Effects of anti-cancer drugs and phyto-metabolites on morphometry, growth and histology of tail regenerate in house lizard

INTRODUCTION

Chemotherapy involves the use of chemical agents to stop the invasive growth of cancer cells. However, chemotherapeutic drugs do not distinguish between cancer and normal cells, and eliminate not only neoplastic cells but also other fast-growing cells that are essential for one’s welfare. In the current scenario, chemotherapeutic regimens are frequently used in conjunction with surgery either preoperatively to decrease the tumor burden prior to surgical resection or postoperatively to help eradicate residual disease. The ultimate goal of this multimodal approach for treating cancer is to eradicate disease while causing the least amount of harm to the patient. However, wound healing in patients with cancer has become a matter of critical importance regardless of whether the wound was surgically created, a complication of radio- or chemotherapy, or due to disease progression (Payne et al., 2008). Therefore, it is crucial that physicians should coordinate their treatment regimens to help optimize wound healing and other side effects by understanding the effects of chemotherapy on wound healing and normally proliferating cells.

Regenerative biology and medicine is an emerging field dealing with restoration of cells, tissues and structures that are lost or damaged after disease, injury or aging (Stocum, 2001; Tsonis, 2002; Brockes and Kumar, 2005; Stoick-Cooper et al., 2007). House lizard, Hemidactylus flaviviridis, has remarkable ability to regenerate its tail by
the process of epimorphic regeneration following three definable stages: wound healing, blastema formation and differentiation stage. The events associated with the progression of regenerate are closure of wound without scarring at the site of injury, generation of inflammatory response, apoptosis of damaged cells, matrix reorganization, activation of stem cells through dedifferentiation, cell proliferation for attaining growth and morphogenesis to resemble the lost structure. Because of involvement of these events, appendage regeneration in adult vertebrates has attracted biomedical interest and served as model system for evaluating toxicity of chemicals and drugs (Bechara et al., 2000, 2003; Zodrow and Tanguay, 2003).

Therefore, in the present study, tail regeneration in house lizard was explored as a model system to evaluate the effects of three anticancer drugs (VCR, MTX and 5-FU) and two phyto-metabolites (CUR and COL) on wound healing and tissue regeneration by evaluating the growth of the regenerating tail (morphometry), its histoarchitecture and the variations in protein and nucleic acid content through different phases of tail regeneration.
RESULTS

Morphometric analysis of tail regenerate administered with VCR:

Administration of VCR caused 4, 7 and 5 days delay in attaining WH, BL and DF stages respectively. The average growth rate of VCR administered animals also decreased significantly at BL (53.87%) and DF (43.73%) stages (Table-1).

Effect of VCR on nucleic acid and protein content of tail regenerate:

WH stage: The DNA and RNA content of regenerating tail in VCR administered animals were found to decline significantly by 39.83% and 34.48% respectively whereas protein content increased by 6.54% as compared to that in control animals. When these animals were allowed to complete wound healing (WH-C group), DNA, RNA and protein contents were significantly higher than that in controls (Table-2).

BL stage: The DNA and protein content decreased significantly during BL stage in VCR administered animals whereas RNA content elevated several folds. However, when these animals were allowed to complete blastema formation (BL-C group), DNA and protein levels were slightly lower than control animals and RNA levels increased enormously by 365.22% as compared to controls (Table-2).

DF stage: VCR administered lizards registered significant decline in DNA and RNA levels whereas 12.47% elevation in protein content was found. At completion of DF stage (DF-C group), VCR administered animals exhibited lower levels of nucleic acids and higher levels of protein content as compared to control animals (Table-2).
**Histological observations of tail regenerate after administration of VCR**

**Localization of nucleic acids:** In WH stage, the nucleic acid content in tail regenerate was less in VCR administered animals (Fig. 1E) as compared to that of control animals (Fig. 1D). However, at completion of wound healing, these animals demonstrated higher amount of both DNA and RNA content (Fig. 1F). In BL stage, DNA content was much less and RNA content was higher in VCR administered animals (Fig. 2E) as compared to control animals (Fig. 2D). At completion of blastema stage, these animals exhibited slightly lower levels of DNA and much elevation in RNA content (Fig. 2F). In DF stage, control animals exhibited higher levels of DNA and RNA (Fig. 3D) as compared to VCR administered animals (Fig. 3E). At completion of differentiation stage, VCR administered animals showed lower levels of both DNA and RNA as compared to control group (Fig. 3F).

