PATHOLOGICAL EFFECTS OF INDUCED STRESS ON THE LYMPHOID ORGANS IN BROILER CHICKEN

By

SURAJ. S.

THESIS

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Centre of Excellence in Pathology
COLLEGE OF VETERINARY AND ANIMAL SCIENCES
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DECLARATION

I hereby declare that the thesis entitled **"PATHOLOGICAL EFFECTS OF INDUCED STRESS ON THE LYMPHOID ORGANS IN BROILER CHICKEN"** is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title, of any other University or Society.

Mannuthy

SURAJ, S.

CERTIFICATE

Certified that the thesis, entitled **"PATHOLOGICAL EFFECTS OF INDUCED STRESS ON THE LYMPHOID ORGANS IN BROILER CHICKEN"** is a record of research work done independently by Dr. Suraj, S., under my guidance and supervision and that it has not previously formed the basis for the award of any degree, fellowship or associateship to him.

30.3.02

Dr. N. Vijayan
(Chairman, Advisory Committee)
Associate Professor
Centre of Excellence in Pathology
College of Veterinary & Animal Sciences, Mannuthy

Mannuthy

CERTIFICATE

We, the undersigned members of the Advisory committee of **Dr. Suraj, S.**, a candidate for the degree of Master of Veterinary Science in Pathology, agree that the thesis entitled **"PATHOLOGICAL EFFECTS OF INDUCED STRESS ON THE LYMPHOID ORGANS IN BROILER CHICKEN"** may be submitted by Dr. Suraj, S., in partial fulfilment of the requirement for the degree.

Dr. N. Vijayan
(Chairman, Advisory Committee)
Associate Professor
Centre of Excellence in Pathology

Dr. K.V. Valsala
Professor & Head and Director
Centre of Excellence in Pathology
(Member)

Dr. C.R. Lalithakumjamma
Associate Professor
Centre of Excellence in Pathology
(Member)

Dr. Leo Joseph
Associate Professor
Centre for Advanced Studies in
Poultry Science
(Member)

External Examiner

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Rain water takes the colour and quality of the soil it falls on;
Wisdom is moulded by quality of one's company.

Verse 452
Thiruvalluvar Circa 2000 B.C.E.

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CONTENTS

LIST OF TABLES

LIST OF GRAPHS

LIST OF PLATES

16	Bursa : Epithelial hyperplasia, cyst formation – Group I: 28th day H&E 250X	74 & 75
17	Bursa: Subepithelial cyst – Group I : 35th day H&E 400X	74 & 75
18	Bursa: Formation of cyst in the follicle – Group I : 42nd day H&E 400X	74 & 75
19	Bursa: Dilatation of inter follicular space – Group II : 28th day H&E 250X	74 & 75
20	Bursa: Necrotic changes in medulla – Group II : 42nd day H&E 250X	74 & 75
21	Bursa: Inter follicular fibrosis and cellular infiltration – Group II : 42nd day H&E 400X	74 & 75
22	Thymus : Tendency for formation of Hassals corpuscles – Group I : 28th day H&E 250X	74 & 75
23	Thymus : Homogenous eosinophilic substance in the cells of the medullary region – Group I : 28th day H&E 400X	74 & 75
24	Thymus : Mild diffuse lymphoid depletion in the cortex – Group I : 35th day H&E 160X	74 & 75
25	Thymus : Moderate degree of lymphoid depletion in the cortex – Group II : 35th day H&E 160X	74 & 75
26	Thymus: Prominent reticular fibres, depletion of lymphocytes – Group II : 35th day H&E 250X	74 & 75
27	Spleen : Sparsely distributed lymphoid cells – Group I : 28th day H&E 400X	74 & 75
28	Spleen : Thickening of blood vessels – Group II : 28th day H&E 400X	74 & 75
29	Spleen : Thickening of blood vessels, moderate degree of lymphoid depletion – Group II : 42nd day H&E 160X	74 & 75

Introduction

1. INTRODUCTION

Indian poultry sector has made significant growth during the last few decades. It has established as an industry contributing nearly 1 per cent of the national GDP with an output of 10 billion rupees. An annual growth rate of 6 to 8 per cent in egg production and 15 to 18 per cent in broilers has made the poultry sector one of the fastest growing areas in Indian agriculture scenario. This spectacular development was achieved by adopting intensive production strategies.

Intensive management practices, however are not free of problems. In the anxiety to produce more and to maximise the profit per unit of input, farmers expose birds to various stressors, overcrowding or high stocking density being a very common one. Stress is an inevitable factor to which the birds get exposed during the production period. There are a wide variety of spontaneous stressors in the environment besides the artificially imposed ones due to mismanagement. Stress is a natural beneficial physiological mechanism that aids the individual to adapt successfully to the environment. However, if stress is severe, consistent and prolonged, it leads to adverse biological effects and triggers a battery of pathological changes in the animal's system and these leads to decline in health status and consequent loss of production. According to Everly and Sobelman (1987) the stress response served as a physiological mechanism of mediation linking the stressor to a target organ and the target

organ response may be negative or positive depending on the effect on the peripheral organ system and the neuroendocrine system.

Among the endocrines, the adrenal plays a significant role in organising the endocrine orchestra responsible for the host responses to stress. The release of the hormones by the adrenal gland during stress particularly corticosteroids induces substantial changes in various organs including the lymphoid organs. Prolonged stress response often leads to sufficient increase in corticosteroid production that causes atrophy of the lymphoid organ and breakdown of immunity and outbreak of diseases. This adverse influence of stress will have a direct bearing on the economics of poultry production. Undue stress, therefore, is a curse to poultry farming.

Considering the importance of stress response and its adverse effects in poultry production, the study was designed to delineate the pathobiological features of physical stress in chicken by limiting the space for housing and chemical stress by administering dexamethasone.

The objective was to critically record, recognise and analyse the stress response in order to suggest suitable criteria to identify these responses and assess the magnitude of its impact on the production system. This will help to focus attention on the need for providing better managemental conditions to avoid stress situations. This awareness among the farmers associated with poultry production will certainly help to avoid undue stress, which shall ensure good economic returns to the farmers.

Review of Literature

2. REVIEW OF LITERATURE

Fraser *et al.* (1975) defined stress as an abnormal or extreme adjustment in the physiology of an animal to cope up with the adverse effects of its environment and management.

The term stress was further qualified by Selye (1980) and Ewbank (1985) to include distress. This was used to identify the extreme responses to adverse stimuli, which caused a damaging pathophysiological reaction in the host, producing associative changes in the behaviour, physiology and disease susceptibility.

According to Everly and Sobelman (1987), the stress response served as a physiological mechanism of mediation linking the stressor to a target end organ and the target end organ effect may be negative or positive. Whether or not the stress was damaging depended on the interaction between the peripheral organ systems and the neuro-endocrine system along with the individual characteristics and properties of the stressor (Bohus *et al.*, 1987).

Siegel (1995) citing various authors suggested that, in birds the link between different anatomical parts of Hypothalamic-Pituitary-Adrenal axis (HPA) was not so essential to find a basal level of corticosteroid in the blood as in mammals. Avian pituitary appears more autonomic and less subject to hypothalamic control.

Elrom (2000) suggested that corticosteroid receptors were present in the hypothalamus and a prior stress may increase the binding of these receptors in a way that will suppress the release of CRF (Corticosteroid releasing factor) by a negative feed back mechanism. These results suggested that end organ responses such as Heterophil Lymphocyte ratio (H:L) or lymphoid organ regression may represent better indicators of chronic stress, whereas plasma corticosteroids or catecholamines were effective indicators of acute stress.

2.1 Types of stressors

Selye (1980) categorised stress into four different components: (a) Overstress (Hyperstress), (b) Understress (Hypostress), (c) Good stress (Eustress) and (d) Badstress (Distress).

Griffin (1989) classified stressors into three: (a) Environmental, which included heat and cold, transportation, novel sounds, odour and taste, (b) Behavioural, which included overcrowding, hierarchical challenge, weaning, changes/restriction in diet (c) Psychological, which encompassed capture of wild animals, physical restraint, etc.

Kettlewell (1989) recommended to classify stress into three subgroups. (a) Mental, (b) Physical and (c) Mixed (mental and physical). He included stress during catching, social group and transportation into mental stress group. Physical stressors included the stress of harvesting, loading and carrying inverted to crate. Many stressors involved mixed factors like transportation and

handling which had both mental (pain, fear, anxiety) and physical (environmental wounds) factors.

Grandin (1998) classified stressors as psychological and physical stress.

2.2 Stress and behaviour

Duncan (1970), Duncan and wood-Gush (1972a,b) consistently observed three behavioural responses of birds to different types of stressors. They were (1) stereotyped back and forward pacing, (2) displacement preening and (3) increased aggression.

Duncan and Wood-Gush (1972b) suggested that birds subjected to low intensity, short-term stress showed increased preening while birds subjected to high intensity, short-term stress showed stereotyped pacing or escape behaviour. Stereotyped back and forward pacing occurred when the level of frustration was higher.

Mauldin and Siegel (1979) suggested that head shaking in chicken might aid in coping with confinement.

Dawkins (1980) opined that some stress elicited abnormal behavioural pattern that might be considered as displacement activity which might be advantageous in ameliorating the psychogenic component of physical stress (Dantzer and Mormede, 1985).

Williams (1984) reported that the alarm reaction in birds was behaviourally manifested by showing attention, pacing the stimulus, elevation

of the hair or feathers around its ear openings and widening its gaze. The stage of resistance was manifested by increased arousal, attack/approach, escape/retreat, appeasement or threat behaviour accompanied by increased sweating, urination and defecation. Animal's behavioural responses to a stressor during the resistance and exhaustion phases seem to be dependent on whether escape, attack, threat or appeasement were possible. Behavioural responses to low intensity short-term stress were characterised by displacement activities and high intensity short term stress by escape behaviour.

Mench *et al.* (1986) and Shea *et al.* (1990) reported that fasting of birds may increase the aggressive pecking behaviour.

Mench *et al.* (1986) reported that behavioural vices like cannibalism, hysteria feather pecking were suggestive of managemental problems. Increase in aggression, changes in pattern of vocalisation, changes in daily feeding practices, stereotyped movements and alterations in spatial distances were all indicative of stress.

Sokolowicz *et al.* (1996) reported that birds responded to thermal stress by often raising their wings, panting and resting singly.

Martrenchar *et al.* (1997) reported that increased stocking density caused decreased standing/lying ratio and higher frequency of pododermatitis.

Andrews *et al.* (1997) reported that when birds were stocked at low rate, they spent more time walking and sitting and less time dozing and keeping. They pecked more at inanimate objects and interacted more with other birds but

this did not include aggressive behaviour. Birds stocked at high density early in rearing periods were more active in the presence of a familiar person and they showed longest period of tonic immobility in response to a fearful stimulus.

2.3 Stress and adrenal

Cannon (1929) identified the role of adrenal medulla in stress. Selye (1946, 1952) contributed to the understanding that both adrenal tissues (cortex and medulla) played a role in stress response.

Selye (1952) observed that in mammals, the resistance phase to general systemic stress was characterised by adrenal cortical hyperactivity as manifested by adrenal cortical hypertrophy and depletion of adrenal cholesterol and ascorbic acid.

Christian (1950, 1955) observed adrenal cortical hypertrophy and progressive atrophy of the gonads and accessory tissue in mice and rats in overpopulated groups.

Adrenal hypertrophy and lymphoid degeneration were noticed in consequence to stress stimuli such as muscular fatigue and exposure to reduced atmospheric pressure and cold (Garren and Shaffner, 1956).

Mild adrenal hypertrophy marked by cortical hyperplasia were noticed in adult female chickens when subjected to higher population density (Siegel, 1959).

Siegel (1960) reported a significant increase in the adrenal weight of cockerels maintained in 0.4 sq ft/bird group. He also reported an increase in the lipid concentration in the adrenal with age along with cortical hypertrophy. There was also a depletion of adrenal cholesterol after prolonged exposure to overcrowding for three to four weeks. He pointed out that the left adrenal was more affected than the right.

Siegel and Siegel (1961) reported that when birds were subjected to increased social competition, there was adrenal hypertrophy and lymphoid degeneration.

Increased adrenal medullary activity was seen with increased population density (Siegel, 1959, 60; Lei *et al.*, 1972; Eskerland, 1978), with acute heat (Nathan *et al.*, 1976; Edens and Siegel, 1973, 1975, 1976; Beuving and Vonder, 1978; Siegel and Gould, 1982), cold (Garren and Shaffner, 1956; Buckland *et al.*, 1974; Nir *et al.*, 1975; Etches, 1976) and handling (Weiss and Brand, 1974; Beuving and Vonder, 1978; Freeman and Flack, 1980). Method of husbandry (Bareham, 1972; Compton *et al.*, 1981), anaesthesia and surgery (Frankel *et al.*, 1967; Scanes *et al.*, 1980), exercise (Conner and Shaffner, 1954); immobilization (Jurani *et al.*, 1972, 1980; Zachariasen and Newcommer, 1974; Beuving and Vonder, 1978; Wodzicka-Tomaszawska *et al.*, 1982); noise (Wildenhahn *et al.*, 1976); excessive day length (Buckland *et al.*, 1976; Freeman *et al.*, 1981); deprivation of food (Weiss and Brand, 1974; Raszyk and Herzig, 1975; Nir *et al.*, 1975; Salen and Jakson, 1977; Scanes *et al.*, 1980;

Freeman *et al.*, 1980, 1981 and Harvey and Klandorf, 1983); attitude (Garren and Shaffner, 1956); social group changes (Candland *et al.*, 1969) and transportation (Freeman *et al.*, 1984a) also resulted in increased adrenal medullary activity.

Atrophy of the adrenal gland, mainly affecting the central regions which is considered equivalent to the mammalian fascicular zone while the periphery (equivalent to mammalian glomerular zone) remained either unchanged or appeared stimulated in adenohypophysectemised pigeons (Miller and Riddle, 1942; Miller, 1967). Frankel *et al.* (1967a,b,c) reported a similar change in adenohypophysectemised cockerels.

Peczely and Muray (1967, 68) reported a functional zonation of the avian adrenal inter-renal tissue (cortex) where in the inner zone of the adrenal inter-renal tissue secreted corticosteroids, while the peripheral zone secreted aldosterone. This corresponded to the ultrastructural difference in the avian adrenal cortex as reported by Kondics and Kjaerheim (1966).

Frankel (1970) reported that the two adrenal inter-renal zones were differentially affected by the experimental treatments, which included administration of prednisolone, adrenocorticotropic hormone, insulin, water loading and deprivation and treatment with sodium chloride and potassium chloride.

Aire (1980) noticed wider inter-renal cord of the subcapsular zone than the inner zone of the gland and this indicated morphologial zonation in the defeathered hens, kept in hot and humid pens for three months.

Simenson *et al.* (1980) observed slight hyperplasia of the adrenal cortical cells and hypertrophy of the nucleus in turkeys maintained at high and low temperatures.

