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Histopathological and Micrometric studies of Diazinon exposure on Thyroid and Parathyroid Tissues in Mice

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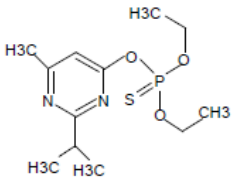
ABSTRACT

Effects of Diazinon (DZ) were studied at 0, 2.5, 5, 10 and 20 $mgkg^{-1}$ in adult male mice (10 in each dose group) for thyroid and parathyroid histopathology. Tissues were recovered following 48 h of DZ treatment. Micrometric data in terms of follicular size of thyroid, cellular, nuclear sizes of the follicular and chief cells were obtained from the histological sections. Statistical analysis has shown significant ($p<0.001$) variations in mean follicular size of thyroid indicating dose dependent depletion of colloid (thyroglobulin). Mean Cross-Sectional Area (MCSA) of follicular cells showed increase in DZ groups as compared to the control with maximum rise at 2.5 and 5mg exposure levels while the MCSA of their nuclei remained more-or-less constant. Statistical analysis has shown significant variation ($p<0.05$) in MCSA of the follicular cells among the groups. Histopathological observations revealed dose dependent apoptotic changes in follicular cells and chief cells of parathyroid. Chief cells MCSA showed slight variations. Nuclear MCSA of the chief cells showed significant ($p<0.001$) dose dependent decline. The present findings suggest that DZ is toxic to endocrine cells of thyroid and parathyroidat 5$mgkg^{-1}$ or more, in mice, bringing about characteristic histopathological and micrometric changes in these tissues.

Key Words: Diazinon, Thyroid, Parathyroid, Histopathology, Micrometry

INTRODUCTION

Diazinon or Diazol (O, O-diethyl O-(2-isopropyl-6-methyl-4-pyrimidinyl phas-phorothioate) first marketed in 1954 as an insecticide (Bartsch, 1974) is an organo-phosphorus (OP) compound. It belongs to a vast heterogeneous group of insecticides (Musilek *et al.,* 2011). Diazinon contains a thiophosphoric ester that acts as the reactive site.



Diazinon ($C_{12}H_{21}N_2O_3PS$)

Diazinon activation takes place in the liver microsomal enzyme system and requires O_2 and NADPH. Mammalian degeneration power for diazinonis is very slow. This group inflicts toxicity mainly by inhibiting acetyl cholinesterase (Papp *et al.,* 2004) leading to neurotoxicity. They also inhibit various metabolic enzymes and cause adverse effects on cardiovascular function (Rush *et al.,* 2010; Adigun *et al.,* 2010).Due to their verified fast action against pests and vector insects, Ops are the most frequently used insecticides in agricultural, industrial and domestic sectors in Pakistan. It is believed that OP exposure can lead to oxidative stress (Giron-Perez *et al.,* 2009; Oruc, 2010).It is observed generally that organochlorine pesticides like lindane and organophosphorus insecticides enhance Thyroid Stimulating Hormone (TSH) level and decrease tri-iodothyronine (T_3)in workers compared with controls (Zaidi *et al.,* 2000). An organochlorine insecticide like malathion affect the catfish and tadpole TSH levels (Fordham *et al.,* 2001). Diazinon in particular has been found to induce chomatin condensation and apoptosis (Rush *et al.*2010).In testes it affects the production of testosterone and causes decline in sperm mortality (Okamura *et al.,* 2009) thus adversely affecting the reproductive functions and capabilities (Casas *et al.,* 2010). DZ has also been designated as an insecticide with endocrine disruptor properties (Ducolomb *et al.,* 2009).

Author's Contribution: K. R. A, conceived the idea; **K.R., T.A,** designed the experimental work; **A.N.,T.A.,** performed experimental work on animal; **S.N.A & M.N.,** performed histological work; **A.Y., S.A.,** executed the micrometric work; **S.N.A,** wrote basic draft; **K.R.A & K.R.,** language & grammatical edits. ***Corresponding author**: k.r.ahmad@gmail.com

