisokaryosis) was also observed. The nucleus of the tumour cells was round to oval in shape and centrally placed. Anisonucleoliosis was prominent in the nucleus of the tumour cells. The nucleoli were basophilic and the number varied from one to three. The nuclear chromatin pattern was coarse to reticulate (Thangathurai *et al.,* 2008) [25]. The composition and quantity of the stroma observed in the regressive transplanted tumours were more evident than in the growing transplanted TVTs, similar to genital TVTs.

The regression of tumourous tissue of the animals of group A at second and third week of study was also supported by the observation of Sethawongsin *et al.* (2018) [21] and Murad *et al* (2019) [17]. The regressed tumour showed vacuolation of cytoplasm. In some cases there was an infiltration of neutrophil and abundant amount of collagen connective tissue. The biopsy of penile or vaginal epithelium at the site of tumour after complete regression of tumour revealed mature connective tissue with no evidence of tumour cells. The submucosa was full of newly formed blood vessels with mature connective tissue.

Hematobiochemical parameters

There was a non-significant (P<0.05) decreased in Hb level subjected to administration of vincristine sulphate in the animals of group A. The Hb level in group A showed a consistent and non-significant gradual decreasing trend up to fourth week (10.24±0.45) from their base value (11.00± 0.23). It was also observed that Hb at third week (10.20±0.46) was non-significantly (P<0.05) lower than the values recorded at second and fourth week time intervals. In the animals of group B subjected to administration of cisplatin, a non-significant (P<0.05) change in the mean value of Hb was observed throughout the period of study. At one week time intervals Hb (11.84±0.39) slightly decreased from base value (12.18±0.98). Thereafter its level decreased and reached to lower level (10.09±0.48) at fourth week interval. Decrease in haemoglobin in both the groups may be due to chronic loss of blood and myelosuppression by the drug (Kumar *et al*., 2018) [12]. The fall in Hb value at various time intervals in CTVT dog treated with vincristine confirms the finding of Dan *et al.* (2017) who have opined that it may be due to depression of reticuloendothelial system.

Significant (P<0.05) decrease was observed in packed cell volume (PCV) throughout the period of study in the animals of group A subjected to the administration of vincristine sulphate. A decreasing trend in PCV level from base value (43.78±3.12) to third week time interval (39.85±2.98) followed by increase upto fourth week (40.85±1.67). In the animals of group B subjected to the administration of cisplatin, the mean PCV value decreased significantly (P<0.05) throughout the period of experiment. PCV level at 0 and first week was significantly (P<0.05) higher than its value at second, third and fourth weeks in the animals of group B. A significant decrease in mean value of PCV in the animals of group A and B, subjected to administration of vincristine and cisplatin respectively may be due to bone marrow depression anaemia. The same results have also been observed by Srivastava *et al.* (2009) [23] in the animals subjected to vincristine treatment.

There was a non-significant (P>0.05) decrease in the level of total leucocytes count (TLC) in the animals of group A up to three-week time interval and then tends to increase towards mean base value at fourth week. In the animals of group B, significant (P<0.05) increase and decrease in TLC value was observed throughout the period of study. Non-significant (P>0.05) change in TLC in animals of groups A, and B in the present study indicated that probably there was side effect of vincristine and cisplatin may be due to depression of reticuloendothelial system along with mild myelosuppression with the use of vincristine sulphate and cisplatine (Dan *et al*. 2018) [3].

In the animals of group A and group B, a non-significant (P>0.05) changes in mean neutrophil values were observed throughout the period of study. The fluctuating increase or decrease in mean neutrophil values at various time intervals was very near to their base value at 0 week. In the animals of group A and B, a non-significant (P>0.05) change in mean neutrophil throughout the period of experiment might be due to myelosuppression. However, no significant changes in neutrophil level were observed by Das and Das (2000) [4]; Sharma, (2011) [22] and Srivastava *et al* (2009) [23] in animals treated with anticancerous therapy.

A significant (P>0.05) change in mean lymphocyte value was observed throughout the period of experiment in the animals of group A subjected to administration of vincristine. The significant increase in mean lymphocyte value was very near to base value at different time intervals throughout the period of study. In the animals of group A, the lowest (27.50±2.74) and highest (37.45±1.55) mean lymphocyte level was observed at 0 and fourth week times interval, respectively. In the animals of group B non-significant (P>0.05) increase in mean lymphocyte value from their base value was observed at different time intervals. In group B, the lowest (31.65±2.43) and highest (34.50±1.29) mean lymphocyte value was observed at 0 and 2 week, respectively. The present finding of lymphocytosis may be due to myelosuppression and was also observed previously by Srivastava *et al.* (2009) [23]. Contrary to lymphocytosis in the present study, the lymphocyte count was in the normal limit throughout the period study in the animals treated with vincristine (Sharma *et al.,* 2011) [22].