**Histology:**

**WH stage:** The control animals at WH stage showed cornified and keratinized wound epithelium (WE) at the apex. Further, mesenchymal cells accumulating under WE could also be seen (Fig. 1G). Administration of VCR inhibited the WE formation in regenerating tail of house lizards. The accumulation of mesenchymal cells under WE could also not prominently seen in the caudal sections of VCR administered animals (Fig. 1H). However, at completion of wound healing, VCR administered animals showed wound epithelium covering stump and mesenchymal cells accumulating underneath WE were evident (Fig. 1I).
**BL stage:** Control sections at BL stage showed few layers thick WE, aptly called apical epithelial cap (AEC) and regenerating muscle bundles were evident as separate aggregates beneath AEC (Fig. 2G). In VCR administered group, tail sections demonstrated thinner AEC with smaller area of proliferating blastemal cells. In addition, the muscle bundles were still not seen (Fig. 2H). At completion of blastema formation, these animals showed thick AEC and larger area of proliferating blastemal cells with muscle bundles aggregated beneath AEC (Fig. 2I).

**DF stage:** In DF stage, the control animals showed well developed epithelium including epidermis and dermis. Pronounced ependymal growth could be seen with well developed cartilaginous tissue surrounding it and regenerating muscles were evident (Fig. 3G). The tissue architecture observed in VCR administered animals at DF stage was poorly organized compared to control animals. The decreased growth of ependyma with relatively decreased number of regenerating muscle cells and hampered growth of cartilaginous tissue surrounding ependyma were marked in VCR administered tail sections. However, regenerating epithelium with separated epidermis and dermis could be seen (Fig. 3H). When VCR administered animals were allowed to complete DF stage, regenerating epithelium with demarcated epidermis and dermis forming prominent epithelium could be seen. In addition, regenerating muscles and cartilaginous tissue were also evident, whereas the growth of ependymal tube was still hampered and smaller ependyma as compared to controls could be evident (Fig. 3I).
Influence of MTX on growth rate of tail regenerate:

The WH, BL and DF stages of tail regeneration were delayed in MTX administered group by 1, 2 and 3 days respectively. Moreover, mean growth rate of tail regenerate in MTX administered animals was significantly decreased by 24.48% and 29.35% at BL and DF stages respectively (Table-3).

Nucleic acid and protein content of tail regenerate administered with MTX:

**WH stage:** MTX administered animals registered 25.75% lower DNA content, whereas RNA and protein levels were significantly higher than control animals. When these animals were allowed to complete wound healing (WH-C group), insignificant elevation of DNA content and a small decline in protein content was registered. However, RNA content was elevated significantly by 30.41% as compared to controls (Table-4).

**BL stage:** The caudal tissues of MTX administered animals registered decreased DNA (22.58%) and protein (5.13%) content and increased RNA (189.27%) content as compared to control group. However, at completion of blastema formation (BL-C group), these animals registered reduction (although insignificant) of DNA content by 3.68%, whereas the amount of RNA and protein content elevated significantly by 23.37% and 5.91% respectively as compared with control group (Table-4).

**DF stage:** MTX administered animals showed significant decline in DNA content (34.40%). On the contrary, RNA and protein contents in experimental animals increased significantly by 59.17% and 4.32% respectively as compared with controls. At
completion of DF stage (DF-C group), significant reduction of DNA (14.21%) content was found in MTX administered animals, whereas the amount of caudal RNA content increased significantly by 26.30% (Table-4).