Main thyroid secretions are tri-iodothyronine (T_3) and tetra iodo-thyronine or thyroxin (T_4). These hormones play important role in various developmental, metabolic and physiological activities. Thyroxin is designated as primitive and most versatile vertebrate hormone. Typical thyroid histology depicts the presence of colloid (thyroglobulin) filled thyroid follicles. Associated anatomically with thyroid, the parathyroid is an independent endocrine structure. Histologically parathyroid contains two types of cells (chief cells and the oxyphil cells). The chief cells secrete PTH (Parathyroid Hormone) and regulate blood calcium level while the function of oxyphil cells is not clearly understood (Ham, 1969). Exposure of OPs has been linked to the serum level of thyroid hormones in floriculture workers. Lacasana *et al.* (2010) claimed an increase in the levels of TSH and T_4 in blood serum with a simultaneous decrease in T_3, on OP exposure. In case of parathyroid, DZ particularly affects the chief cells causing de-granulation and vacuolations. This loss of secretory granules was further associated with degenerative changes in endoplasmic reticulum, Golgi complex and mitochondria (Rangoonwala *et al.,* 2005). There is relatively little information available about the histopathological and micrometric changes of thyroid and parathyroid on DZ exposure. The present research work was planned to obtain initial insights into histopathological impairments of parathyroid and thyroid in laboratory mice on DZ exposure.

MATERIALS AND METHODS

Fifty young male laboratory mice of the Swiss Webster strain weighing 30-35gwere distributed in equal number (n=10) in the four diazinon (2.5, 5, 10 and 20mgkg^{-1}) and a vehicle (corn oil) treated group. The ambient housing conditions were maintained at 12h dark-light cycles, 40-45% humidity and 25±2^{o}C temperature. The animals were well acclimatized as they were born and maintained in the same animal house. All animals were allowed free access to food and water throughout the experimental period. The respective dose concentrations of DZ were prepared by appropriate dilutions in corn oil. All treatments were applied orally by gavage. Animals of each experimental group were euthanized after 48h of the respective exposures. Intact thyroid glands were carefully removed and immediately placed in alcoholic Bouin's fluid (for 24h) for fixation. The tissues were then processed for wax embedding and microtomy. Histological sections (8 microns thick) were obtained on a rotary microtome (ERMA TOKYO 422).The serial histological sections were carefully stretched on warm water (45-47^{0}C) and affixed on albumenized glass slides. De-waxing, staining (hematoxylin and eosin) and mounting (Canada balsam) were carried out according to the standard protocol to obtain permanent histological sections for further study.

Sections were examined and photographed on 100, 400 and 1000× magnifications for histopathological outcomes and micrometry on a stereoscopic electric research trinocular (Labomed CXR2) microscope mechanically attached with a high resolution (7.2mega pixels) digital camera (Sony DSC-W35). To highlight histological abnormalities, photographs of selected sections were processed in CorelDRAW11 and CorelPHOTOPAINT11 for brightness / contrast adjustments and digital cropping. Digital photographs of these sections were also used for computer assisted micrometry. In this process calibrated measurements of the cell, nuclear and follicular sizes of the parathyroid and thyroid tissues were obtained from the projected images using calibrated wire-frame mode in CorelDRAW11. Microscopic images of the stage micrometer (obtained on the same optical and digital specifications used for histological photography) were used for calibrations.

Cross-sectional areas of 100 randomly selected thyroid follicles from the photo-micrographs of each experimental group were obtained. Sizes of the follicular and chief cells of thyroid and parathyroid respectively and their nuclei were also obtained in the similar way. To estimate the level of significance of variation these size data were subjected to various statistical (Mean Standard Error, ANOVA and Duncan's Multiple Range Test) analyses and presented in the result section as histograms.

RESULTS

Size of Thyroid Follicles

The cross-sectional area of the follicles of thyroid gland has shown dose dependent sensitivity to the DZ exposure. Analysis of variance of the data showed an overall highly significant difference among the groups ($p>0.001$) {$F= 9.55$: F critical = 2.58}.Post hoc analysis of the means indicate that 2.5 mgkg^{-1} dose group did not differ significantly {Duncan's Multiple Range Test (DMRT) ($p\leq0.05$)} with control whereas the MCSA in 5, 10 and 20mgkg^{-1} exposure groups varied significantly with each other and the 2.5mgkg^{-1} and Control groups (Fig. 1).

Size of follicular Cells

The mean follicular cell sizes of the thyroid in DZ treated groups have shown a dose dependent increase in MCSA as compared to the control (Fig. 2). Analysis of variance has shown significant difference ($p>0.05$) {F=2.748: F critical= 2.539}. Post hoc analysis of the means showed a significant rise ($p\leq0.05$) in 2.5 and 5mgkg^{-1} groups to that of the control and 20mgkg^{-1} groups.