A gradual and non-significant (P>0.05) decrease in the serum protein was observed up to fourth weeks in the animals of group A, and B. In both the groups, lowest level was at fourth week time interval. The decrease in the serum protein was within normal physiological limit. The decreased concentration of total protein (TP) indicates the possibility of mild interference in absorption and assimilation of nutrient owing to intestinal disorders in dogs as a sequel to vincristine therapy. This is mainly because of primary action of antineoplastic drugs on fast dividing cells. The same observation has been reported by Dan *et al*., 2018 [3]. However in group C and D, the effect of vincristine and

cisplatin might be neutralize by action of scaffolds. Varughese *et al.* (2012) [27] and Ganguly *et al.* (2016) [6] have also observed low concentration of total protein in canine venereal granuloma like other benign and malignant tumour as compare to normal dogs. However, Sharma *et al*. (2011) [22] did not observe any change in the level of total protein in CTVT dog treated with vincristine.

A non-significant (P>0.05) change in the level of serum albumin was observed throughout the period of experiment in the animals of groups A and B subjected to administration of vincristine sulphate and cisplatin respectively. The levels of serum albumin remained in the normal physiological limit in all the animals of both the groups at different time intervals throughout the period of the study. Non-significant change in albumin level at various time intervals in all the groups of animals does not indicates the harmful effect of cisplatin and vincristine on the hepatocytes of the liver (Antonov, 2017 and Murad *et al.*, 2019) [1, 17].

In the animals of group A, a non-significant (P>0.05) decrease in mean glucose level was observed up to second week (61.50±3.58) followed by increase in its values at fourth week time intervals. In group A the mean glucose level at second week (61.50±3.58) was significantly different from their mean base value (74.40±2.85) as well as the value at fourth week (75.25±3.18). The mean glucose level at first, second, and third week intervals did not vary significantly (P<0.05). In the animals of group B, subjected to administration of cisplatin, the fluctuating mean glucose values at various time periods were close to their base value (81.85±4.85). Non-significant change in blood glucose level in animals of groups A, and B subjected to administration of vincristine sulphate and cisplatin indicates that no considerable effect of these drug in the body. However, a significant decrease in the mean blood glucose level was a characteristic feature of venereal granuloma (Tella, 2004) [24].

In the animals of group A, a slight and gradual decrease in serum urea nitrogen was observed throughout the period of study. However its level was in the normal physiological limit. In the animals of group B, slight non-significant (P>0.05) increase in serum urea nitrogen was observed upto fourth week. Non-significant change in serum urea nitrogen at various time intervals in all the animals of group A indicates the no harmful effect of drugs on renal, hepatic system and the other related system, while significant increase in BUN level of group B indicate harmful effect on renal system. Tella *et al.* (2004) [24] and Sharma *et al.* (2011) [22] have also observed similar changes in BUN level in CTVT dog.

There was no non-significant (P>0.05) difference in the serum creatinine level in animal of group A and significant increase in animals of group B at respective time interval throughout the period of this study. A slight increase or decrease in the level of serum creatinine throughout the period of observation was within the normal physiological limit and it was near to their base value. Non- significant change in creatinine level at various time intervals in group A subjected to administration of vincristine sulphate indicates the no harmful effect of drugs on renal and other related organs, while significant increase in BUN level of group B indicate harmful effect on renal system Sharma *et al.* (2011) [22] have also observed similar change in creatinine level in canine CTVT dog.

There was non-significant (P>0.05) change in the level of serum aspartate amino transferase (AST) and alanine amino transferase (ALT) throughout the period of experiment in all the animals of group A and the values of serum aspartate amino transferase and alanine amino transferase were in the normal physiological limit in all the animals of different groups at different time intervals throughout the period of this study, However there is significant increase in the level of serum aspartate amino transferase (AST) and alanine amino transferase (ALT) throughout the period of experiment in all the anima.ls of group B.

AST is present in hepatocytes as well as in cardiac and skeletal muscles in high concentration. Non-significant change in AST during successive weeks of treatment in animals of group A subjected to administration of of vincristine sulphate are in accordance with the observation of Mello *et al*. (2013) [16]; Dan *et al.* (2018) [3] who have reported that vincristine has no harmful effects on liver and muscles. Contrary to the present study, Sharma *et al.* (2011) [22] observed a significant (P<0.05) increased in AST value in CTVT dog following treatment with vincristine up to second week of treatment. ALT is liver specific enzymes and present in large quantities in cytoplasm of the hepatocytes. The liver is one of the main metabolic centers of the body and the cells contain a multiplicity of vital enzymes. When the liver damage occurs, the cell membrane may become more permeable or the cell wall may rupture so that these enzymes diffuse into blood stream and increased levels are found in circulating blood. Thus the measurement of the serum activity of these enzymes can reflect the integrity of the walls of liver cells and can provide an important method of assessing liver damage. The cytoplasmic enzymes such as alanine amino transferase and aspartate amino transferase are affected by permeability of cell membrane.