**Histological features of tail regenerate after administration of MTX**

**Localization of nucleic acids:** The tail sections of MTX administered animals at WH stage exhibited lower DNA content and higher RNA content (Fig. 4E) as compared to controls (Fig. 4D). However, when these animals were allowed to complete wound healing, DNA content was more or less similar to control group, whereas RNA content was higher than control animals (Fig. 4F). At BL stage, the tail sections of MTX administered animals exhibited lower amounts of DNA content (Fig 5E) as compared to control animals (Fig. 5D), whereas, the RNA content was much higher in MTX administered animals (Fig. 5E). When these animals completed blastema formation, DNA content was more or less similar to control animals and RNA content was higher than control group (Fig. 5F). At DF stage, the experimental animals (Fig. 6E) exhibited higher amount of RNA and declined DNA content as compared to controls (Fig. 6D). Even after these animals were allowed to complete DF stage, DNA content was lower and quantum of RNA was higher than that of the control group (Fig. 6F).

**Histology:**

**WH Stage:** The cornified and keratinized wound epithelium (WE) was evident at the apex of the caudal sections of control animals. In addition, the mesenchymal cells could be evident as accumulated cell mass under WE (Fig. 4G). The MTX administered animals
showed an incomplete WE formation with fewer mesenchymal cells under WE (Fig. 4H). When these animals were allowed to complete wound healing, they exhibited mesenchymal cells covered by well developed WE covering entire stump region (Fig. 4I).

**BL Stage:** At BL stage, apical epithelial cap (AEC) was evident in the tail sections of control animals with aggregates of muscle bundles (MB) beneath AEC indicating initiation of myogenesis in tail regenerate (Fig. 5G). The tail sections of MTX administered animals demonstrated thinner AEC with decreased number of proliferating blastemal cells and the muscle bundles were still not evident (Fig. 5H). At completion of blastema formation, these animals exhibited muscle bundles aggregated underneath thick AEC (Fig. 5I).

**DF stage:** In DF stage, the caudal sections of control animals demonstrated well developed epithelium where epidermis and dermis were clearly demarcated. The prominent growth of ependymal tube could also be seen with well developed cartilaginous tissue surrounding it. Further, the muscles appeared to regenerate (Fig. 6G). The organization of tissue architecture at DF stage was adversely affected in MTX administered animals which showed hampered growth of ependyma with relatively decreased rate of myogenesis and decreased mass of cartilaginous tissue (Fig. 6H). When these animals were allowed to complete DF stage, the histoarchitectural organization of tail regenerate was found well developed as ependymal tube and surrounding cartilaginous tissue were evident. In addition, the regenerated muscles were seen beneath epithelium with demarcated epidermis and dermis (Fig. 6I).
Chapter: 3

**Progression of tail regenerate under the influence of 5-FU:**

The animals of 5-FU administered group registered 2, 3 and 2 days delay in attainment of WH, BL and DF stages respectively as the growth was significantly decreased at BL (34.45%) and DF (23.79%) stages (Table-5).

**Nucleic acid and protein content of tail regenerate administered with 5-FU:**

**WH stage:** 5-FU administered animals registered significantly decreased levels of DNA (27.85%) and RNA (61.46%) content. The quantum of total protein in 5-FU administered animals was significantly elevated by 17.40% as compared to control group. When these animals were allowed to complete WH stage (WH-C group), 21.18% lower RNA content and 5.32% higher protein content were found as compared to controls (Table-6).

**BL stage:** DNA and RNA content of tail regenerate in 5-FU administered animals were declined by 22.07% and 79.90% respectively. However, significant elevation of 11.32% in protein content was also registered in experimental animals. At completion of BL stage (BL-C group), these animals exhibited more or less similar amount of nucleic acid and protein content as compared to control group (Table-6).