Freeman *et al.* (1980) studied the responses of immature fowl to withdrawal of a stressor (corticotropin 30 IU/kg) and noted the hypertrophy of the adrenal and increased relative adrenal weight even after 14 days of recovery. The adrenal cholesterol which got depleted at the end of the treatment was moderately depleted after seven days but significantly depleted again at 14 days.

Freeman *et al.* (1983, 84a), Freeman and Manning (1984), Williamson *et al.*, (1985), Davison *et al.* (1985) and Freeman (1987) opined that certain stressors or noxious stimuli did not always affect the adrenal gland.

Depletion of the adrenal cholesterol, and other lipids and a variable degree of hyperplasia in the cortical cells during summer in layers were reported by Ghodasara *et al.* (1990).

Ghosh *et al.* (1992) on histological examination observed extensive increase in the area of the adrenal medulla, enlargement, vacuolation and degranulation of the adrenal medullary cells. The cortical area appeared to be smaller on treatment of birds with formalin. However, cold wet immobilization

increased the glandular norepinephrine along with corticosterone depletion and heightened activity of the adrenal cortex.

Ghodasara et al. (1995) observed that in broiler birds subjected to summer stress, there was extreme vacuolation of the adrenal cortical cells which indicated lipid depletion along with hyperplasia of the cells.

2.3.1 Stress and adrenal hormones

Brown and Nestor (1974) reported that adrenal response to stressor depended on the strain of the bird and that the high response strain of birds had elevated plasma corticosterone response which in turn caused an elevated plasma catecholamines.

Acute stress response characterised by the flight-fight reaction is a result of physiological change due to the production of catecholamines by the sympathetic adreno-medullary system (Axelrod and Reisine, 1984).

Birkenhoch (1983) and Levine (1985) noted that different types of physiological and physical stimuli produced varying levels of norepinephrine (NE) and Epinephrine (E). Axelrod and Reisine (1984) opined that elevation of plasma epinephrine levels resulted from the activation of adreno-medullary tissues and was closely associated with corticosteroid production within the adrenal cortex following stimulation by HPA system.

Carmichael and Winkler (1985) went on further to suggest that cortical hormones apparently ensured that the synthesis of adrenaline was maintained.

Levine (1985) observed that acute stress in rats caused simultaneous increase in plasma catecholamine and corticosteroid levels. However, following termination of stress, catecholamine levels returned to normal levels immediately, whereas corticosteroid levels remained high for 30 minutes. Adaptation to chronic stress resulted in persistent elevation of plasma catecholamines while plasma corticosteroids returned to normal levels early.

Gross and Siegel (1983) opined that plasma concentration of corticosteroids may not be a good indicator of chronic stress. They pointed out that end organ responses like H:L ratio might be useful as a bioassay for chronic stress.

Failure of sympathetic adreno-medullary system to resolve stress (chronic stress) leads to the activation of hypothalamic-pituitary-adreno cortical response which is manifested behaviourally by the conservation withdrawal reaction. (Griffin, 1989; Siegel, 1995; Elrom, 2000).

Siegel (1995) stated that the avian adrenal was more autonomic and less subject to the control of the pituitary gland. He reported in many reports in which hypophysectemised birds responded to stress with elevated blood corticosterone levels. He proposed extrapituitary areas that might be responsible for Adreno-cortico-tropic-hormone (ACTH) production.

Korte *et al.* (1997) observed a higher corticosterone and noradrenaline response in high adrenal response strains while plasma adrenaline levels showed no difference.

Romero *et al.* (1998a, b) observed that modulation of corticosterone release was controlled at multiple sites in the hypothalamic-pituitary-adrenal axis.

Heath and Duffy (1998) observed that birds in good physical condition responded more quickly to stressor by increasing the corticosteroid levels and adapted physiologically to stress more rapidly than birds in poor physical condition.

Elrom (2000) reported that avian leukocytes (lymphocytes and macrophages) might produce ACTH or ACTH like substances.

2.4 Stress and lymphoreticular system

Foglia and Selye (1938) and Leblond *et al.* (1939) reported thymico lymphatic involution as a stress response.

The direct effects of corticosteroids and indirect effects of ACTH or stress on the lymphoid tissues were well documented (Dougherty and White, 1944; Glick, 1967; Siegel and Latimer, 1970). The effects included reduction in the lymphocytic tissue mass.

Hill (1983) observed that all responses to stress may not occur simultaneously as pointed out by Brown (1967). He confirmed the different observations obtained in experiments with stress like the observation made by Schindler (1962) and Siegel (1960) who noticed that some stressors only affected adrenal and the lymphatic system, whereas pituitary weights remained unchanged. Garren and Barber (1955) and Wolford and Ringer (1962) who

reported changes in leukocytic counts and regression of the bursa but no change in the adrenal size.

Garren and Shaffner (1956), Glick (1967) and Siegel (1980, 1983) opined that stress may cause involution of the thymus, spleen and the bursa of Fabricius which are responsible for the reduced circulating lymphocyte numbers. Direct lysis or apoptosis (Munck and Guyre, 1991) and delay in maturation (Sapolsky, 1992) of lymphocytes due to stress induced increase in corticosteroids were considered to exacerbate the problem further.

Cell culture studies showed that corticosteroids bond to specific protein receptors in the cytoplasm of the lymphoid cells and the steroid receptor complex passes into the nucleus of the cell and latter altered the enzymatic activity and influenced the nucleic acid metabolism (Thompson and Lippman, 1974 and Sullivan and Wira, 1979). As a consequence, glucose uptake and protein synthesis was suppressed and the cell proliferation factor interleukin II produced by the T helper cells in response to the antigenic stimulation was reduced (Gillis *et al.*, 1979).

Comsa *et al.* (1982) opined that steroid may have a direct effect on the lympho-reticular system (LRS) through their interaction with receptors on the mononuclear cells.

In vivo studies in the fowl have recently showed that injection of ACTH or exposure to high environmental temperature increased the amount of

endogenously produced corticosteroids that was bound in the lymphocytes (Gould and Siegel, 1981and Siegel and Gould, 1982).

Glucocorticoids have been shown to suppress the expression of class II MHC molecules by the monocytes (Snyder and Unanue, 1982) and decreased the production of interleukin I (Mac Dermott and Stacey, 1981). They inhibited the transcription of IL-I genes and post transcriptional expression of RNA (Knudsen *et al.*, 1987). Production of TNF (Tumor Necrosis Factor) by murine (Beutler *et al.*, 1986) and human (Waage and Bakke, 1988) leukocytes was also inhibited by steroids. Steroids also act as potent IL2 inhibitors (Gillis *et al.*, 1979). Naïve T cells appeared much more sensitive to steroids than antigen primed memory cells (Cupps and Fauci, 1982).

Siegel (1987) reported that the primary immunological effects of the corticosteroids in chicken was on the T cell population as reported by Meyer *et al.* (1964), Sato and Glick (1970) and Pardue and Thaxton (1984).

Siegel (1960) observed that increased population density caused reduction in the weight of the bursa. However, no changes were observed histologically.

Siegel (1961) observed that the administration of ACTH caused a significant reduction in the weight of the bursa and spleen.

Frankel (1970), Freeman (1971) and Siegel (1971) reported the lymphatic involution particularly in the bursa of Fabricius that could be used as an indicator of stress.

Gross *et al.* (1980) observed that feeding 5 to 50 mg of corticosterone per kg body weight to chicken resulted in dose related changes which included reduced size of the lymphoid organs.

Muhmed and Hanson (1980) reported that repeated injection of cortisol caused both the thymus and bursa to loose about 95 per cent of their mass in 10 days in chicken.

Siegel and Gould (1982) noted that exposure to environmental temperature of 42°C for 1 hour increased the concentration of endogenously produced corticosteroids in the nucleus of the lymphoid cells of the bursa of Fabricius, thymus and spleen of chicken.

Pesti and Howarth (1983) found that the percentage of bursa weight to body weight had decreased to 0.31 per cent when reared under 116 cm^2/bird as compared to 0.37 per cent when reared in 697 cm^2/bird.

Munck and Guyre (1991) reported that direct lysis or apoptosis may be responsible for the reduction in the number of circulating lymphocytes and involution of the lymphoid organs due to stress induced increase in corticosteroids.

Sapolsky (1992) suggested that corticosteroids may cause a delay in the maturation of the lymphocytes which may further exacerbate the problem of reduced lymphocyte number in circulation.

Poon *et al.* (1994) observed that injection of cortisol at the rate of 1 mg/day for seven days in ducks caused reduction in the size of the bursa of Fabricius and absolute weights of the primary lymphoid organs but had no effect on the weight of the spleen.

Siegel (1995) observed that injection of ACTH increased endogenous production of corticosteroid in the cytoplasm and nucleus of sensitive lymphatic tissues such as the thymus and the bursa of Fabricius.

Siegel (1995) pointed out that high levels of corticosteroids released in acute stress might decline as the stressor continues and there was a negative feed back on the hypothalamus which causes inhibition of secretion of the corticotrophin releasing factor (CRF). Birds under consistent stress showed lympholytic effect of ACTH as avian leukocytes produced ACTH or ACTH like substances. This regression included depletion of the lymphocytes from the germinal centres, lymphopenia and heterophilia (Siegel, 1995; Elrom, 2000).

Vijayan and Lalitha (1997) reported bursal enlargement in chicken under acute stress. Histologically the medulla of the follicles showed mild depletion, whereas the cortex was closely packed with lymphocytes and mild degree of interfollicular oedema was also observed.

Zhang-LeCui *et al.* (1998) studied the effect of short-term heat stress on the lymphoid organs and reported that the absolute weights and relative weights of the spleen, bursa of Fabricius and thymus were significantly lower in the

heat stressed groups. Stannius follicles of the bursa in the test group were smaller and few.

Manisha De and Ghosh (1998) reported that in the epinephrine treated chicken absolute and relative bursa weights had decreased and the lymphoid follicles in the bursa were slightly decreased in size. The compactness of the medullary cells decreased by parenchymal disorder and forming irregular spaces. Some amount of nuclear pyknosis was also observed in both epinephrine and norepinephrine treated groups. Also the total number of both cortical and medullary nuclei of both epinephrine and norepinephrine treated groups exhibited a numerical reduction.

Wang-Shu Bai *et al.* (1999) reported oedema, atrophy and lymphocyte necrosis of the bursa of Fabricius and spleen of all heat stressed birds while the thymus showed no change.

Puvadolpirod and Thaxton (2000a) described a model of physiological stress by using continuous administration of adrenocroticotropic hormone using physiological miniosmotic pumps and observed reduction in the relative weight of the major immunobiological organs like the spleen, thymus and bursa of Fabricius.

Puvadolpirod and Thaxton (2000b) reported that the order of response to stress was elevated plasma cortiosterone level by 2 hours, decreased relative weight of the spleen by 24 hours and decreased relative weight of the thymus and bursa of Fabricius by four days.

2.5 Stress and immunity

Gross and Siegel (1965) observed that short term social stress (mixing unrelated cockerels for short time as one day) induced resistance to air sac on intravenous challenge with pathogenic *E. coli*. However, this resistance declined with increase in duration of the stressor.

Gross and Colmano (1970) reported that birds with relatively high levels of corticosterone were relatively more susceptible to viral and mycoplasmal infection. While birds with low plasma corticosterone was relatively more susceptible to bacterial diseases.

Thaxton and Siegel (1970) reported depression of agglutinin antibodies within 12 hours after exposure to heat or injection of ACTH.

Experimental results of stress induced immuno suppression have not been consistent (Siegel (1995). Stress induced immunosuppression, although, usually evident in cell-mediated immunity (CMI) was not always observed when humoral antibodies such as those represented by agglutinin or precipitin reactions were measured (Thaxton and Briggs, 1972; Subba Rao and Glick, 1977 and Regnier *et al.*, 1980).

Carew (1976) reported that higher cage densities resulted in greater mortality due to Marek's disease and lymphoid leukosis.

Subba Rao and Glick (1977) reported that chronic cold exposure significantly increased the antibody titres while birds at 37.2°C and above had

signifiantly depressed agglutinin levels. Brief exposure to cold two or four times increased IgM antibody production and markedly reduced IgG antibody. Elevation of antibody titre was related to the time of cold treatment and antigen injection.

Heller *et al.* (1979) reported higher antibody titres in chicks (1-2 h) heat stressed for short term.

Regnier *et al.* (1980) reported that an acute intermittent heat or cold stress did not much alter the antibody formation in the fowl. They also observed that an acute, intermittent heat or cold stress did not significantly alter the ability of chicken to synthesize antibodies.

There is a plethora of literature suggesting the potential role of stressors in eliciting numerous deleterious effects. They may impede production of antibodies and an effective cell-mediated immunity, thereby increasing the susceptibility to viral diseases, tumors and mycoplasmal infections (Gross and Colmano, 1969; Gross, 1972 and Zulkifli *et al.*, 1994a,b).

Gross and Siegel (1981) studied long term responses of chicken to three different levels of social strife. They observed that though, humoral immune responsiveness was not affected in chicken maintained under low social strife, they were more susceptible to *E. coli* challenge, but more resistant to mycoplasmal infection than those maintained in moderate and higher strife settings. They opined that moderate social strife allowed maximum manifestation of genetic potential for disease resistance, antibody responses to

various antigens and productivity. High social strife though caused only minor changes in the feed intake caused reduction in weight gain and feed efficiency. Defence against *E. coli* and Mycoplasma infection was contrary to the chicken maintained under low social strife.

Jamadar and Jalnapurkar (1994) reported lower serum antibody titres to New Castle disease virus in heat stressed chicken based on the haemagglutination titres obtained.

Pierson *et al.* (1997) reported that social stress on the birds on the 2nd, 3rd, 4th and 5th day and especially on the 4th and 5th day significantly increased the protection against coccidiosis.

Peterson and Siegel (1998) reported that cage density had no significant effect on the HI titres to sheep RBC.

Al-Bisher *et al.* (1998) reported that heat stress, stimulated antibody production during primary immunization and suppressed during secondary immunisation.

Elrom (2000) opined that corticosteroids, produced in stress, in addition to depressing antibodies and cell-mediated immunity also reduced macrophage migration and also phagocytosis. These also reduced monocyte accumulation at a site in response to macrophage inhibiting factor. IL2 also depressed IL2 promoted T cell division and release of mediators like interferon (IFN) γ, B cell growth and division, and monocyte and NK cell activation. IFN γ was shown

to be a potent inducer of macrophage activation and MHC II molecules in tissues and had antiviral and antiproliferative effects.

Variation in the susceptibility to different pathogens should be attributed to the effect of glucocorticoids either on the particular pathology involved or on the immunological defence mechanism. The anti-inflammatory action of glucocorticoids reported to be useful against bacterial diseases where the major pathology involved local or generalised inflammation. On the contrary, viral infections, which caused direct invasion of tissue and inflammation was required to localise the invasion. The increase in the number of heterophilic granulocytes and a decrease in the lymphocytes could also be attributed to the same reason (Siegel, 1995; Zulkifli and Siegel, 1995 and Elrom, 2000).

Lazarevic *et al.* (2000) reported that long term sound stress decreased the cutaneous hypersensitivity to phytohaemagglutinin and caused a decrease in the leucocyte number which suggested that long term stress could result in immunosuppression.