Size of the Thyroid Cells Nuclei

The MCSA of nuclear size of follicular cells of the experimental groups have shown slight variations (Fig. 1). Statistical analysis (ANOVA) of the data showed no significant difference {F=0.277: F critical = 2.578}.

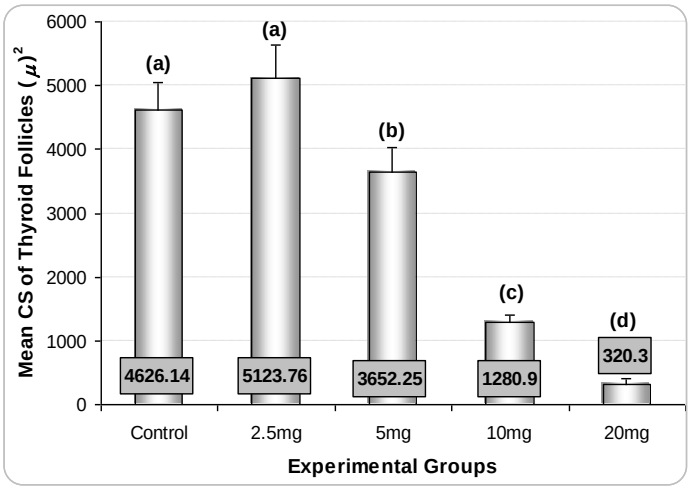


Fig 1: Effect of different doses of diazinon (Zero, 2.5, 5 10 and 20mgkg^{-1} dose levels) on mean CS of thyroid follicles {Lowercase letters shown within brackets above each column indicate Post hoc level of significant variation among the groups(any two groups not sharing a common lowercase letter differ significantly from each other)}**.**

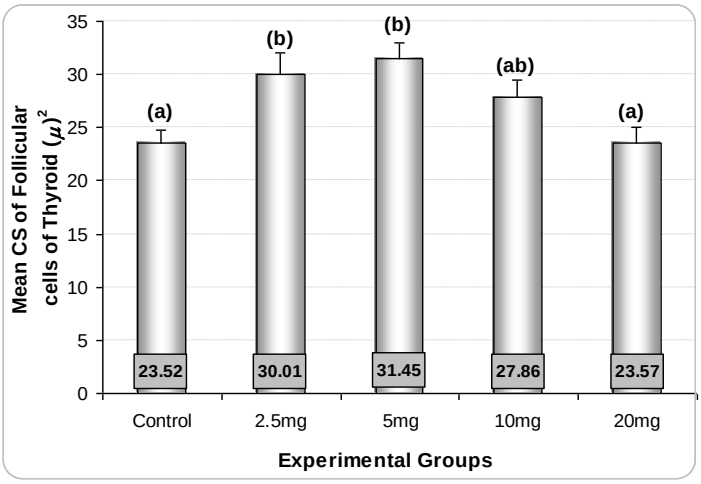


Fig 2: Effect of different doses of diazinon (Zero, 2.5, 5 10 and 20mgkg^{-1} dose levels) on mean size of follicular cells. {Lowercase letters shown within brackets above each column indicates

Post hoc level of significant variation among the groups (any two groups not sharing a common lowercase letter differ significantly from each other)}.

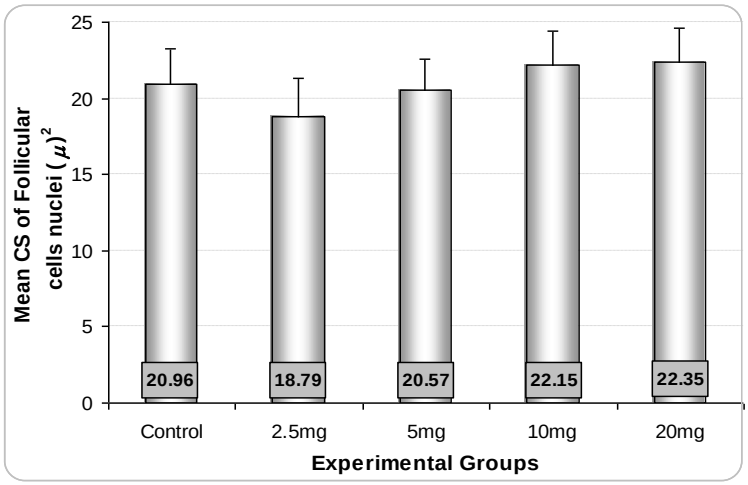


Fig 3: Effect of different doses of diazinon (zero, 2.5, 5 10 and 20mgkg^{-1} dose levels) on mean nuclear size of the follicular cells.