**DF stage:** 5-FU administered animals registered significantly lowered levels of DNA (14.92%) and higher levels of RNA (14.73%) and protein (5.03%) as compared to controls. However, when these animals completed DF stage (DF-C group), the increase in the DNA content was insignificant (1.29%) whereas the RNA and protein contents elevated significantly (10.41%, 9.84% respectively) as compared to controls (Table-6).
Histological features of tail regenerate of 5-FU administered house lizards

Localization of nucleic acids: In WH stage, the control animals exhibited higher amounts of DNA and RNA in regenerating tail (Fig. 7D) as compared to 5-FU administered animals (Fig. 7E). When these animals were allowed to complete wound healing, DNA content was more or less similar to control group whereas RNA content was found to be lower than controls (Fig. 7F). At BL stage, both DNA and RNA were much less in 5-FU administered animals (Fig. 8E) as compared to control animals (Fig. 8D). At completion of BL stage, these animals exhibited more or less similar amount of nucleic acid contents as compared to control animals (Fig. 8F). The tail tissue sections of control animals at DF stage (Fig. 9D) exhibited higher amount of DNA and lower amount of RNA as compared to 5-FU administered animals (Fig. 9E). At completion of DF stage, these animals showed similar amount of DNA and slightly higher amount of RNA content as compared with controls (Fig. 9F).

Histology:

WH stage: The caudal sections of control animals at WH stage showed cornified and keratinized wound epithelium (WE) covering the apex of the wound with a mass of mesenchymal cells under WE (Fig. 7G). However, the 5-FU administered animals demonstrated delayed formation of WE with fewer mesenchymal cells under WE (Fig. 7H). At completion of wound healing, the experimental animals exhibited well-developed WE with accumulated mesenchymal cells under WE (Fig. 7I).
**BL stage:** The control animals showed few layers thick WE forming apical epithelial cap (AEC) at BL stage of progression of regenerate. The initiation of myogenesis was evident as aggregated muscle bundles could be seen beneath AEC (Fig. 8G). On the other hand, in 5-FU administered animals, tail sections demonstrated a thin AEC with decreased number of proliferating blastemal cells. The myogenesis was also hampered as very few muscle bundles were seen under AEC (Fig. 8H). When these animals were allowed to complete blastema, AEC and aggregated muscle bundles under it were evident with larger population of proliferating blastemal cells (Fig. 8I).

**DF stage:** In DF stage, the control animals showed a well developed epithelium with separated epidermis and dermis. The ependymal tube was evident with well developed cartilaginous tissue surrounding it. The regenerating muscle tissue could also be seen under dermis (Fig. 9G). The histological features of 5-FU administered animals demonstrated disorganized tissue architecture at DF stage as compared to control group. The ependyma was relatively small with decreased rate of myogenesis and hampered growth of cartilaginous tissue (Fig. 9H). However, when these animals were allowed to complete DF stage, well organized tissue architecture could be evident which was similar to that of control animals (Fig. 9I).
Effect of CUR on progression of tail regenerate:

The time taken for attainment of WH stage in CUR administered animals was similar to that of control animals. However, administration of CUR was found to delay the attainment of BL stage by 2 days with 24.68% reduction in growth rate. Moreover, attainment of DF stage was also delayed by 1 day in CUR administered animals although the growth rate of tail regenerate was not affected (Table-7).

Quantitative analysis of caudal nucleic acid and protein content of house lizards administered with CUR:

WH stage: As time taken to heal the wound in CUR administered animals and control animals were same (5th day after amputation), WH-C group could not be formed in this experiment. CUR administered animals exhibited insignificant decline in protein and nucleic acid content as compared with controls (Table-8).

BL stage: The group of CUR administered animals registered significantly lower levels of DNA (32.48%) and RNA (70.59%) content, whereas a small increase in the protein content was observed (2.11%). When these animals were allowed to complete BL stage (BL-C group), significant reduction in DNA content was noted, whereas the RNA content was almost similar to that found in controls (Table-8).

DF stage: The RNA content in CUR administered animals was 7.95% lower than that of control animals, while both the DNA and protein contents exhibited small decline in the former and a small elevation in the latter (-3.87%; + 0.22% respectively). When these
animals were allowed to complete DF stage (DF-C group), no significant changes occurred in the quantities of nucleic acid and protein contents when compared with control group (Table-8).