2.6 Effect of stress and blood parameters

Winter (1935) found that haemoglobin content of the blood increased in winter significantly.

Shapiro and Schechtman (1949) reported that a single injection of adrenocortical extract in the adult fowl caused transient lymphopenia and leukocytosis.

Stamler *et al.* (1950) reported no effect on the number of eosinophils after several ACTH injections.

Huble (1955) reported that treatment of cockerels with ACTH or corticosterone produced relative lymphopenia, a relative and absolute rise in heterophils and a relative and absolute eosinopenia while no change was observed in the absolute number of lymphocytes and erythrocytes.

Huston (1960, 65) observed that packed cell volume increased in the birds exposed to cold.

Deaton *et al.* (1969) observed a higher haematocrit and haemoglobin levels in birds reared under cold environments.

Brake *et al.* (1982) observed a significant basophilia and eosionophilia in 70 week old fowl in induced moulting.

Gross and Siegel (1983) observed that the number of lymphocytes in the chicken blood samples decreased and number of heterophils increased in response to stressor and also to increasing levels of corticosterone in chicken feed. They observed that the ratio of heterophils to lymphocytes was less variable and it was, therefore, a more reliable indicator of stress. They also concluded that HL ratio was more reliable indicator of the perceived magnitude of stressor than plasma corticosterone values in the avian species.

Hoffman and Leighton (1985) reported that captivity stress caused non regressive type of anaemia in Herring bulls.

Gray *et al.* (1989) noted significant heterophilia, monocytosis, eosinophilia and basophilia in 7-8 month old laying fowl after daily injection of ACTH.

Maxwell *et al.* (1990) reported that feed restriction for long periods of time (4-20 weeks) caused a significant increase in the number of basophils despite no variation in HL ratio. Further studies on food restriction on broiler revealed highly significant basophilia (Hocking *et al.*, 1993 and Savory *et al.*, 1993).

Maxwell *et al.* (1992) reported that heat stress induced ultra structural changes in the broiler chicken blood cells. Granules in the basophils were reduced, heterophil lobulation increased, occasionally cytoplasmic fragmentation was evident in the heterophils and increased cytoplasmic lipid droplets were seen within the monocytes suggesting early fatty degeneration. The eosinophils did not exhibit much change. The erythrocytes and thrombocytes were longer and thinner. These changes could alter their function affecting the immune response.

Maxwell (1993) reported that although HL ratio may be a reliable measure of stress response in the avian species, however its value during periods of extreme stress was limited. During such periods basophilia and heteropenia predominated. He concluded that in some poultry, heterophilia may be a response to mild or moderate stress but basophilia may result from severe stress.

Gross and Siegel (1993) observed that HL ratio of about 0.2, 0.5 and 0.8 characterized low, optimum and high levels of stress respectively. They suggested that in birds, HL ratio was a good measure of long term stress (hours to weeks).

Zulkifli *et al.* (1994b) reported that starvation increased H:L ratio within 24 hours.

Mitchell *et al.* (1996) observed that in birds crated, where temperature generated could result in life threatening increase in body temperature significantly increased basophil counts in approximately 25 per cent of surviving birds.

Maxwell and Robertson (1995) reported that climatic and food restriction produced significant basophilia in some types of poultry. Although, HL ratio was a less variable indicator of avian stress during extreme stress as in life threatening situation, a heteropenia and basophilia often developed. Thus in some stress situations, a biphasic cellular reaction might be present.

Vijayan and Rema (1997) reported that heterophil : lymphocyte ratio was a useful measure of stress in chicken.

Yahav *et al.* (1997) reported that a significant decrease in packed cell volume occurred in birds exposed to higher temperatures, while acute exposure of chicks to high temperatures did not have any significant effect on the packed cell volume or plasma volume.

Peterson and Siegel (1998) observed no significant changes in the percentage of heterophils, lymphocyte or HL ratio in birds subjected to higher cage density.

Furlan *et al.* (1999) reported that acute heat stress was associated with a decrease in RBC number, PCV, pCO2 and increase in pH of blood and pO2 in many strains of broiler birds.

Lazarevic *et al.* (2000) who studied on long term sound stress on the broilers reported that leukocyte count decreased and H:L ratio increased after exposure to sound stress.

Puvadolpirod and Thaxton (2000b) reported that following continuous infusion of ACTH (8 IU/kg/day for 7 days) which was established as a model for studying stress, the H:L ratio increased by the second day.

2.7 Stress and production

Tomhave and Seegar (1945) found that decreased floor space resulted in lower body weight, increased mortality and poor feed conversion.

Heishman *et al.* (1952) reported no significant difference in the number of culls, mortality, cannibalism or dressed quality as area was reduced from 1.0 to 0.5 sq ft/bird. But body weights were reduced as population density increased.

Siegel and Coles (1958) found no significant difference in the weight or feed conversion of broilers grown at floor space levels ranging from 0.5 to 1.25 sqft/bird.

Kubena *et al.* (1974) indicated a direct relationship between carcass fat content and temperature over the range of 7-32°C. Increased abdominal fat was noticed in birds reared in thermal stress.

Gross *et al.* (1980) observed that feeding corticosterone from 5 to 50 µg/kg greatly decreased the feed efficiency, increased the appetite and there was a great increase in the body and liver fat.

Gross and Siegel (1981) observed that in birds housed individually with no sight of other birds exhibited superior growth and feed efficiency during the first three months. Thereafter, they became anorectic, emaciated and flabby.

Pesti and Howarth (1983) observed that body weight gain during the first week in broiler chicken kept on 697 cm^2/bird was significantly less than those kept at 348 cm^2/bird although the chicks at 697 cm^2 ate more feed. During weeks two and three, chicks kept at 116 cm^2/bird did not grow as well as those kept at 232 cm^2/bird or more.

Narayanankutty and Ramakrishnan (1992) observed in birds reared in California type cages (60 x 45 x 45 cm) at densities 3, 4 and 5 birds per cage that birds in three birds/cage showed higher weight, carcass yield and lower mortality while feed efficiency was better in birds from 5 birds/cage group.

Joseph and Ramachandran (1993) observed that induced hypocorticalism by dexamethozone retarded the body weight gain of chicken.

Sokolowicz *et al.* (1996) observed that stress significantly decreased the body weight and carcass yield and increased the mortality rate of broiler chicken.

Kannan *et al.* (1997) pointed out that higher pre slaughter stress levels in broilers influenced the colour of thigh meat, although overall meat quality was not affected.

Martrenchar *et al.* (1997) reported that higher stocking density decreased live weight, increased the prevalence of foot and pad dermatitis and breast blisters.

Cooper and Washburn (1998) observed a lower body weight gain in birds under thermal stress.

Peterson and Siegel (1998) observed a greater body weight gain in birds with lower stocking density than with higher stocking densities.

Al-Batshan and Hussein (1999) observed that hot cycling temperature reduced the body weight, weight gain, feed intake, carcass weight and breast meat while it increased the feed conversion, carcass yield, drumstick and thigh weight of broiler birds.

Elrom (2000) reported that muscle depletion and fat accretion occurred in corticosteroid release due to stress. Corticosteroid treated birds had poor

absorptive efficiency and showed poor growth and skeletal development despite increased food consumption.

Puvodolpirod and Thaxton (2000b) observed reduction in body weight in birds administered ACTH continuously using miniosmotic pump at the rate of 8 IU/kg/day for seven days.

Sorensen *et al.* (2000) observed that high stocking density (435 cm^2/bird) was associated with reduced live weight, poor walking ability and more foot and hock burns.

3. MATERIALS AND METHODS

3.1 Experimental design

The study was conducted on one hundred and two, healthy day-old Hubbard strain broiler chicks during the period of November-December, 2000. The chicks were randomly divided into three equal groups I, II and III. The chicks were tagged individually and maintained on deep litter (wood shavings). All the birds were fed *ad libitum* on standard broiler feed tested and found free of aflatoxin and ochratoxin. Water was provided *ad libitum* to all the birds.

Birds of group I were administered dexamethazone (Dexona, Cadila) powdered and uniformly mixed in the feed at the rate of 50 ppm on day 20 and at 25 ppm on days 27, 34, 41 and 44.

Birds of group II were subjected to physical stress by providing a floor space to 0.25 ft^2/bird till the fourth week and to 0.5 ft^2/bird thereafter.

Birds of group I and III were maintained in a floor space of 1 ft^2/bird.

Birds of group III which were maintained on standard management conditions served as control.

All the birds were vaccinated against New Castle disease with Lasota strain one drop administered intranasal and another intra ocular on the seventh day.

All the birds were brooded at 35°C, 32°C and 30°C during the first, second and third week respectively. Thereafter, the birds were reared at room temperature, which varied from 25-30°C.

3.2 Observations

All the birds were observed for behavioural changes and clinical manifestations throughout the experimental period.

The body weight of all the day-old birds were recorded and also at weekly intervals, starting from the 21st day. Feed intake of each group was measured weekly and weekly feed conversion ratio (FCR) was calculated.

$$\text{FCR (weekly)} = \frac{\text{Weekly feed intake}}{\text{Weekly body weight gain}}$$

3.2.1 Haematological studies

Blood was collected from the wing vein with ethylene diamine tetra acetate (EDTA) as anticoagulant on the 21st, 28th, 35th, 42nd and 45th day for haematological analysis.

Volume of packed red blood corpuscle (VPRC) was estimated by the method described by Wintrobe (1981).

Haemoglobin was estimated by the cyanmethemoglobin method described by Miale (1972) and the final reading was taken in an Exma photometer.

Total leukocyte count (TLC) and Total red blood cell count (RBC) were determined as per the method described by Sastry (1976). The differential leukocyte count (DLC) was done with copper peroxidase method of Sato and Sekio (1965).

3.2.2 Immunological tests

3.2.2.1 Haemagglutination Inhibition Antibody titre (HI titre)

HI titre against New Castle disease virus was determined by the method described by Gulka *et al.* (1982).

3.2.2.2 Enumeration of T-lymphocytes

T-lymphocytes were enumerated on the 21st, 35th and 42nd day using Alpha naphthyl acetate esterase (ANAE) activity. Mononuclear cells were separated using density gradient centrifugation with Ficoll-Paque (Pharmacia, Uppsala, Sweden) and thin smears were made on clean grease free glass slides and the smears were fixed by the method of Giorno and Beverly (1981). They were stained following the method of Knoles *et al.* (1978).

The slide preparations were examined for ANAE activity in the cells using 1000X magnification to work out the percentage of ANAE positive T-lymphocytes. A total of 200 cells per slide were examined and counts were made.

3.2.2.3 Leukocyte migration inhibition test (LMIT)

LMIT was conducted on the 35th and 42nd days of age. The test was carried out according to the method described by Bendixen (1977) with minor modifications.

Leukocytes were separated from the whole blood by gradient centrifugation in Ficoll-Paque technique as described by Blaxhall (1985).

The cell concentration was adjusted to $2x10^7$ viable leukocytes per ml. They were then divided into two equal portions and to one portion, eight haemagglutination units of New Castle disease antigen was added (Vijayan, 1998) and to the other portion, an equal volume of sterile normal saline was added. The contents of each tube were thoroughly mixed and then incubated for one hour at 37°C with occasional shaking to avoid cell clumping. The contents of each tube were filled in six wells of three millimetres diameter, cut eight millimetres apart in agarose gel on a glass plate. The charged plates were incubated at 37°C in a humid chamber for 20 hours. Then the cells were fixed to the glass surface by flooding the plates with methanol acetic acid fixative (7 parts of methanol, 1 part of acetic and 2 parts of distilled water). The agar gel in the plates were partially dried to facilitate peeling of the agar gel from the plates.

Migration area of the leukocytes was measured by taking average diameter of the opaque zone around the wells. The migration index (MI) was calculated as

$$MI = \frac{\text{Average area of migration of cells treated with antigen}}{\text{Average area of migration of cells treated with normal saline}}$$

3.2.3 Gross and histopathological studies

Six birds from each group were randomly chosen, weighed and sacrificed on 21st, 28th, 35th, 42nd and 45th days. The carcasses were subjected to detailed gross examination for the presence of bruises, blisters or other abnormalities. Adrenal, bursa, thymus, spleen, pancreas, liver and thyroid were collected, weighed immediately to prevent weight loss due to desiccation and examined for gross lesions, and abnormalities. One adrenal of each bird was fixed in Wood's fixative (Wood, 1963). The other adrenal, bursa, thymus, spleen, liver and kidney were fixed in 10% neutral buffered formalin (NBF) for histopathological studies.

Tissues were processed by routine paraffin embedding technique (Luna, 1968). Paraffin sections were cut at three to five microns thickness and stained with Haematoxylin and Eosin (H&E) method as described by Sheehan and Hrapshack (1980).

3.2.3.1 Special stains

Sections of adrenal fixed in Wood's fixative were stained with Wood's stain as described by Wood (1963) to differentiate between the medullary epinephrine and nor epinephrine producing cells and cortical cells. Some of the sections fixed in woods fixative were stained with routine H&E stain also.

Sections fixed in Orth's fluid were stained for chromaffin cells as described by Sheehan and Hrapshack (1980).

Sections of the adrenal fixed in NBF were stained with

(a) Van Giesons fast green as described by Lillie (1954)

(b) Phosphotungstic acid haematoxylin (PTAH) for the demonstration of ganglion cells

Formalin fixed sections of liver cut using cryostat were used to stain for fat as described by Sheehan and Hrapshack (1980).

3.3 Statistical analysis

Covariance completely randomised design (Cov. CRD) was used for analysing the effect of stressors on the final body weights of the birds taken on the 21st, 28th, 35th, 42nd and 45th days by taking initial body weight as covariate, since the initial body weight had effect on the final body weight.

Organ weights were also subjected to covariance analysis (Cov. CRD) taking the final body weight as the covariate, since organ weights depended on the body weight of the birds.

Analysis of Variance (ANOVA) technique was used for analysing body weight to organ weight ratio.

Body weights, organ weights, blood parameters and HI titre were subjected to split plot in time analysis by taking treatments as the main plot treatment and time of observation as the subplot treatment.

3.4 Stress scores

Stress scores were calculated as per the method of Puvadolpirod and Thaxton (2000e) with minor modifications for all the quantitative parameters using either one of the formulae. Formula A was used whenever the mean treatment value for that parameter was greater than the mean control value and formula B was used when the mean control value was greater than the mean treatment value.

$$\text{Formula A} = \frac{\text{Mean treatment value}}{\text{Mean control value}} \times P$$

$$\text{Formula B} = \frac{\text{Mean control value}}{\text{Mean treatment value}} \times P$$

'P' was arbitrarily assigned the following values

4 - when probability for that parameter was less than or equal to 0.01 per cent

2 - when probability for that parameter was less than or equal to 0.02 per cent and more than 0.01 per cent

1 - when probability for that parameter was less than or equal to 0.05 per cent but greater than 0.02 per cent

0 - when probability for that parameter was greater than 0.05 per cent

Results

4. RESULTS

4.1 Behavioural changes

Birds of Group I developed diarrhoea, a day after the initial administration of dexamethazone. The birds were drowsy and lethargic. Eventhough they recovered from diarrhoea, many of the them developed tracheal rales and mild conjunctivitis. Feed consumption was reduced drastically.