Thyroid Histology

The histological sections obtained from control group indicate well placed thyroid follicles containing colloid surrounded by the follicular cells. Compared to the control the follicles in DZ groups (except 2.5mgkg^{-1} group) have shown a dose dependent size reduction (Fig 4), as also evident in the micrometric estimations of MCSA of the follicles (Fig1). The mean cross-sectional area of the follicular cells was significantly higher in 2.5 and 5mgkg^{-1} DZ groups than the control as well as to the 10 and 20mgkg^{-1} groups (Fig 2 and 4), on the other hand no significant change in the MCSA of the nuclei of these groups was noted (Fig 3). The DZ exposure might have led to the rapid removal of the thyroglobulin form the thyroid follicles as evident from the appearance of fluid filled spaces between the follicular cells and the central follicular collide in DZ treated groups thyroid sections; moreover, leakage of contents and dislocation of individual follicular cells from the basement connective tissues was evident in 10 and 20mg dose exposure groups respectively (Fig 4).

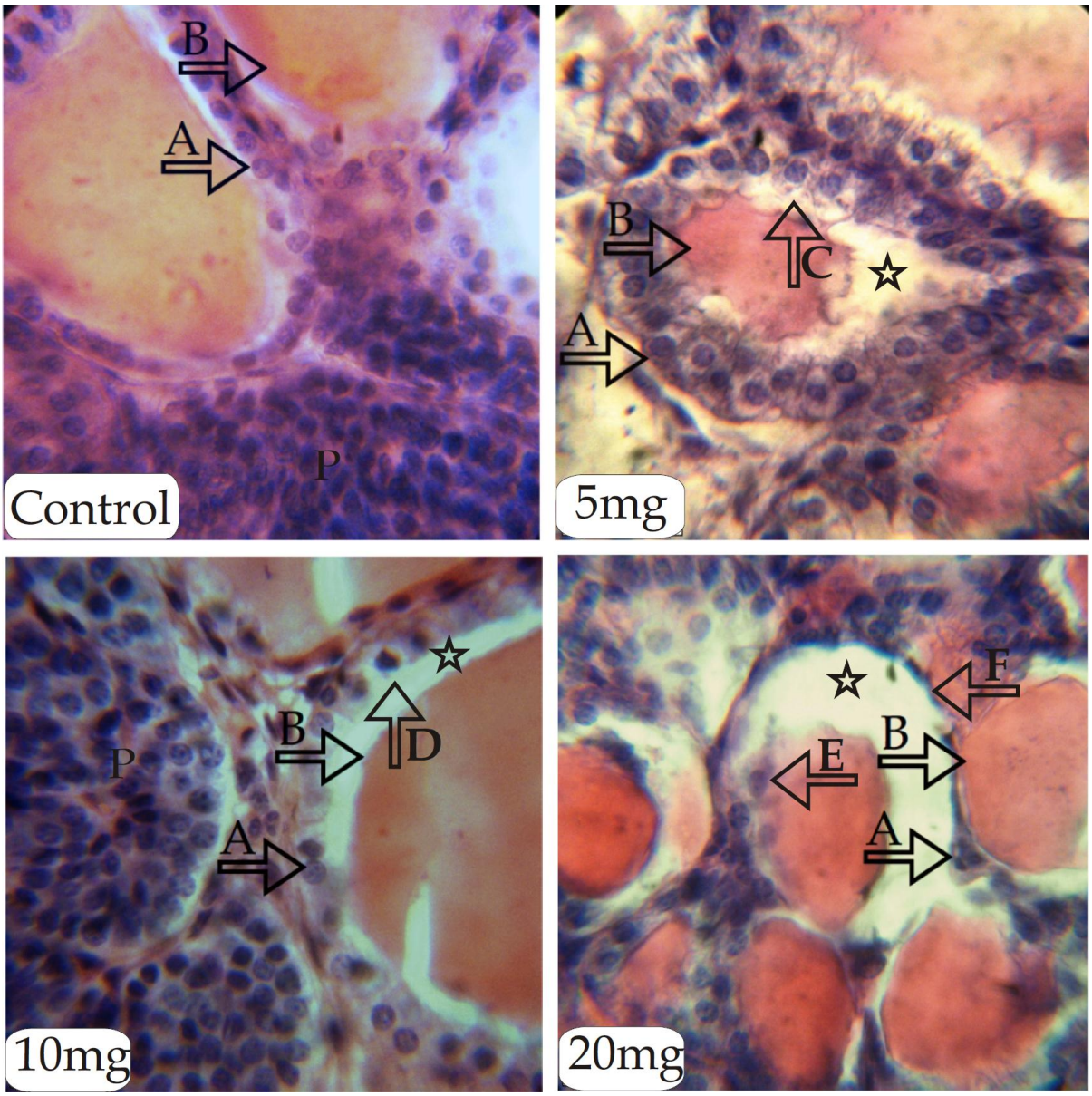


Fig 4: Sections of thyroid exposed to different doses of diazinon (H&E stained) (1000x) (A: Follicular cell; B: Colloid; C: enlarged cytoplasmic contents; D: cytoplasmic leakage; E: Dislocated follicular cell; F: Fibroblast of the devoid of follicular cells; *: Fluid filler para-colloidal space).