**Histological observations of tail regenerate after administration of CUR**

**Localization of nucleic acids:** In WH stage, the nucleic acid content in caudal tissues of CUR administered animals (Fig. 10D) was more or less similar to that of control animals (Fig. 10C). In regeneration blastema (BL) of CUR administered animals (Fig. 11E), the DNA and RNA contents were found lesser than control animals (Fig. 11D). However, when these animals were allowed to complete blastema formation, the nucleic acid content was more or less similar to that of controls (Fig. 11F). In DF stage, tail tissue sections of control animals (Fig. 12D) exhibited higher levels of RNA as compared to CUR administered animals (Fig. 12E), whereas the amount of DNA was similar in control (Fig. 12D) and experimental animals (Fig. 12E). When these animals completed DF stage, the levels of DNA and RNA did not alter significantly from that of control group (Fig. 12F).

**Histology:**

**WH stage:** In WH stage, the regenerating tails of control and CUR administered animals showed cornified and keratinized wound epithelium (WE) at the apex. The mesenchymal cells accumulating under WE could also be seen (Fig. 10E and 10F).

**BL stage:** Control animals at BL stage showed few cellular layers in the apical epithelial cap (AEC). The regenerating muscle bundles were also evident as separate aggregates
beneath AEC (Fig. 11G). The tail sections of experimental group demonstrated similar AEC as seen in controls. However, the area of proliferating blastemal cells was smaller and aggregated muscle bundles were less as compared to control animals (Fig. 11H). At completion of BL stage, CUR administered animals showed well developed muscle bundles covered by thick AEC (Fig. 11I).

**DF stage:** In DF stage, the control sections exhibited well developed epithelium containing demarcated layers of epidermis and dermis. The regenerating muscles could be evident under dermis. A well-developed ependymal tube surrounded by regenerating cartilaginous tissue was also seen (Fig. 12G). The tissue architecture of tail regenerate at DF stage in CUR administered animals showed regenerated muscle surrounded by epithelium containing epidermis and dermis. However, the growth of ependyma was relatively less as compared with control sections (Fig. 12H). When these animals were allowed to complete DF stage, the organization of tissue architecture was similar to that of controls (Fig. 12I).
Effect of COL on morphometric growth of tail regeneration in House lizard

Administration of COL resulted in 4, 5 and 5 days delay in attainment of WH, BL and DF stages respectively. The average growth rate of tail regenerate in COL administered lizards were also decreased significantly at BL (45.57%) and DF (41.92%) stages (Table-9).

Nucleic acid and protein content of tail regenerate in COL treated house lizards:

WH stage: COL administered animals registered a significant decline in DNA (47.44%) and RNA (64.74%) content whereas the amount of protein content increased significantly by 8.38%. Even after these animals were allowed to heal the wound (WH-C group), the DNA and RNA levels remained significantly lower (28.75% and 17.89% respectively) than control animals (Table-10).

BL stage: The nucleic acid content significantly declined in COL administered animals as compared to controls. However, the quantum of total protein in tail regenerate was elevated by 21.71%. When these animals were allowed to complete blastema formation (BL-C group), the amount of DNA and RNA content was found to decline (16.88% and 42.36% respectively) but the protein content increased by 12.67% as compared to that of controls (Table-10).

DF stage: The regenerating tail of COL administered animals exhibited lower levels of DNA (52.63%) and RNA (62.74%), whereas significant elevation of 5.34% in protein content was found. When these animals were allowed to complete DF stage (DF-C
group), DNA and RNA contents were found to be declined by 21.76% and 33.59% respectively as compared with controls (Table-10).