Birds in the overcrowded group (Group II), though appeared normal and active in the initial stages, showed poor development of feathers and reduction in feed intake subsequently. They became aggressive and hyper responsive to external stimuli after the 28th day, when the floor space was increased to 0.5 ft^2 per bird. However, as the treatment progressed, birds became dull with ruffled feathers and showed less social interaction and preening behaviour. Litter got caked easily compared to the other two groups. Some of the birds showed lameness.

Birds of Group III remained apparently normal.

4.2 Body weight

Mean body weights of the birds are set out in Table 1 and illustrated in Graph 1. Birds of Group I weighed less when compared to the control group and the difference was significant ($P<0.01$) throughout the experiment. Mean body weight of Group I on the 45th day was 1391.4 7 ± 22.94 g which was 38

per cent less compared to the control group. The effect of administration of dexamethazone on the body weight was not uniform at different periods of observation. For instance, the body weight on the 21st and 45th day showed a reduction of 12 per cent and 38 per cent respectively (Table 2). This effect of 'Duration of treatment' on the body weight was statistically significant ($P<0.01$).

Birds of Group II showed a 7.3 per cent increase in body weight on the 21st day as compared to the control which was significant ($P<0.01$). On the 28th, 35th, 42nd and 45th day, there was a reduction in body weight compared to the control. However, the reduction was not significant on the 28th day. The effect of overcrowding on the body weight was not uniform at different periods of observation. The effect was maximum on the 45th day, when a 29 per cent reduction was noticed and least on the 28th day, when only 2 per cent reduction was recorded.

4.3 Feed efficiency

Feed efficiency of the birds is presented in Table 4 and illustrated in Graph 2. Birds in Group II showed a higher feed efficiency on the 21st day. However, from the 28th day to the end of the period of experiment, feed efficiency remained lower than the control group. Feed efficiency of Group I birds was lower than the control group throughout the period of the experiment.

Table 1. Mean body weight of treatment and control groups on different days of experiment

Group	21st day	28th day	35th day	42nd day	45th day
	Body wt. (g) ± SE	Body wt. (g) ± SE	Body wt. (g) ± SE	Body wt. (g) ± SE	Body wt. (g) ± SE
Control	536.99 ± 8.28	933.3 ± 9.17	1332.86 ± 11.93	1788.23 ± 41.87	2235.62 ± 22.94
I	472.01 ± 8.28	656.98 ± 9.17	976.07 ± 11.93	1333.6 ± 41.87	1319.47 ± 22.94
II	575.99 ± 8.28	917.22 ± 9.17	1230.24 ± 11.93	1510.67 ± 41.87	1594.59 ± 22.94

Table 2. Percentage difference of body weights of treated groups in comparison with the control on different days of observation

Group	21st day	28th day	35th day	42nd day	45th day
	Per cent difference	Per cent difference	Per cent difference	Per cent difference	Per cent difference
I	-12.10	-29.61	-26.77	-25.42	-40.98
II	+7.26	-1.72	-7.77	15.52	-28.67

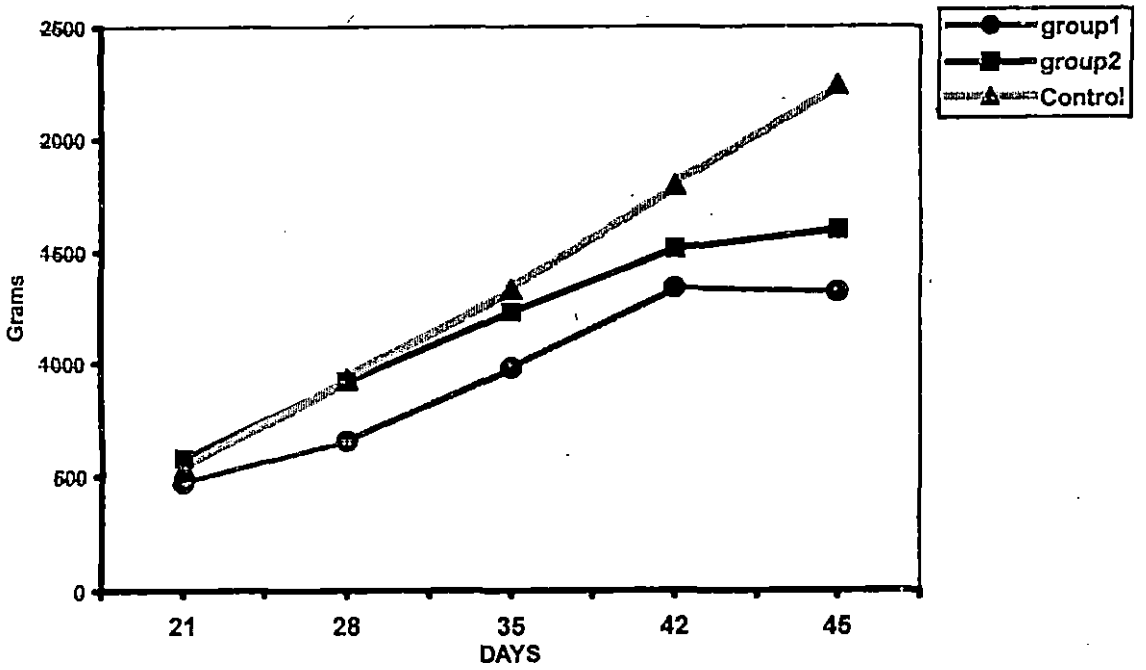
Table 3. Stress scores for body weights for treated groups during different periods of observation

Group	21st day	28th day	35th day	42nd day	45th day
I	4.55	5.68	5.46	5.36	6.43
II	4.55	4.07	4.33	4.73	5.61

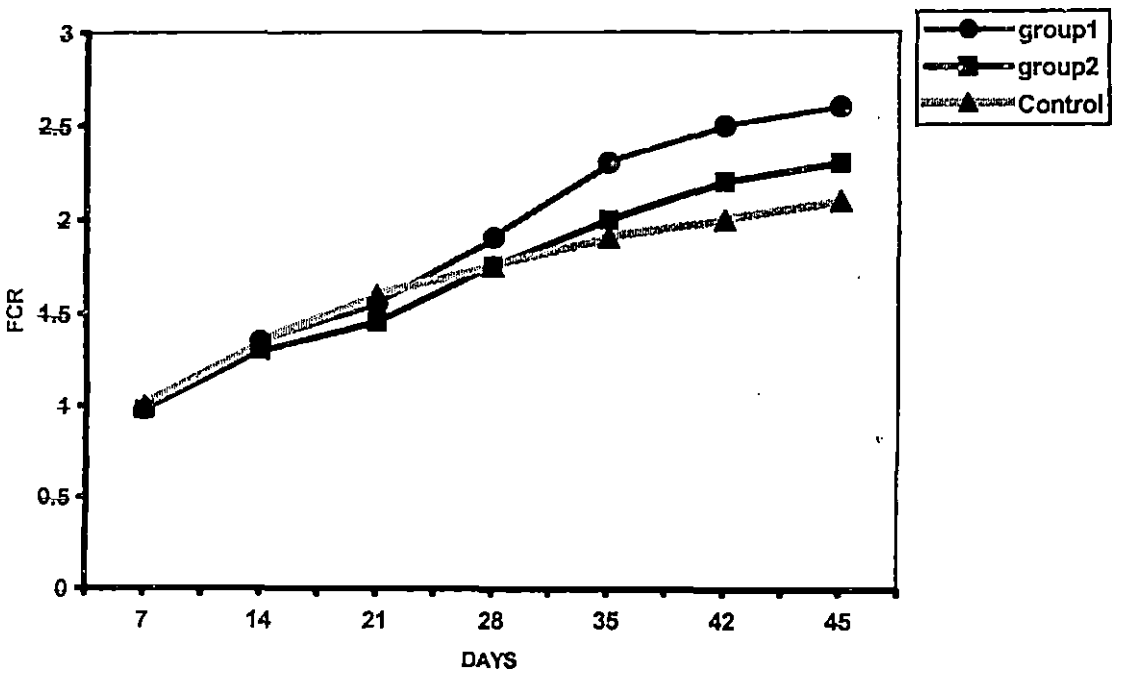
Table 4. Weekly feed conversion ratio of treatment and control groups during different days of experiment

Group	7th day	14th day	21st day	28th day	35th day	40th day	45th day
Control	1.00	1.35	1.60	1.75	1.90	2.00	2.10
I	0.98	1.35	1.55	1.90	2.30	2.50	2.60
II	0.98	1.30	1.45	1.75	2.00	2.20	2.30

Graph 1 Mean body weight of treatment and control groups during different days of experiment



Graph 2 Weekly feed conversion ratio of the treatment and control groups during different days of experiment



4.4 Haematological parameters

4.4.1 Total leucocyte count (TLC)

Mean TLC values are set out in Table 5 and illustrated in Graph 3. Birds of Group I and II showed significant increase in TLC as compared to the control. The increase was maximum on the 28th day in Group I and 35th day in Group II which were 43 per cent and 12 per cent respectively.

4.4.2 Total red blood cell count (RBC count)

Mean RBC counts of the birds are presented in Table 6. Birds of Group I and II showed a significantly lower ($P<0.01$) RBC count than the control group.

4.4.3 Volume of packed red blood corpuscles (VPRC)

Mean VPRC of birds are presented in Table 7. VPRC of both the treatment groups were higher on the 21st day compared to the control group. On the subsequent period, VPRC remained lower for both the groups than the control. Duration of treatment showed a significant ($P<0.01$) variation of the effect of stressors on VPRC. The effect was higher on the 35th day, when 12 per cent and 11 per cent drop in VPRC were recorded for Group I and II respectively.

4.4.4 Haemoglobin concentration

The mean haemoglobin values are presented in Table 8. Group I and II showed significant difference ($P<0.01$) in the mean haemoglobin value all

Table 5. Mean total leukocyte count (TLC) and stress scores of the treatment and control groups during different days of experiment

Group	21st day		28th day		35th day		42nd day		45th day	
	TLC	Score	TLC	Score	TLC	Score	TLC	Score	TLC	Score
Control	24.50^{c}		24.67^{c}		31.83^{c}		25.17^{b}		24.33^{c}	
I	32.67^{a}	**5.3**	35.33^{a}	**5.7**	38.00^{a}	**4.8**	34.50^{a}	**5.5**	32.00^{a}	**5.3**
II	29.83^{b}	**4.9**	29.17^{b}	**4.8**	35.50^{b}	**4.5**	34.17^{a}	**5.4**	29.50^{b}	**4.8**

TLC – Total leukocyte count in thousands

Figures having atleast one common superscript do not differ significantly (P<0.01)

Table 6. Mean Red blood cell count (RBC) and stress scores of the treatment and control groups during different days of experiment

Group	21st day		28th day		35th day		42nd day		45th day	
	RBC	Score	RBC	Score	RBC	Score	RBC	Score	RBC	Score
Control	2.83^{b}		3.30^{c}		2.98^{c}		2.83^{b}		1.87^{b}	
I	2.40^{ab}	**4.7**	2.07^{a}	**6.4**	1.92^{a}	**6.2**	1.88^{a}	**6.0**	2.97^{a}	**6.3**
II	2.73^{a}	**4.1**	2.92^{b}	**4.5**	2.56^{b}	**4.6**	2.48^{b}	**4.6**	2.62^{a}	**5.6**

RBC – Total Red blood cell count in million

Figures having atleast one common superscript do not differ significantly (P<0.01)

Table 7. Mean value of packed red blood corpuscles (VPRC) and stress scores of the treatment and control groups during different days of experiment

Group	21st day		28th day		35th day		42nd day		45th day	
	VPRC	Score	VPRC	Score	VPRC	Score	VPRC	Score	VPRC	Score
Control	27.33^{b}		28.83^{b}		30.83^{b}		31.67^{b}		30.67^{b}	
I	35.67^{a}	**0**	27.00^{a}	**0**	27.00^{a}	**0**	29.00^{a}	**0**	29.67^{ab}	**0**
II	28.67^{b}	**0**	28.67^{b}	**0**	27.33^{a}	**0**	28.67^{a}	**0**	28.67^{a}	**0**

Figures having atleast one common superscript do not differ significantly (P<0.01)

Table 8. Mean haemoglobin (Hb) and stress scores of the treatment and control groups during different days of experiment

Group	21st day		28th day		35th day		42nd day		45th day	
	Hb	Score	Hb	Score	Hb	Score	Hb	Score	Hb	Score
Control	9.33^{a}		10.32^{b}		11.03^{c}		11.00^{b}		10.97^{b}	
I	8.35^{a}	**4.5**	9.17^{a}	**4.5**	8.83^{a}	**5.0**	8.93^{a}	**4.9**	9.40^{a}	**4.7**
II	8.87^{a}	**4.2**	9.90^{ab}	**4.2**	9.95^{b}	**4.4**	9.97^{ab}	**4.4**	9.18^{a}	**4.8**

Figures having atleast one common superscript do not differ significantly (P<0.01)

through the experiment. Duration of treatment modified the effect of stressors in a significant way. Maximum decrease of haemoglobin values in Group I was observed on the 35th day (20% reduction), whereas in Group II, the decrease was observed maximum on the 45th day (16% reduction).

4.4.5 Differential leucocyte count (DLC)

Mean values of differential leucocyte counts of the birds are set out in Table 9 and 9a and illustrated in Graphs 4, 5 and 6.

Groups I and II showed a statistically significant ($P<0.01$) higher mean heterophil count (Graph 4) throughout the period of experiment. Duration of treatment caused a significant ($P<0.01$) variation in the effect of treatments (stressors) on the heterophil count. Group I showed 27 per cent increase on the 21st day, while it was lowest on the 42nd day when only 5.6 per cent increase was recorded.

Groups I and II showed a significant ($P<0.01$) decrease in lymphocyte count (Graph 5) throughout the experiment. Duration of treatment showed no significant variation on the effect of stressors on lymphocyte count.