There was non-significant (P>0.05) change in the level of serum gamma glutamyl transpeptidase (IU/L) serum aspartate throughout the period of experiment in both the groups of animals and the values of serum were in the normal physiological limit in all the animals of different groups at different time intervals throughout the period of this study. Serum GGT is found in many tissues but primary source is liver. Microsomal (ribosomal and mitochondrial) enzymes like gamma glutamate dehydrogenase need severe damage of liver cells before their elevation. The increased level of the serum GGT in animals suffering from hepatic disorders observed in this study confirms the findings of Kaneko *et al.* (2008) [9]. The decrease in serum GGT level after the initiation of treatment in different groups indicates the beneficial effect of the therapy.

Table 1: Mean±SE of heamtobiochemical parameters after administration of vincristine sulphate in animals of group A and cisplatin in animals of group B at different time intervals.

Parameters (Mean±SE)	group	Time intervals				
		0week	1week	2week.	3week	4 week
Haemoglobin (gm/dl)	1	11.00±0.23	10.86±0.47	10.22±0.55	10.20±0.46	10.24±0.45
	2	12.18±0.48	11.84±0.39*	10.90±0.81**	10.70±0.69*	10.09±0.48*
PCV (%)	1	43.78±1.58	42.25±2.15	40.60±2.45*	39.85±2.98*	40.85±1.67
	2	47.25±2.56	44.50±1.83*	43.75±1.78	43.60±1.98	42.35±1.30*
TLC (10^3/µl)	1	8.68±0.49	7.90±0.59	7.85±0.56	7.52±0.54	7.58±0.58
	2	8.20±0.44	7.83±0.55*	8.98±0.52*	9.10±0.52*	9.15±0.43
Lymphocyte (%)	1	27.50±2.74	31.75±0.44	35.15±2.73*	37.28±1.28*	37.45±1.55
	2	31.65±2.43	32.36±3.15	34.50±2.65	33.75±2.69	34.25±1.58
Neutrophils (%)	1	70.50±3.27	71.76±1.75	68.00±2.32	69.00±2.22	70.00±1.81
	2	62.75±2.99	65.50±3.89	63.25±1.85	66.25±2.42	62.50±0.81
Total Protein (g/dl)	1	5.98±1.51	5.52±1.46	5.48±1.40	5.23±0.28	5.27±0.88
	2	5.83±1.72	5.69±1.71	5.60±1.50	5.55±1.52	5.48±1.49
Albumin (g/dl)	1	3.15±0.40	3.00±0.46	3.20±0.50	3.32±0.61	3.35±0.44
	2	2.19±0.28	2.16±0.44	2.17±0.616	2.11±0.53	2.33±0.71
Glucose (mg/dl)	1	74.40±2.85	69.75±2.462	61.50±3.58	70.65±2.28	75.25±3.18
	2	84.85±4.85	82.40±6.94	82.25±5.76*	81.40±4.75*	81.85±3.06
Blood urea nitrogen (mg/dl)	1	29.50±2.35	29.75±1.68	30.15±2.56	30.21±1.57	31.01±1.32
	2	28.45±1.86	32.00±2.33	33.25±1.64	33.60±1.49*	34.50±2.96*
Creatinine (mg/dl)	1	1.16±0.19	1.20±0.44	1.26±0.47	1.25±0.84	1.27±0.32
	2	1.12±0.35	1.60±0.08	1.75±0.04*	1.72±0.21	1.70±0.28*
AST (IU/L)	1	37.25±1.44	37.65±3.29	38.10±3.05	38.22±1.05	38.25±1.02
	2	38.75±1.65	39.25±3.25	42.50±2.67	42.25±2.78	45.75±2.98
ALT(IU/L)	1	29.50±3.13	28.50 ± 3.03	28.75± 3.46	30.00± 2.45	31.25± 3.12
	2	30.00± 3.56	31.29± 3.63	32.15± 2.15	32.00± 3.45	32.75± 3.96
GGT (IU/L)	1	4.25± 0.54	4.15± 0.75	3.45± 0.60	3.95± 0.50	4.50± 0.80
	2	4.00± 1.29	4.20± 1.28	4.35± 1.45	3.95± 0.95	4.30 ± 1.29

* Significant at $p<0.05$ difference with 0-week time interval
** Significant at $p<0.01$difference with 0-week time interval

Conclusion

On the basis of parameter observed in this study, it was concluded that extent of apoptosis in CTVT cell at first and second week interval was more in the animals treated with vincristine sulphate as compare to cisplatin as indicated by physical, clinical, hematological, biochemical and histopathological (H&E staining) examination, studies. The early and best regression of the CTVT was observed in the animals treated with vincristine. Cisplatin regressed the CTVT masses upto some extent by apoptosis; however vincristine sulphate is more effective when it is used in appropriate dose. Vincristine sulphate is an effective drug for the treatment of CTVT even in metastatic conditions, as compared to Cisplatin.

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