**Histological observations of tail regenerate after administration of COL**

**Localization of nucleic acids:** In WH stage, the control animals (Fig. 13D) exhibited higher amount of both DNA and RNA content as compared to COL administered animals (Fig. 13E). Even though these animals were allowed to heal the wound, DNA and RNA contents were still lower than control animals (Fig. 13F). At BL stage, the quantum of nucleic acids declined in COL administered animals (Fig. 14E) as compared to control animals (Fig. 14D). At completion of BL stage, these animals exhibited lower amounts of nucleic acids (Fig. 14F). In DF stage, caudal sections of control animals (Fig. 15D) demonstrated higher levels of both DNA and RNA contents as compared to COL administered animals (Fig. 15E). At completion of DF stage, the experimental animals showed lower levels of DNA and RNA content (Fig. 15F) as compared to controls (Fig. 15D).

**Histology:**

**WH stage:** The histological features of control tail sections at WH stage demonstrated cornified and keratinized wound epithelium (WE) covering the entire apex region of the wound. The mesenchymal cells could also be seen under WE as accumulated cell mass (Fig. 13G). However, the WE sheet could not be seen around apex of wound in caudal sections of COL administered lizards. Moreover, the accumulation of mesenchymal cells under WE could not be observed (Fig. 13H). When these animals were allowed to
complete WH stage, the stump region was covered by cornified WE layer with accumulated mesenchymal cells underneath it (Fig. 13I).

**BL stage:** At BL stage, control animals showed thick WE forming apical epithelial cap (AEC) beneath which the aggregated regenerating muscle bundles could be seen (Fig. 14G). However, in COL administered group, the tissue sections demonstrated thinner AEC with smaller area of proliferating blastemal cells. In addition, very few muscle bundles could be evident under AEC (Fig. 14H). At completion of BL stage, the tail sections of COL administered animals demonstrated aggregated muscle bundles covered by well developed AEC (Fig. 14I).

**DF stage:** In DF stage, the tissue architecture observed in control tail sections were well organized compared to COL administered group. The control sections showed well developed epithelium including epidermis and dermis. Well-defined ependymal tube could be seen with well developed cartilaginous tissue surrounding it. The regenerating muscles could also be evident under dermis (Fig. 15G). On the other end, the COL administered animals showed smaller ependyma with relatively decreased rate of myogenesis and hampered growth of cartilaginous tissue (Fig. 15H). When these animals were allowed to complete DF stage, well developed epidermis and dermis along with regenerated muscles and cartilaginous tissue could be seen. However, the ependymal tube was smaller than that of control animals (Fig. 15I).
DISCUSSION

The treatment of neoplastic diseases has advanced into a multimodal approach incorporating surgery with chemotherapy. Managing wounds in this population has become a challenging task as controversial observations suggesting that chemotherapeutic agents interfere with the normal course of wound healing. Further, chemotherapeutic agents also exert cytotoxic effects on normally proliferating non-neoplastic cells. Therefore, the present investigation was undertaken to study the effects of anti-cancer drugs (VCR, MTX and 5-FU) and phyto-metabolites (CUR and COL) on wound healing and tissue regeneration using tail regeneration in house lizards as a study model by evaluating morphometric growth, histological features, protein content and nucleic acid profile of tail regenerate.

Vincristine sulphate (VCR) is a dimeric alkaloid isolated from periwinkle plant, *Catharanthus roseus*, and used as treatment regimen for a wide variety of neoplastic diseases. The cytotoxicity of VCR is based on well-established pharmacologic properties that include impairment of microtubule polymerization by inhibiting addition of tubulin monomer to the growing end of microtubule (Jordan et al., 1985). Effect of VCR on wound healing studied by Cohen and co-workers (1975) showed that mice administered with 3 mg/kg of vincristine at the time of surgery resulted in a transient decrease in wound tensile strength. In the present experiment, administration of VCR found to delay wound healing in house lizards. VCR might have bound to microtubules and inhibited growth of wound epithelium by altering the dynamics of tubulin addition.
required for polymerization of microtubules. Further, decreased levels of caudal nucleic acids in VCR administered animals also indicated reduced cell proliferation in the wound region. The observed alteration in blastemal architecture and decreased growth rate of blastema in VCR administered animals could be attributed to cell cycle inhibitory activity of VCR. In addition to microtubule depolymerization, VCR also suppresses cyclin dependent kinases (cdk), a group of proteins which are required for the progression and regulation of the cell cycle, by inducing upregulation of cdk suppressor gene p21 (Shinwari et al., 2008). Reduced cell proliferation in blastemal area of VCR administered house lizards was further confirmed by declined levels of caudal DNA content. VCR also exerted detrimental effect during DF stage. The differentiation of myoblasts involves their withdrawal from the cell cycle along with onset of synthesis of specific proteins (Devlin and Emerson, 1979). Several reports have emphasized the potential role of microtubules in the myofibrillar organization (Antin et al., 1981; Toyama et al., 1982). Further, rearrangement of microtubular framework was noticed during terminal differentiation of chondrocytes (Farquharson et al., 1999). Delayed myogenesis and declined regeneration of cartilaginous tissues observed in VCR administered animals might be due to adverse effect of VCR on microtubular architecture of myoblasts and chondrocytes. In addition, decreased levels of caudal nucleic acids at DF stage and alteration in structural architecture are accountable for the decreased growth of tail regenerate in VCR administered house lizards.