Mean basophil counts (Table 9; Graph 6) of Group I, II and III did not show any significant difference upto day 35. However a significant ($P<0.01$) increase in the basophil count was observed on the 42nd and 45th day in Group II

Table 9. Differential leukocyte count (%) of treatment and control groups during different days of experiment

Group	21st day					28th day					35th day					42nd day					45th day				
	H	L	M	E	B	H	L	M	E	B	H	L	M	E	B	H	L	M	E	B	H	L	M	E	B
Control	32.1^{b}	64.6^{c}	1.33^{a}	1.3^{a}	0.5^{a}	32.8^{b}	64.5^{b}	1.8^{a}	1.1^{a}	0.5^{a}	32.8^{c}	66.3^{c}	2.1^{a}	0.8^{a}	0.3^{a}	32^{c}	65.1^{c}	1.8^{a}	0.6^{a}	0.6^{a}	31.0^{c}	64.0^{c}	1.1^{a}	0.3^{a}	0.5^{a}
I	41.0^{a}	55.3^{a}	1.5^{a}	0.8^{a}	1.0^{a}	40.5^{a}	56.1^{a}	2.0^{a}	1.0^{a}	0.3^{a}	37.8^{a}	55.5^{a}	1.8^{a}	1.1^{a}	0.3^{a}	34.6^{a}	52.8^{a}	1.8^{a}	0.3^{a}	0.8^{a}	34.6^{a}	54.1^{a}	1.6^{a}	0.8^{a}	1.1^{a}
II	40.5^{a}	56.0^{b}	2.0^{a}	0.8^{a}	0.6^{a}	39.6^{a}	56.5^{a}	1.8^{a}	1.3^{a}	0.6^{a}	41.3^{b}	53.8^{b}	2.1^{a}	0.6^{a}	0.3^{a}	39.8^{b}	54.0^{b}	1.5^{a}	0.5^{a}	2.5^{b}	40.0^{b}	52.6^{b}	1.3^{a}	1.3^{a}	2.5^{b}

Figures having atleast one common superscript do not differ significantly ($P<0.01$)

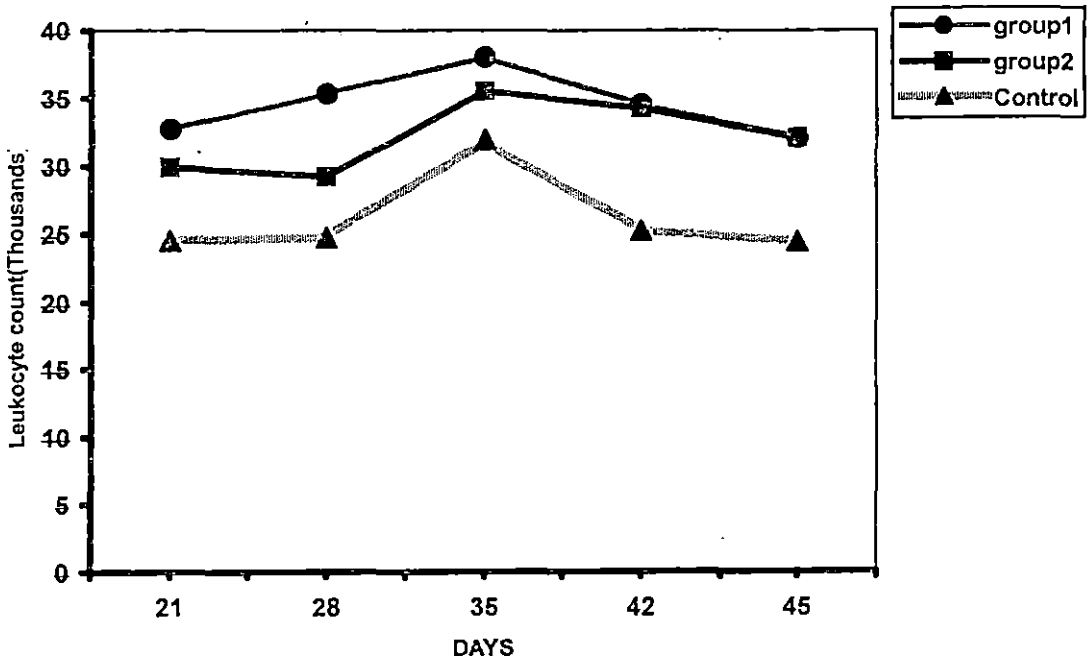
Table 9a. Stress scores for differential leukocyte count (%) of treatment and control groups during different days of experiment

Group	21st day					28th day					35th day					42nd day					45th day				
	H	L	M	E	B	H	L	M	E	B	H	L	M	E	B	H	L	M	E	B	H	L	M	E	B
I	5.1	4.7	0	0	8.0	5.0	4.6	0	0	6.1	4.6	4.8	0	0	4	4.2	4.7	0	0	4	4.5	4.6	0	0	9.4
II	5.0	4.6	0	0	5.4	4.8	4.6	0	0	5.4	5.0	4.9	0	0	4	4.8	4.8	0	0	4	5.2	4.9	0	0	20.0

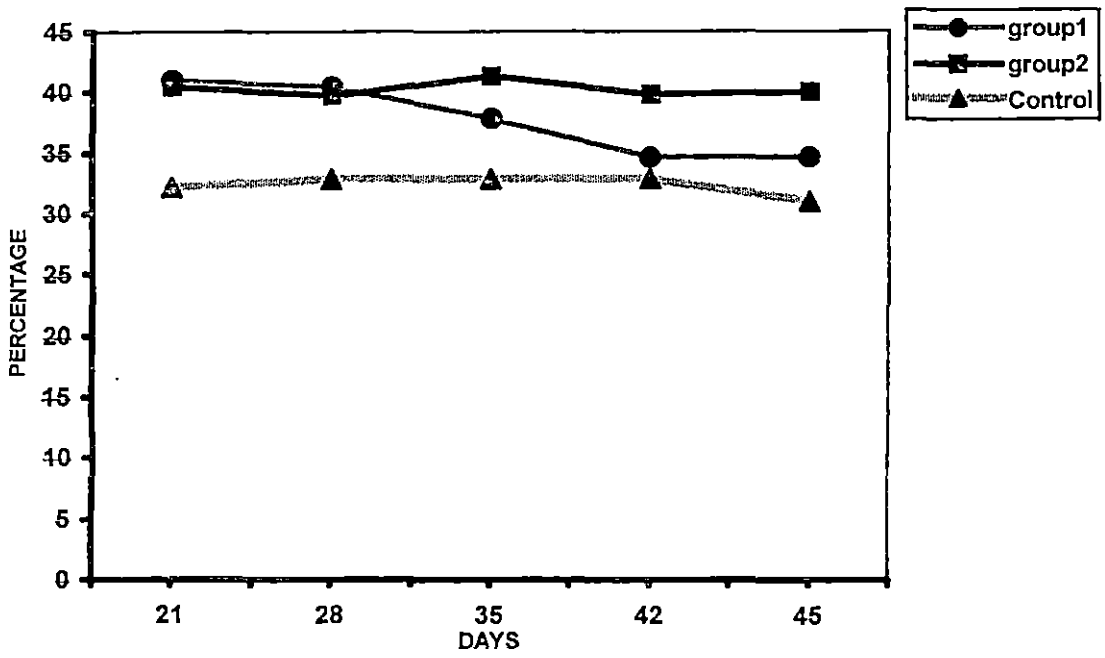
H – Percentage heterophil count L – Percentage lymphocyte count M – Percentage monocyte count

E – Percentage Eosinophil count B – Basophil count

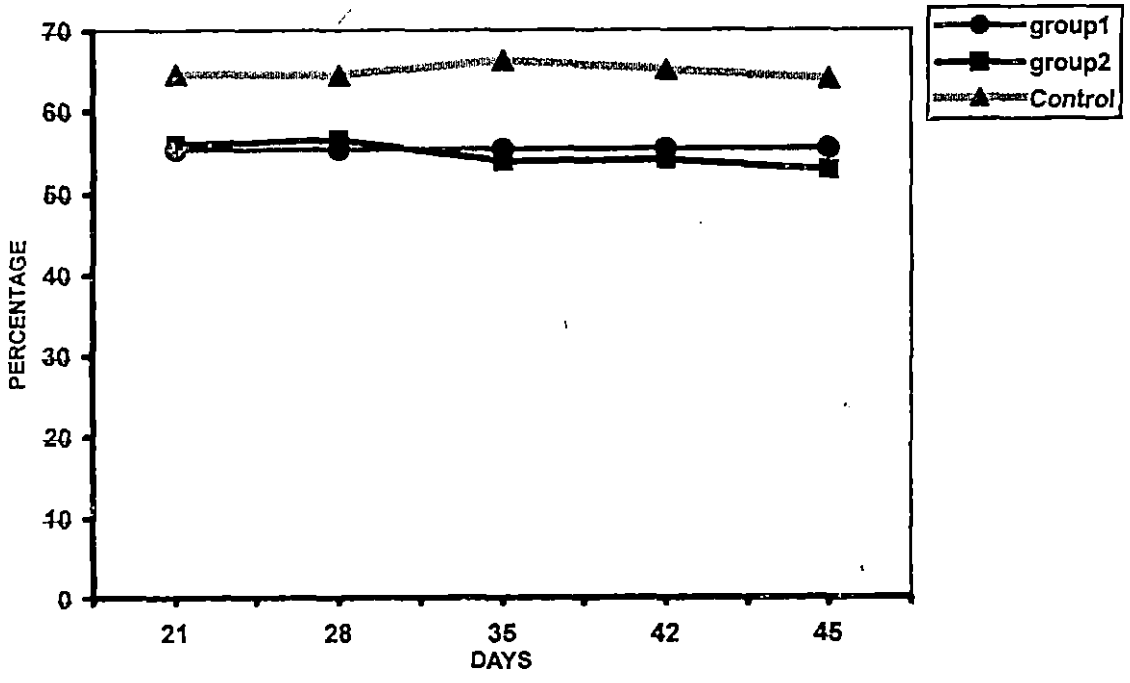
Graph 3 Mean Total leukocyte count of the treatment and control groups during different days of experiment



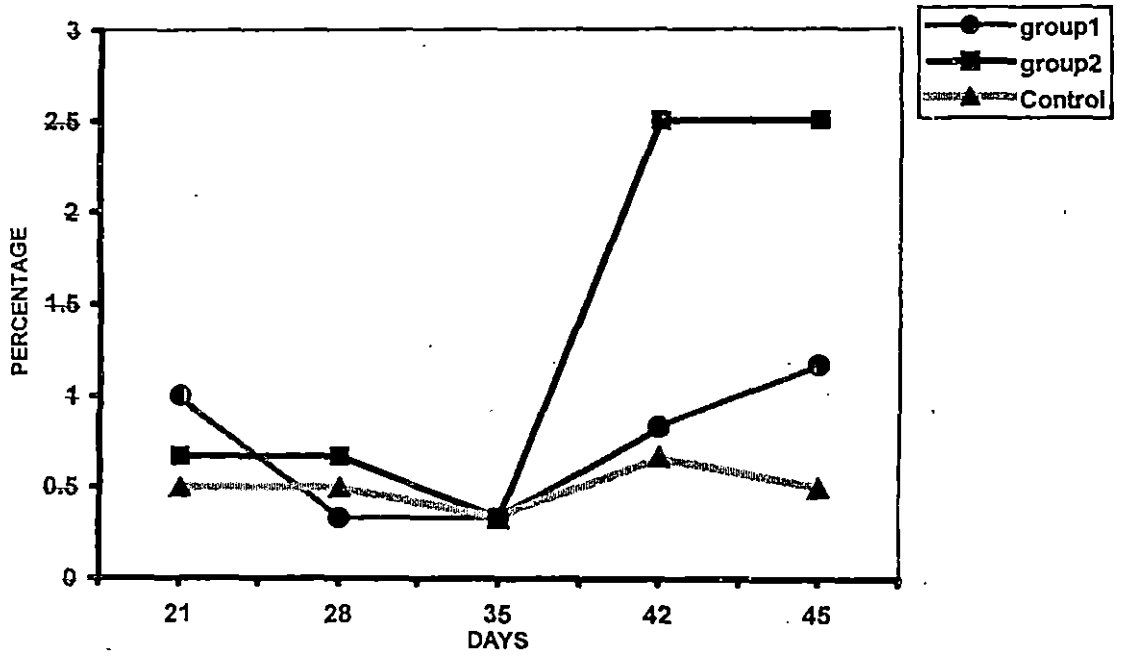
Graph 4 Mean Heterophil count (percentage) of the treatment and control groups during different days of experiment



Graph 5 Mean lymphocyte count (percentage) of the treatment and control groups during different days of experiment



Graph 6 Mean Basophil count (percentage) of the treatment and control groups during different days of experiment



Mean eosinophil and monocyte counts of Groups I, II and III though varied slightly, was not significant. Neither the period of the experiment individually nor the combined effect of the period of experiment and treatments showed any significant difference on analysis.

4.4.6 Heterophil-lymphocyte ratio

Mean *heterophil to lymphocyte ratio* (HL ratio) of Group I and II showed a significant ($P<0.01$) increase over the control group (Table 10; Graph 7). The duration of treatment modified the effect of the stressor in a statistically significant ($P<0.01$) way. Group II showed an increase in HL ratio during 21st, 28th and 35th day, while it dipped marginally on the 42nd and 45th day.

4.5 Immunological parameters

4.5.1 *Haemagglutination inhibition titres*

The geometric mean of haemagglutination inhibition (HI) titres expressed as $\log_2$ values against New Castle disease virus of the birds are presented in Table 11 and graphically represented in Graph 8. HI titres in both the treatment groups were lower than the control group. The duration of treatment had modified the effect of the stressors on HI titres significantly ($P<0.01$). The trend for lower values of HI titres continued till the 45th day when it had decreased to 44 per cent in Group I and 15 per cent in Group II.