Size of Chief Cells

In parathyroid MCSA of the chief cells had shown slight variations among the experimental groups (Fig.5). Analysis of variance of the data brought about no significant result {F=0.461: F critical=2.578}.

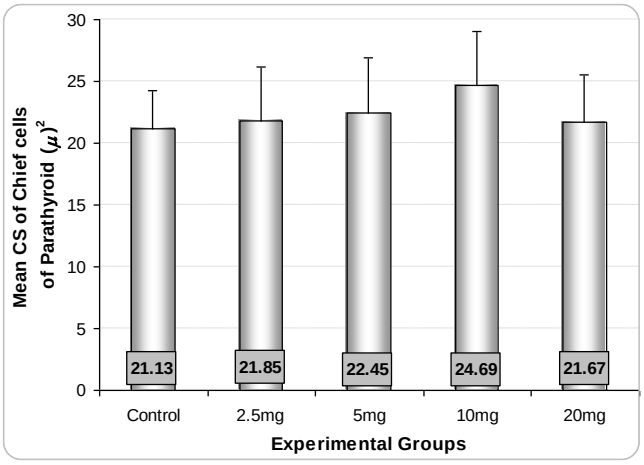


Fig.5: Effect of different doses of diazinon (zero, 2.5, 5 10 and 20mgkg^{-1} dose levels) on mean chief cell size of the parathyroid.

Nuclear size of Chief Cells

The mean nuclear sizes have shown a dose dependent decrease in DZ treated groups to that of the control group (Fig.6). Analysis of variance indicates significant difference ($p<0.001$) {F= 6.42: F critical= 2.58}. Post hoc analysis has shown that Control and 2.5mgkg^{-1} groups were significantly different from 10 and 20mgkg^{-1} groups; while 5mgkg^{-1} group with intermediate mean nuclear size did not show significant difference with any other group.

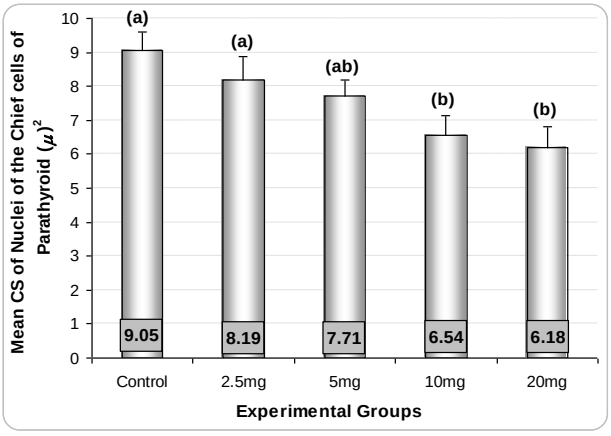


Fig.6 Effect of different doses of diazinon (zero, 2.5, 5 10 and 20mgkg^{-1} dose levels) on mean nuclear size of chief cells of the parathyroid{Lowercase letters shown within brackets above each column indicate Post hoc level of significant variation among the groups(any two groups not sharing a common lowercase letter differ significantly from each other)}**.**