Methotrexate (MTX), a structural analogue of folicacid, is widely used as a chemotherapeutic agent for leukemia and other malignancies. MTX inhibits de novo
synthesis of nucleoside thymidine and thus suppresses proliferation of rapidly dividing cells (Goldman, 1974). While studying the effect of MTX on wound healing, Calnan and Davies (1965) found decreased wound tensile strength in MTX administered rats. In the present study, MTX administered lizards showed delayed wound healing with disintegrated wound epithelium. The reduction in cell proliferation rate of MTX administered animals were further confirmed by lower levels of DNA content in caudal tissues of MTX administered lizards. The efficacy of MTX is often limited by its toxicity which causes severe side-effects on the haematopoietic system (Weinblatt and Fraser, 1989). MTX also inhibits proliferation of non-neoplastic cells such as endothelial cells and osteoblasts (Yamasaki et al., 2003; Annssek et al., 2012). In the present context, MTX administration delayed the attainment of BL stage in house lizards with inhibition of myoblast aggregation underneath AEC. Further, MTX treatment is also associated with skeletal growth arrest and osteoporosis (Wheeler et al., 1995). Xian and co-workers (2007) showed chondrocyte apoptosis and suppressed chondrocyte proliferation in rats treated with MTX. In the present experiment, MTX administered animals showed architectural impairment of ependyma and hampered myogenesis in tail regenerate during DF stage.

The anti-metabolite 5-fluorouracil (5-FU) is widely used in the treatment of patients with colon cancer and other cancer types. 5-FU is a pyrimidine analogue and inhibits thymidylate synthase (Peters et al., 1994). It was suggested that 5-FU can be incorporated into DNA and this may contribute to the cytotoxicity of 5-FU (Schuetz et al., 1984). After incorporation into DNA, 5-FU can be excised by uracil-DNA-glycosylase
followed by a purinic-apyrimidinic end nucleolytic cleavage, resulting in DNA strand breaks (Mauro et al., 1993). In addition, 5-FU is also incorporated into RNA and inhibits rRNA processing (Ghoshal and Jacob, 1994) and mRNA splicing (Lenz et al., 1994). The non-neoplastic cells such as osteoblasts are also potent target of 5-FU treatment (Xian et al., 2004). Studies of 5-FU on wound healing revealed that 5-FU causes detrimental effects on wound healing in laboratory animals (Fellenbaum et al., 1994; Weiber et al., 1994). In the present experiment, administration of 5-FU resulted delayed wound healing along with cytoarchitectural impairment of wound area as evident by histological features. Further, 5-FU administration also caused delay in attainment of BL and DF stages with structural impairments of myoblast aggregation underneath AEC, ependymal growth, regeneration of cartilaginous tissue and myogenesis. The sustained decline in nucleic acid content of tail regenerate also showed decreased cell proliferation and in turn hampered growth of regenerate in 5-FU administered house lizards.