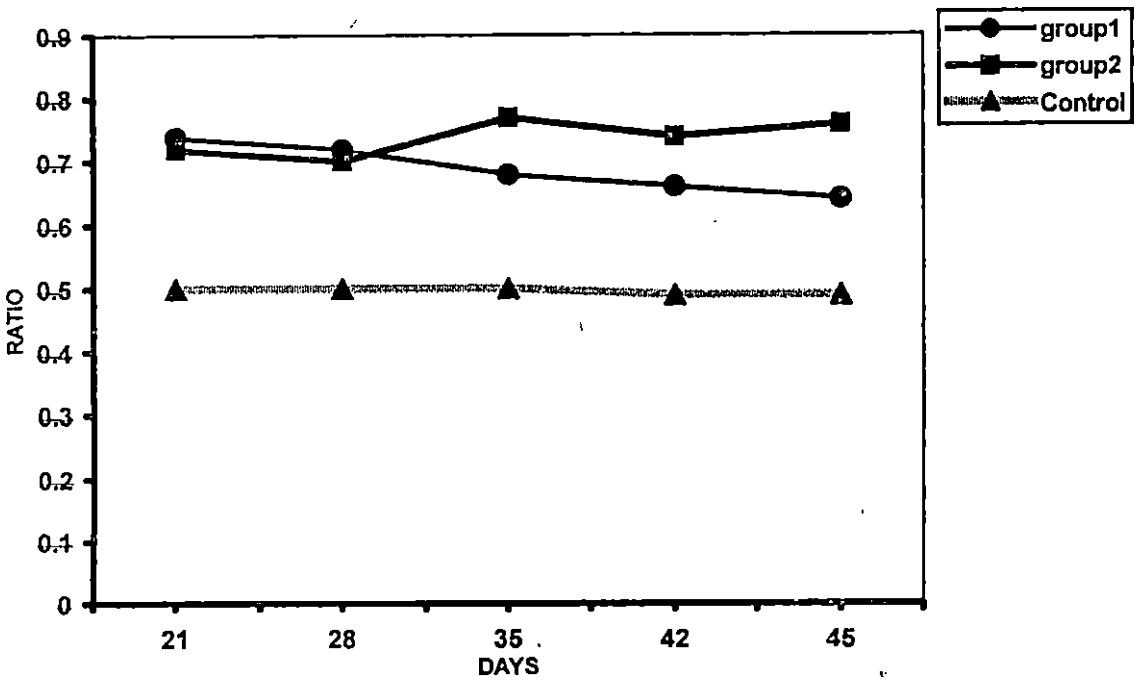
Table 10. Mean heterophil to lymphocyte ratio of treatment and control groups during different days of experiment

Group	21st day	28th day	35th day	42nd day	45th day
Control	0.5	0.5	0.5	0.49	0.49
I	0.74	0.72	0.68	0.66	0.64
II	0.72	0.70	0.77	0.74	0.76

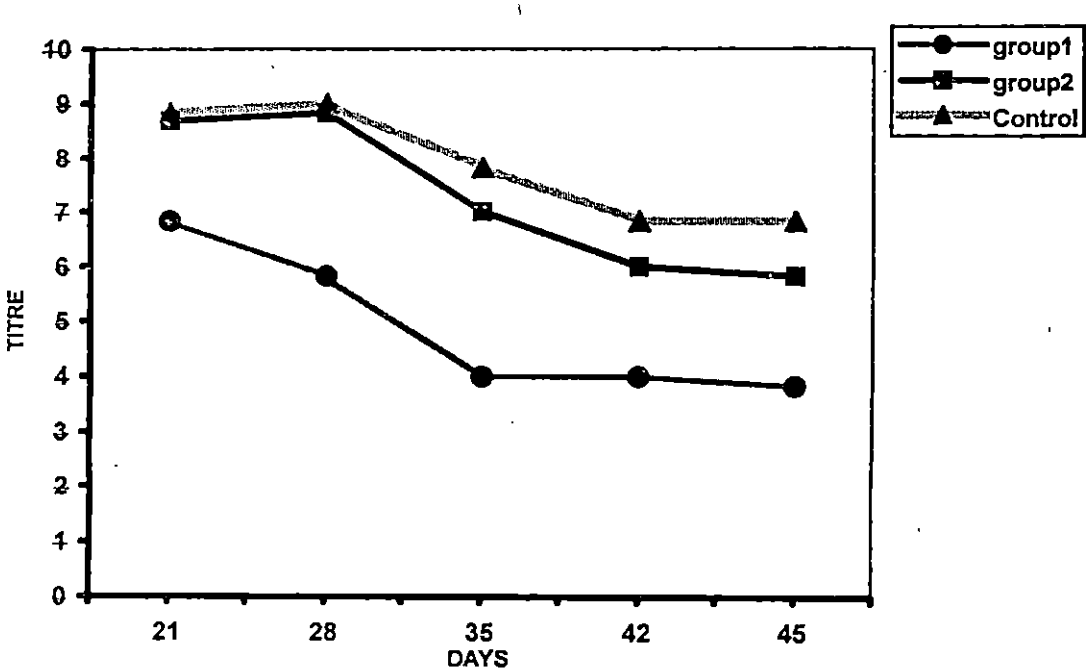
Table 11. Geometric mean of haemagglutination inhibition titres expressed as $\log_2$ values against New Castle disease of treatment and control groups on different days of experiment

Group	21st day		28th day		35th day		42nd day		45th day	
	Titre	Stress score	Titre	Stress score	Titre	Stress score	Titre	Stress score	Titre	Stress score
Control	8.83		9.00		7.83		6.83		6.83	
I	6.83	5.17	5.83	6.17	4.00	7.83	4.00	6.83	3.83	7.13
II	8.67	4.07	8.83	4.08	7.00	4.47	6.00	4.55	5.83	4.69

Graph 7 Mean Heterophil Lymphocyte ratio of treatment and control groups during different days of experiment



Graph 8 Geometric Mean of Hemagglutination Inhibition titres expressed as log2 values against New Castle disease virus of the treatment and control groups on different days of the experiment



4.5.2 Enumeration of T-lymphocytes

The mean percentages of T-lymphocyte counts are presented in Table 12 and Graph 9. The percentage of T-lymphocytes were significantly lower in both Group I and II than the control Group. They continued to be progressively lower as the duration of treatment advanced.

4.5.3 Leukocyte migration inhibition test (LMIT)

The percentage of leukocyte migration inhibition (LMI) values are presented in Table 13. Lower migration indices were recorded in both Group I and II than the control. Birds of Group I were more severely affected than Group II.

4.6 Organ weights

4.6.1 Weight of the adrenal

The mean weights of the adrenals are presented in Table 14 and illustrated in Graph 10. Birds in the overcrowded group (Group II) showed higher adrenal weights in all the periods of the experiment. The difference in weight was significant ($P<0.01$). Duration of the experiment had caused a significant variation in the effect of stressors on the adrenal weight. During the first week, there was a mean 11.98 per cent increase in the adrenal weight over the control group, whereas during the 45^{th} day, 66.29 per cent increase in weight was recorded. Adrenal to body weight ratio (Table 15) was also increased in Group II in all the periods of the experiment. The ratio of the adrenal to body weight had increased by 90 per cent during the 35^{th} day.

Table 12. Mean percentage T-lymphocyte count of the treatment and control groups during different days of experiment

Group	21st	35th	42nd
Control	44.5	45	40
I	30	26	22
II	42	35	32

Table 13. Mean percentage of leucocyte migration inhibition values of the treatment and control groups during different days of experiment

Group	35th day	42nd day
Control	46.5%	42.0%
I	29%	26%
II	35%	32%

Table 14. Mean adrenal weights of treated and control groups on different days of observation

Group	21st day	28th day	35th day	42nd day	45th day
	Adrenal wt. mg ± SE	Adrenal wt. mg ± SE	Adrenal wt. mg ± SE	Adrenal wt. mg ± SE	Adrenal wt. mg ± SE
Control	71.2^{a} ± 0.4	84.7^{a} ± 0.61	110.2^{bc} ± 1.39	121.5^{a} ± 1.94	114.7a ± 1.24
I	70.9^{b} ± 0.4	73.8^{b} ± 0.61	114.0^{b} ± 1.39	112.1^{b} ± 1.94	115.7^{b} ± 1.24
II	79.7^{a} ± 0.4	106.9^{c} ± 0.61	127.0^{a} ± 1.39	176.7^{c} ± 1.94	190.8^{a} ± 1.24

Figures bearing atleast one common superscript do not differ significantly ($P<0.01$)

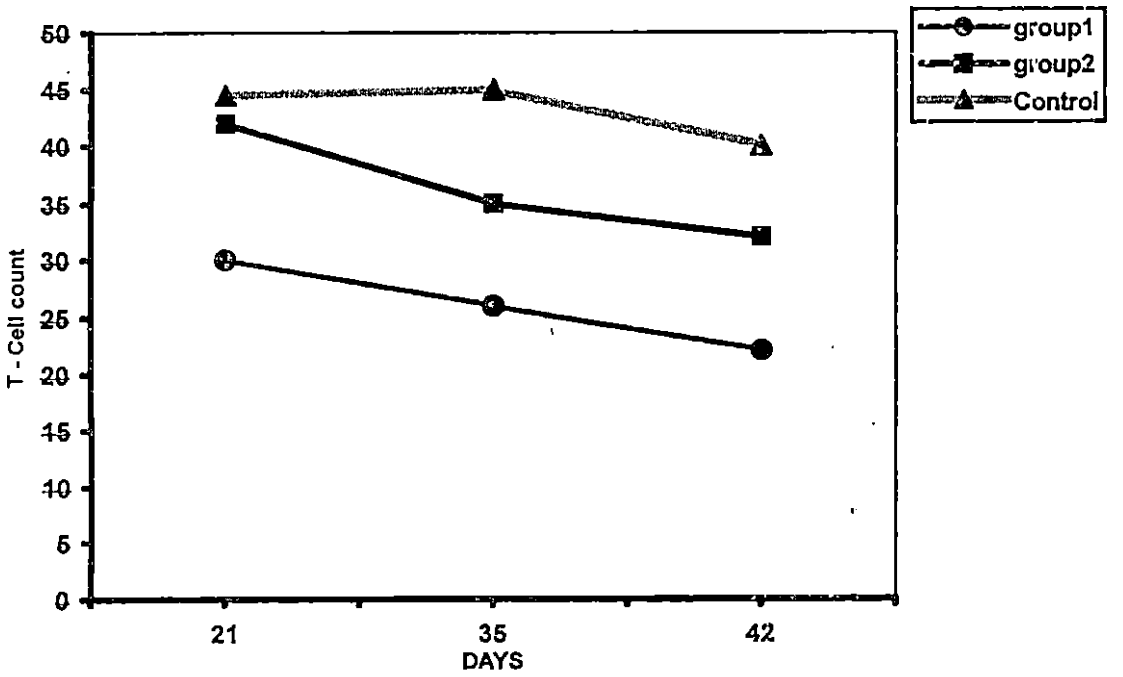
Table 15. Mean adrenal weights to body weight ratio of treated and control groups on different days of observation

Group	21st day	28th day	35th day	42nd day	45th day
	Adrenal wt. to body wt. ratio mg/ 100 g	Adrenal wt. to body wt. ratio mg/ 100 g	Adrenal wt. to body wt. ratio mg/ 100 g	Adrenal wt. to body wt. ratio mg/ 100 g	Adrenal wt. to body wt. ratio mg/ 100 g
Control	13.46 ± 0.17	9.91 ± 0.13	8.94 ± 0.19	6.99 ± 1.05	6.07 ± 0.22
I	13.57 ± 0.06	9.15 ± 0.26	10.49 ± 0.43	8.28 ± 0.30	7.30 ± 0.30
II	14.86 ± 0.27	12.32 ± 0.23	10.57 ± 0.33	11.76 ± 0.45	11.61 ± 0.46

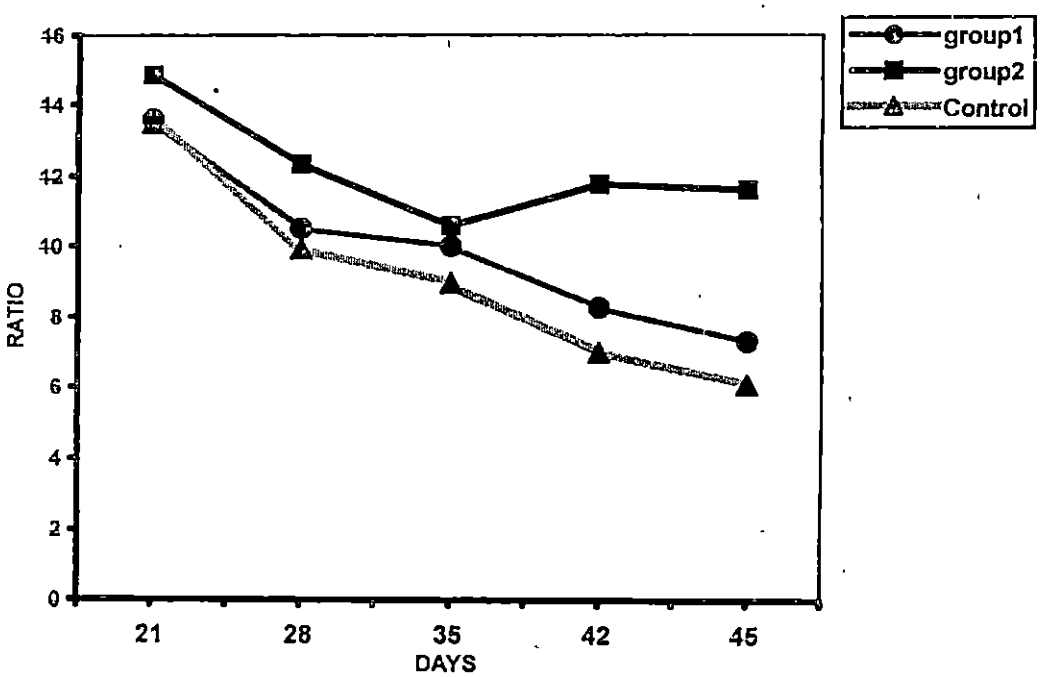
Table 16. Stress scores for adrenal weights and adrenal weight to body weight ratio for treated groups during different periods of observation

Group	21st day		28th day		35th day		42nd day		45th day	
	Organ wt.	Ratio	Organ wt.	Ratio	Organ wt.	Ratio	Organ wt.	Ratio	Organ wt.	Ratio
I	4.02	4.03	4.59	4.33	4.14	4.69	4.34	4,74	6.65	4.83
II	4.47	4.42	5.05	4.97	4.61	4.73	5.81	6.73	6.65	7.68

Graph 9 Mean T- Lymphocyte count of the treatment and control groups during different days of the experiment



Graph 10 Mean adrenal to body weight ratio of the treatment and control groups during different days of experiment



Birds of Group I recorded lower adrenal weights than Group III in all the periods of the experiment. This difference was significant ($P<0.01$). The duration of treatment had modified the effect of stressors on the adrenal weight in a statistically significant way. The adrenal weight was lowest in the second week (12.86 per cent decreased), when compared with the control. The ratio of adrenal weight to body weight (Table 15) was however higher all through the experimental period. It was 0.82 per cent higher in the first week and was 20.66 per cent higher on the 45th day of the experiment.

4.6.2 Weight of the Bursa of Fabricius

Mean weights of the Bursa of Fabricius is presented in Table 17 and illustrated in Graph 11. Birds in Group II recorded higher weights for the Bursa of Fabricius during all the periods of the experiment. This difference in weight was statistically significant ($P<0.01$). Duration of the treatment caused a variation in the effect of treatment on the bursal weight. There was 17 per cent increase in the bursal weights during the 21st day of the experiment whereas in the fifth week, 36 per cent increase in the weight was recorded. The increase in the bursal weight to body weight ratio (Table 18) was consistent all through the experimental period. It was increased by 66 per cent on the 45th day of the experiment compared to the control group. The weights of bursa from birds of Group I were less than the control. Duration of the treatment caused a significant ($P<0.01$) variation in the effect of treatment on the bursa weight. Bursal weight showed 32 per cent decrease on the 21st day of experiment.