Parathyroid Histology

Apart from the slight increase in the mean size of the chief cells and significant decline in the nuclear size at 10 and 20mgkg^{-1}, the nuclear dis-morphogenesis (change of shape from rounded to elongated, polygonal and ultimately sickle shaped) was obvious particularly in 20mgkg-1 group. Moreover, the apoptotic signs in terms of enlarged cytoplasmic contents (obviously due to increased vacuolations) along with rupture and release of cytoplasmic contents were also seen (Fig.7).

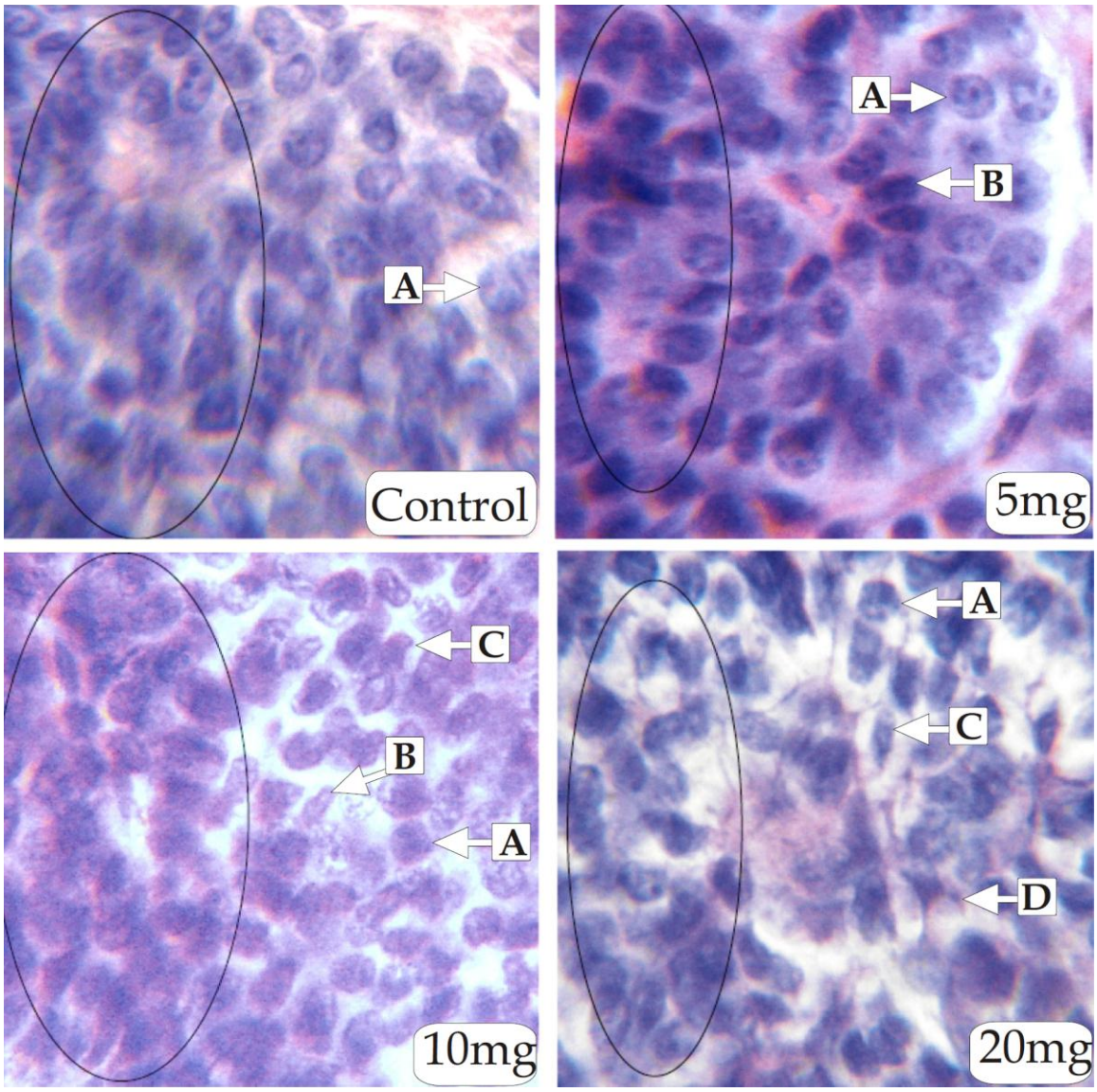


Fig 7: Sections of parathyroid exposed to different doses of diazinon (1000x) (Encircle: cluster of chief cells; A: Functional chief cell with rounded nucleus; B: ellipsoidal nucleus; C: Sickle shaped nucleus; D: necrosis and leakage of the cellular contents).