Curcumin is a yellow-orange pigment obtained from the rhizome of the plant, *Curcuma longa*, and has many benefits to human health including cancer chemoprevention, anti-inflammation and reduction of reactive oxygen species (Soudamini and Kuttan, 1989; Menon and Sudheer, 2007). In addition, despite insufficient recognition, evidence strongly suggests that curcumin can exert toxic effects under certain specific conditions. For example, an in vitro study revealed that curcumin is cytotoxic to chondrocytes and synoviocytes (Clutterbuck et al., 2008). Moreover, embryotoxic and teratogenic effects of curcumin on the development of zebrafish
embryo were also evaluated by Wu et al. (2007). Using tail regeneration in house lizard as study model, we found that CUR administration exerted neither positive nor negative influence on WH and DF stages of tail regeneration. The histoarchitecture and nucleic acid profiles of CUR administered animals and control animals were more or less similar. However, at BL stage, decreased morphometric growth and nucleic acid content were found in CUR administered house lizards which could be attributed to its DNA damaging activity. Although many studies have demonstrated that curcumin can prevent DNA damage, it has also been found to cause DNA damage in human gastro mucosa cells and lymphocytes (Blasiak et al., 1999), and in Chinese hamster ovary cells (Araujo et al., 1999).

Colchicine is extracted from *Colchicum autunale*. Studies of the effect of colchicines on wound healing obtained contradictory results as Stanisstreet and Panayi (1980) found no significant alteration of wound healing in Xenopus embryo treated with colchicines, whereas Chvapil et al. (1980) showed reduced breaking strength of wound tissues in rats treated with colchicine. In the current study, a 4 day delay was recorded in attainment of wound healing in house lizards treated with colchicine. The decreased caudal nucleic acid content and inhibition of WE formation in tail regenerate of colchicine administered house lizards clearly indicates detrimental effect of COL on wound healing. Colchicine treatment was found to inhibit myogenesis in blastema of tadpole tail by disturbing longitudinal anisometry in the myoblasts and myotubes (Warren, 1968). Similarly, COL administration also inhibited aggregation of myoblasts in regeneration blastema of house lizards. Further, proliferation of blastemal cells was
hampered and in turn attainment of BL stage was delayed in COL administered lizards. Colchicine was also found to inhibit cell proliferation in several cell types including osteoblasts (Davis, 1990; Salai et al., 2001). In the present experiment, administration of COL resulted in decreased growth of ependymal tube and cartilaginous tissue. The reduced morphometric growth of COL administered tail regenerate could be attributed to its anti-mitotic capacity. Microtubules are labile structures that lengthen or shrink by elongation at one end and dissolution at the other. Colchicine does not enhance the rate of microtubule dissolution but inhibits the process of microtubule self-assembly in a substoichiometric fashion by binding β-tubulin with the formation of tubulin-colchicine complexes (Sackett and Verma, 1993; Vandecandelaere et al., 1997).

In conclusion, the present study indicated that administration of anti-cancer drugs (VCR, MTX and 5-FU) and phyto-metabolite (COL) impaired the progression of tail regeneration by causing delay in attaining WH, BL and DF stages. Administration of these compounds also exerted reduction of DNA content in tail regenerate and caused fluctuations in RNA and protein contents. In addition, several events of epimorphosis such as WE formation, accumulation of mesenchymal cells underneath WE, proliferation of blastemal cells, myogenesis, ependymal tube formation and regeneration of supporting cartilaginous tissues surrounding ependyma were also adversely affected in experimental groups. However, Curcumin exerted adverse effect only during BL stage whereas WH and DF stages remained relatively unaffected. Therefore, emphasis must be placed on the usage of phyto-metabolites such curcumin in treating cancer patients due to having efficacy in removal of neoplastic cells along with exertion of lesser side
effects than synthetic drugs. Further, it can be suggested from the present study that the detrimental effects exerted by anti-cancer drugs and phyto-metabolites on wound healing and tissue regeneration should be kept under serious consideration before its implementation as chemotherapeutic regimen. Additionally, tail regeneration in house lizards can be explored successfully as model system to evaluate pharmacological properties of drugs.