Table 17. Mean weights of bursa for treated and control groups on different days of observation

Group	21st day	28th day	35th day	42nd day	45th day
	Bursa wt. mg ± SE	Bursa wt. mg ± SE	Bursa wt. mg ± SE	Bursa wt. mg ± SE	Bursa wt. mg ± SE
Control	1195.11 ± 24.00	2292.07 ± 35.00	3327.02 ± 70.07	3959.04 ± 49.95	3727.12 ± 30.69
I	814.27 ± 24.00	978.28 ± 35.00	1205.44 ± 70.07	1163.83 ± 49.95	1396.79 ± 30.69
II	1399.96 ± 24.00	2814.65 ± 35.00	4047.22 ± 70.07	4831.76 ± 49.95	5069.47 ± 30.69

Table 18. Mean bursa weights to body weight ratio of treated and control groups on different days of observation

Group	21st day	28th day	35th day	42nd day	45th day
	Bursa wt. to body wt. ratio mg/ 100 g	Bursa wt. to body wt. ratio mg/ 100 g	Bursa wt. to body wt. ratio mg/ 100 g	Bursa wt. to body wt. ratio mg/ 100 g	Bursa wt. to body wt. ratio mg/ 100 g
Control	226.35 ± 3.61	259.32 ± 14.26	270.75 ± 10.43	230.46 ± 28.54	187.05 ± 8.80
I	144.45 ± 10.57	117.25 ± 6.22	85.64 ± 21.23	78.62 ± 2.80	78.44 ± 3.10
II	261.73 ± 9.90	315.98 ± 5.69	336.40 ± 9.27	320.67 ± 11.14	309.78 ± 5.24

Table 19. Stress scores for bursa weights and bursa weight to body weight ratio for treated groups during different periods of observation

Group	21st day		28th day		35th day		42nd day		45th day	
	Organ wt.	Ratio	Organ wt.	Ratio	Organ wt.	Ratio	Organ wt.	Ratio	Organ wt.	Ratio
I	5.87	6.27	9.37	8.85	11.04	12.64	13.60	11.73	10.67	9.54
II	4.69	4.62	4.91	4.87	4.86	4.97	4.88	5.57	5.44	6.62

Reduction in the weight was more pronounced on day 42, where 71 per cent reduction in the weight was observed. Bursa weight to body weight ratio (Table 18) too had reduced significantly ($P<0.01$). The ratio had decreased by 36 per cent during the 21st day. The drop was greatest on the 35th day (68 per cent), while the ratio showed a 58 per cent reduction on the 45th day.

4.6.3 Weight of the thymus

The mean weights of the thymus is presented in Table 20 and illustrated in Graph 12. The weight of the thymus was less in birds of Group I compared to the control. But the difference was significant ($P<0.01$) from the 35th day onwards. In Group II, birds showed statistically significant ($P<0.01$) decrease in the weight of the thymus on all the days of observation. The drop in weight of the thymus when compared to the control was more pronounced on 28th and 42nd day, when 50 per cent and 67 per cent reductions respectively were noticed over the control group. The thymus weight to body weight ratio (Table 21) too were significantly lower in both the Groups I and II compared to the control group.

4.6.4 Weight of the spleen

Mean weights of the spleen of Group II on the 21st day (358.26 ± 7.77 mg) was more than the control (355.92 ± 7.77 mg) (Table 23; Graph 13). But this increase was not significant. During the rest of the experiment, Group II recorded lower weights for the spleen. The difference became statistically significant ($P<0.01$) from the 42nd day. The effect of the stressors showed a

Table 20. Mean weight of thymus for treated and control groups on different days of observation

Group	21st day	28th day	35th day	42nd day	45th day
	Thymus wt. mg ± SE	Thymus wt. mg ± SE	Thymus wt. mg ± SE	Thymus wt. mg ± SE	Thymus wt. mg ± SE
Control	1022.98 ± 10.38	2485.98 ± 38.66	3366.09 ± 41.03	4194.87 ± 36.49	4103.5 ± 26.43
I	724.62 ± 10.38	1233.46 ± 38.66	1807.69 ± 41.03	1405.92 ± 36.49	1405.18 ± 26.43
II	981.90 ± 10.38	2325.72 ± 38.66	2785.55 ± 41.03	3065.46 ± 36.49	3138.58 ± 26.43

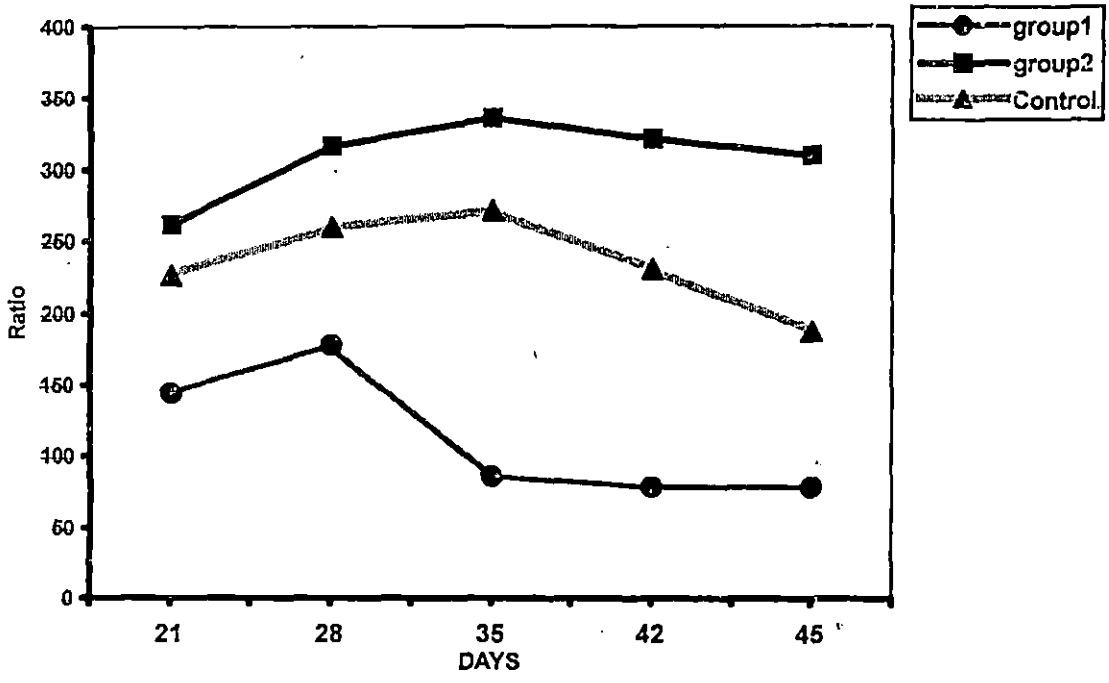
Table 21. Mean thymus weights to body weight ratio of treated and control groups on different days of observation

Group	21st day	28th day	35th day	42nd day	45th day
	Thymus wt. : body wt. ratio mg/100 g	Thymus wt. : body wt. ratio mg/100 g	Thymus wt. : body wt. ratio mg/100 g	Thymus wt. : body wt. ratio mg/100 g	Thymus wt. : body wt. ratio mg/100 g
Control	191.57 ± 7.12	276.95 ± 13.02	264.57 ± 7.79	237.55 ± 34.6	195.84 ± 11.35
I	143.19 ± 6.11	165.52 ± 19.09	163.82 ± 8.96	104.58 ± 10.11	88.44 ± 4.90
II	178.30 ± 7.64	2325.72 ± 38.66	230.68 ± 9.93	204.34 ± 7.86	192.30 ± 6.10

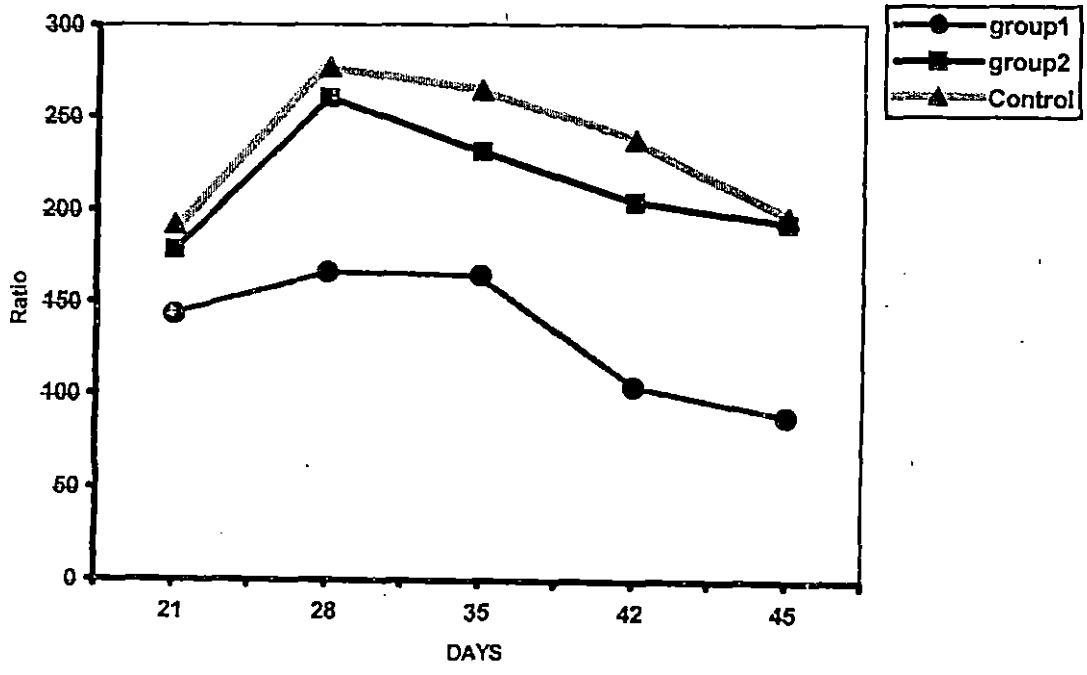
Table 22. Stress scores for thymus weights and thymus weight to body weight ratio for treated groups during different periods of observation

Group	21st day		28th day		35th day		42nd day		45th day	
	Organ wt.	Ratio	Organ wt.	Ratio	Organ wt.	Ratio	Organ wt.	Ratio	Organ wt.	Ratio
I	5.65	5.35	8.06	6.69	7.45	6.46	11.93	9.08	11.68	8.86
II	4.17	4.30	4.28	4.26	4.83	4.59	5.47	4.65	5.23	4.07

Graph 11 Mean bursa to body weight ratio of the treatment and control groups during different days of experiment



Graph 12 Mean thymus to body weight ratio of the treatment and control groups during different days of experiment



variation from week to week. This variability was statistically significant ($P<0.01$). Spleen to body weight ratio (Table 24) also showed a similar trend. It was lowest on the 42nd day when it showed 38 per cent decrease in Group I and 19 per cent reduction in Group II over the control group.

4.6.5 Weight of the pancreas

Mean weights of the pancreas showed no significant variation between the groups during the period of the experiment. The organ weight to body weight ratio however, showed an increase in both the Groups I and II with Group I recording 52 per cent increase and Group II 32 per cent increase by the 45th day.

4.6.6 Weight of the liver

The mean liver weights are set out in Table 26 and graphically represented in Graph 14. Weight of the liver of Group II birds showed significant reduction on the 21st day (15287.26 ± 155.62 mg) than the control (16180.74 ± 155.62 mg). However, during the rest of the experiment, Group II showed significant increase in the weight of liver. The stressors were found to have a variation in its effect depending on the period of the experiment. The effect was greatest (94% increase) during day 35 for Group I and on day 45 for Group II (47% increase). Liver weight to body weight ratio (Table 27) also showed a similar increasing trend. The ratio showed greatest effect on day 45 for both Group I (93% increase) and Group II (45% increase.)

Table 23. Mean weight of spleen for treated and control groups on different days of observation

Group	21st day	28th day	35th day	42nd day	45th day
	Spleen wt. mg ± SE	Spleen wt. mg ± SE	Spleen wt. mg ± SE	Spleen wt. mg ± SE	Spleen wt. mg ± SE
Control	355.92 ±7.77	812.90 ±9.24	1078.61 ±14.34	1561.25 ±21.96	1440.03 ±11.70
I	238.48 ±7.77	501.81 ±9.24	699.77 ±14.34	780.14 ±21.96	851.59 ±11.70
II	358.26 ±7.77	690.46 ±9.24	971.78 ±14.34	1109.36 ±21.96	1129.89 ±11.70

Table 24. Mean spleen weights to body weight ratio of treated and control groups on different days of observation

Group	21st day	28th day	35th day	42nd day	45th day
	Spleen wt. : body wt. ratio mg/100 g	Spleen wt. : body wt. ratio mg/100 g	Spleen wt. : body wt. ratio mg/100 g	Spleen wt. : body wt. ratio mg/100 g	Spleen wt. : body wt. ratio mg/100 g
Control	66.97 ±3.09	92.72 ±3.41	83.14 ±2.53	90.52 ±12.45	71.70 ±3.25
I	44.74 ±3.49	62.92 ±2.94	67.81 ±5.22	55.70 ±1.98	53.38 ±2.89
II	66.22 ±4.30	79.49 ±2.71	79.81 ±2.36	73.40 ±2.39	67.96 ±1.98

Table 25. Stress scores for spleen weights and spleen weight to body weight ratio for treated groups during different periods of observation

Group	21st day		28th day		35th day		42nd day		45th day	
	Organ wt.	Ratio	Organ wt.	Ratio	Organ wt.	Ratio	Organ wt.	Ratio	Organ wt.	Ratio
I	5.97	5.99	6.48	5.89	6.16	4.90	8.00	6.50	6.76	5.37
II	4.03	4.05	4.71	4.67	4.44	4.17	5.63	4.93	5.09	4.22

Table 26. Mean weight of liver for treated and control groups on different days of observation

Group	21st day	28th day	35th day	42nd day	45th day
	Liver wt. g ± SE	Liver wt. g ± SE	Liver wt. g ± SE	Liver wt. g ± SE	Liver wt. g ± SE
Control	16.18 ±0.16	18.32 ±0.15	26.93 ±0.13	40.93 ±0.71	35.40 ±0.59
I	28.07 ±0.15	29.48 ±0.15	52.24 ±0.13	54.74 ±0.71	64.74 ±0.59
II	15.29 ±0.16	21.86 ±0.15	33.89 ±0.13	45.56 ±0.71	51.92 ±0.59

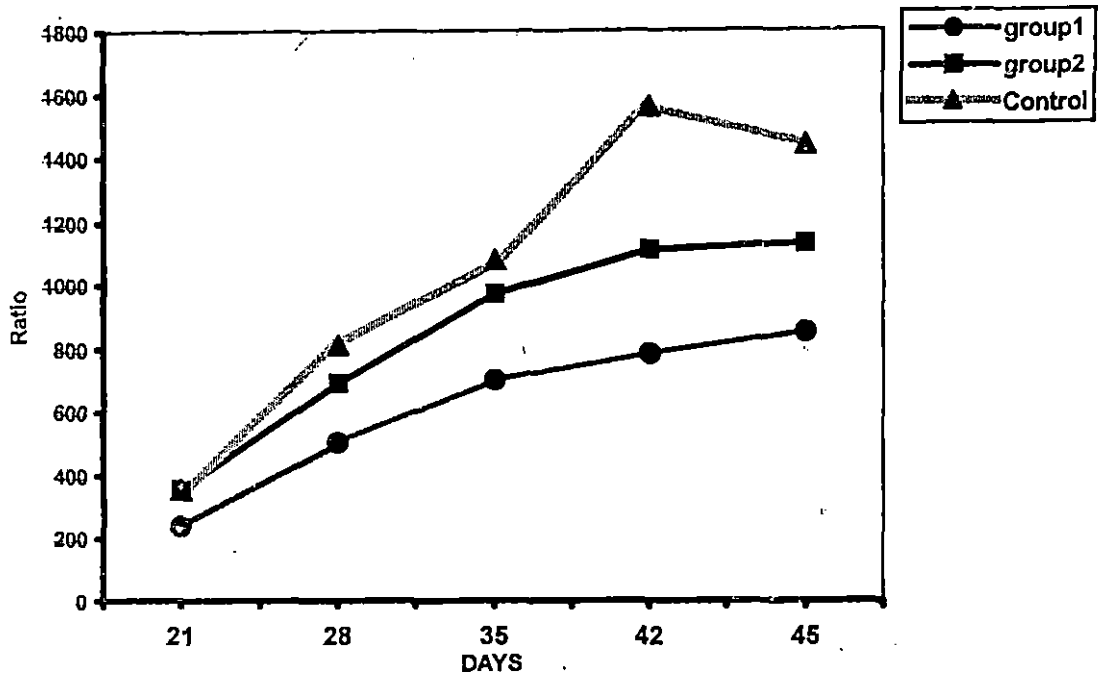
Table 27. Mean liver weights to body weight ratio of treated and control groups on different days of observation