DISCUSSION

Although organophosphate insecticides are proven endocrine disruptors and reproductive toxicants (Jeong *et al.,* 2006; Slotkin, 2004; Sarkar *et al.,* 2000), little is known about their effect on endocrine glands other than testis and ovaries, particularly thyroid and parathyroid (Meeker and Stapleton, 2010). Thyroid and parathyroid secretions are vital from the stand point of metabolic and blood calcium regulation. Thyroid hormones are particularly needed for the regulation of general metabolism, reproductive, developmental, neuro-endocrine and cardiac functions (Miller *et al.,* 2009). Organo- phosphate pesticides have a potential to disturb the thyroid function (Lacasana *et al.,* 2010).

As insecticide related histo-pathological studies on the thyroid and parathyroid are scarce thus the results presented here are not directly comparable to the available literature. The significant reduction in MCSA of the thyroid folliclesin 5, 10 and 20mgkg^{-1} groups indicate increased resorption of colloid under probable rise of TSH for up-regulation of T_4 levels on DZ

exposure (Lacasana 2010) and a simultaneous incapacitation of the follicular cells of thyroid to rapidly synthesize and replace thyroglobulin (Fig. 1). This argument is further supported by the persistent observation of a rapid shrinkage of colloid as compared to the decline in MCSA of the follicles thus leaving a fluid filled space between the follicular cells and the thyroglobulin mass in a dose dependent manner (Fig. 4).

As thyroid falls among the most highly vascularized organs {destined to produce the physiologically most dynamic vertebrate hormones (T_3 and T_4)} thus it is logically liable to the exposure of circulating toxins. Dislocations of the follicular cells and the appearance of apoptotic signs in them (like nuclear and cytoplasmic enlargement and leakage of the nuclear contents) were thus considered as the consequence of dose dependent metabolic and oxidative stresses of DZ exposure.

Rangoonwala *et al.* (2005) have shown that intramuscular injection of diazinon at dose level 150mgkg^{-1} and 225mgkg^{-1} for 14 days has induced hypocalcaemia without altering serum phosphate levels in rats (*Rattus norvegicus*).Moreover, this response was found to be duration and dose dependent. The chief cells in parathyroid tissues of experimental animals showed simultaneous de-granulation and vacuolation along with chomatin losses and degenerative changes in Golgi complex, endoplasmic reticulum and the cisternae of mitochondria. In present study we have seen dose dependent increase in MCSA of the chief cells of parathyroid except in 20mgkg^{-1}group where a secondary decline in MCSA was noted (Fig.5). Even so the MCSA in 20mgkg^{-1} group remained higher to that of the control. This decline in chief cells size at 20mg DZ exposure was attributed to the most rapid removal of storage granulesat this dose as compared to the other dose groups where this response seemed to be less severe. The dose dependent increase in MCSA indicates vacuolation and apoptotic changes in the chief cells especially when seen in association with a dose dependent significant decline in nuclear size in these cells. Thus our findings are well in line with parathyroid cellular finding of Rangoonwala *et al.*(2005). Apparently misleading sign of secondary decline in the MCSA of the parathyroid chief cells in 20mgkg^{-1}group is also justifiable if seen in conjuncture with the histopathological sings where the endocrine cells show disfigured nuclei and cytoplasmic enlargements causing leakage of cellular contents and consequently size reduction in the individual necroting chief cells and thus adding towards secondary decline in MCSA in the overall perspective.

Based upon our findings we conclude that thyroid and parathyroid are highly sensitive to DZ exposure and a dose level of 5mgkg^{-1}or more, in mice can bring about many histopathological and micrometric changes in these endocrine tissues. To have a deeper insight into these histopathological findings a separate research dealing with the estimation of the relevant hormones and oxidative stress on DZ exposure in these tissuesis recommended.

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