Group	21st day	28th day	35th day	42nd day	45th day
	Liver wt. : body wt. ratio mg/100 g	Liver wt. : body wt. ratio mg/100 g	Liver wt. : body wt. ratio mg/100 g	Liver wt. : body wt. ratio mg/100 g	Liver wt. : body wt. ratio mg/100 g
Control	3069.76 ±30.38	2276.89 ±25.15	2352.21 ±18.17	2379.58 ±353.72	2101.26 ±71.16
I	5577.79 ±46.5	3647.56 ±8.16	4748.73 ±25.29	4040.88 ±20.76	4060.57 ±26.44
II	2929.27 ±26.52	2659.97 ±29.9	2874.08 ±14.96	3023.34 ±82.74	3042.13 ±22.43

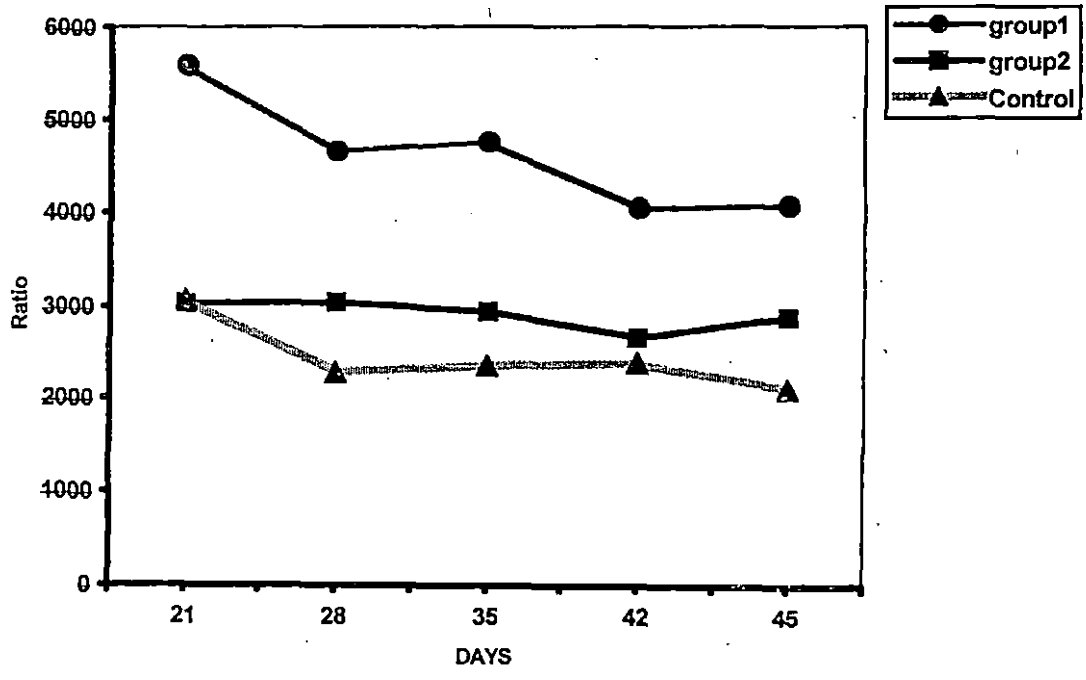
Table 28. Stress scores for liver weights and liver weight to body weight ratio for treated groups during different periods of observation

Group	21st day		28th day		35th day		42nd day		45th day	
	Organ wt.	Ratio	Organ wt.	Ratio	Organ wt.	Ratio	Organ wt.	Ratio	Organ wt.	Ratio
I	6.94	7.27	6.43	6.40	7.76	8.08	5.35	6.79	7.31	7.73
II	4.23	4.19	4.77	4.67	5.03	4.89	4.45	5.08	5.87	5.79

Graph 13 Mean spleen to body weight ratio of the treatment and control groups during different days of experiment



Graph 14 Mean liver to body weight ratio of the treatment and control groups during different days of experiment



4.6.7 Weight of the thyroid

Mean weights of the thyroid did not show any significant variation between the treatments. The ratio of the thyroid weight to body weight also did not show any statistically significant variation.

4.7 Gross pathological changes

Carcasses of the birds in Group I were icteric. Deposition of fat in the abdominal viscera was prominent. The muscles showed moderate degree of wasting. The liver was markedly enlarged, friable and greasy to touch with rounded borders. The surface showed yellowish streaks. The bursa, thymus and spleen were smaller in size (Fig.1, 2 and 3). The kidneys appeared pale and swollen. The heart muscles were pale. The keel bone was prominent and the breast muscles were emaciated.

Carcasses of Group II had bruises and scratches on the breast muscles. Pododermatitis and blisters seen in the foot pads increased significantly with age. The bursa of the birds in Group II showed a marked enlargement than the control group. The bursa was soft to touch and the folds appeared to fill the entire lumen. The thymus and the spleen were smaller, when compared to the control group. A moderate increase in the abdominal fat was also noticed. The control group did not show any gross pathological changes.

4.8 Histopathological changes

4.8.1 Adrenal

Group I

The adrenals from Group I birds sacrificed on day 21 showed predominantly medullary cells. The medullary cells were enlarged and had a swollen appearance. Increased proportion of ephinephrine producing cells were found in Wood's stained sections (Fig.4).

Medullary cells showed mild to moderate degree of cytoplasmic vacuolar changes on the 28th day (Fig.5). In contrast to the adrenals on the 21st day, there was a predominance of cortical cells. However some of the cortical cells had hyperchromatic nuclei and showed mild degree of degeneration.

Adrenals collected on the 35th day showed progressive changes. Medullary cells showed various degrees of degenerative and necrotic changes. Cortical cells showed vacuolations and condensation of nucleus. The arteries showed various degrees of degenerative changes including thickening and hyaline changes. The ganglion cells showed vacuolation and pyknosis of the nuclei (Fig.6). Hyperchromasia of the cells was also evident.

On day 42, multiple vacuoles were found in the cytoplasm of the cells in the cortical and medullary areas (Fig.7) The vacuoles varied in size. Cortical cell clusters showed various degrees of degenerative changes such as vacoulation and swelling of nucleus. Most of the cortical cells lost their intense

staining properties and only an outline with remnants of condensed chromatin could be seen.

On day 45, the lesions were a progression of what was observed on day 42.

Group II

Sections of adrenals collected on day 21 from Group II birds showed hypercellularity. Moderate degree of hyperplasia of the medullary cells with scanty cytoplasm was noticed. Cortical cells also showed hyperplastic changes with tendency to form dense aggregates (Fig.8). The cortical cells in these aggregates were smaller in size with scanty cytoplasm. The nucleus of few cortical cells had become more prominent and hyperchromatic. On the 28th day, the cells at the periphery formed large spherical clusters (Fig.9). These clusters had both cortical and medullary cells in varying proportion. In some of these clusters, the cells were swollen and their cytoplasmic boundaries were indistinct. Nucleus was hyperchromatic showing a tendency to loose their vesicular structure and some of them showed condensation of chromatin appearing as darkly stained particles in the nucleus. The aggregates of cortical cells progressively increased in number and size, with individual cortical cells becoming shorter, hyperchromatic with scant cytoplasm. On day 35, adrenals showed tendency for dilatation and cyst formation in the peripheral cell clusters (Fig.10). The medullary cells showed increased granularity of cytoplasm. Severe degree of hyperplasia in the cortical cell aggregates was the most

striking feature. The cells in the aggregates showed hyperchromatic nucleus and a narrow rim of cytoplasm

On day 42, there were extensive areas of rapidly multiplying cortical cell aggregates (Fig. 11). The aggregates now had spread diffusely and no longer had the spherical appearance. Cysts formed in the peripheral area of the adrenal were larger as compared to those of 35th day (Fig.12). Adrenals, on the 45th day showed progressive lesions as it was observed on day 42. Wood's staining and haematoxylin and eosin staining after Wood's fixation showed predominantly nor epinephrine producing cells (Fig.13). Van Gieson's Fast green staining method (Fig.14) and Phosphotungstic acid haematoxylin (PTAH) staining (Fig. 15) gave a clear demarcation between adrenal cortical and medullary cells. The adrenal from the control group showed almost equal proportion of cortical and medullary cells which did not show any significant pathological changes.

4.8.2 Bursa of Fabricius

Group I

Sections of bursa collected on 21st day showed depletion of lymphoid cells especially from the medullary areas. The lymphoid cells showed variation in the size and shape of nucleus and cytoplasm. Marked reduction in the number of plasma cells and significant increase in cells with pyknotic nuclei suggestive of early necrotic changes were evident. Moderate degree of hyperplasia of interfollicular ciliated columnar epithelial cells and goblet cells

was also observed. On the 28th day, there was depletion of lymphoid cells in the lymphoid follicles, which were reduced in size when compared to the control group. Due to reduction in size of lymphoid follicles, the plical epithelium appeared corrugated (Fig.16). Rupture of the mucosal goblet cells into the lumen was more frequent and prominent. Fibroplasia was pronounced in the inter follicular connective tissue. Individual follicles revealed a washed out appearance with very less lymphoblastic activity and increased number of degenerating and necrotic lymphocytes in the medulla. On day 35, bursa of some birds of Group I showed necrotic changes in the medullary areas which showed a tendency to become hyalinised. The cortical area appeared greatly reduced in size and was pushed to the periphery. The follicles were comparatively smaller in size. The epithelial mucosa showed cysts of varying shape and size. Some of the cysts contained a homogenous pink staining fluid lined by a thin epithelial layer (Fig.17).

On the 42nd day, mucosal foldings and cysts were more prominent. A few cysts had ruptured resulting in erosions of the mucosa. Hyalinisation of follicular mass which varied in intensity starting from hyalinisation of the medullary region to hyalinisation of the entire follicles resulting in formation of cysts were also observed (Fig. 18).

On the 45th day, the lesions were progressive with depletion of lymphoid cells and stromal proliferation. The reticular framework became more prominent along with mucosal folds and erosion into the lumen.

Group II

On the 21st day, sections revealed mild degree of inter follicular oedema. The individual follicles appeared larger in size. The lymphoid cells were loosely arranged and did not show any evidence of degenerative changes. Significant increase in the lymphoblasts and lymphocytes with nucleoli or prominent euchromatin, indicating active lymphoblastic activity was also noticed. The epithelial cells of the mucosal lining appeared to be intact with moderate number of goblet cells.

On the 28th day, the cortex appeared to be increased in thickness which was packed with lymphocytes, while the medullary area appeared to be smaller with few lymphocytes. The lymphocytes were in various stages of degeneration and were loosely arranged. The dilatation of the inter follicular space was more pronounced (Fig.19). Congestion and haemorrhage in inter follicular area was also noticed. On day 35, the changes were progressive in nature and mild degree of stromal hyperplasia was also evident.

On day 42, the cortex and medulla showed a loose arrangement of lymphocytes suggestive of intra follicular oedema. Necrotic changes were noticed in the medulla (Fig. 20). Diffused inter follicular fibrosis with mild cellular infiltration mainly of mononuclears and some heterophils was evident (Fig.21). The lesions on the 45th day were more severe than on the 42nd day.

The bursa from the control group did not show any significant pathological changes on any of the days.

4.8.3 Thymus

Group I

On day 21, the lesions include depletion of the lymphocytes revealing prominent connective tissue and reticular framework. Degenerating and necrotic lymphocytes increased in proportion by day 28. Tendency for formation of Hassalls corpuscles like bodies could be seen (Fig.22). The medullary region revealed many vacuoles spherical in shape, limited by a bounding membrane. Some of them had homogenous eosinophilic substance inside (Fig. 23).

The lobules of the thymus became progressively smaller in size with prominent septae on the 35th day (Fig.24). Thymus appeared to have a washed out appearance with a few thymocytes arranged loosely amidst degenerating and necrotic lymphocytes.

Group II

Thymus of Group II were comparable to the control on day 21. However, as the days progressed, thymus started showing depletion of lymphoid cells in the cortex (Fig.25). The reticular cells in the background started to become more prominent (Fig.26). However these lesions were less severe than Group I.

The thymus from the control group did not show any significant pathological changes except for mild degree of congestion.

4.8.4 Spleen

Group I

Moderate degree of vacuolar degenerative changes were noticed in the lymphoid cells. The lymphoid cells were sparsely distributed (Fig.27). The sinusoids were engorged. Lymphoid collection in the periarteriolar sheath became progressively reduced as the treatment prolonged. There was diffuse lymphoid depletion in the cortical and paracortical areas along with degeneration and necrosis of the lymphocytes. The walls of the arteries were sclerosed.

Group II

Spleen of Group II birds were comparable with the control during the 21st and 28th day except for mild degree of lymphoid depletion and thickening of the blood vessels (Fig.28). As the treatment prolonged, the vessels became increasingly thicker with reticular and connective tissue framework. There was slight degree of swelling of the vascular endothelial cells. Lymphoid cells were seen loosely distributed in the parenchyma. Mild to moderate degree of lymphoid depletion was also observed (Fig.29).

The spleen from the control group did not reveal any significant pathological changes.

4.8.5 Liver

Group I

Birds of Group I revealed extensive damage to the liver. Moderate degree of vacuolar degeneration was observed in the hepatocytes. These vacuoles stained negatively for fat. Isolated cells with pyknotic nuclei was seen distributed in the parenchyma. Moderate degree of kupffer cell reaction along with congestion of portal and central vessels were also seen. The lesions were more pronounced as the treatment prolonged. The cord like arrangement of the hepatocytes were disrupted at many places and showed a ductular or acinar pattern.

Group II

Liver of Group II birds did not show any significant pathological changes, when compared to the control on 21st and 28th day. As the treatment prolonged, mild to moderate vacuolar degenerative changes, congestion of the vessels, sinusoidal dilatation, hepatocytomegaly along with focal areas of regeneration of the hepatocytes were also noticed.

Liver form birds of group III did not reveal any significant pathological changes.

4.8.6 Kidney

Group I

Moderate degree of vacuolar degenerative and necrotic changes in the tubular epithelial cells were noticed. Tubules showing regenerating epithelium were seen in clusters amidst the necrotic tubules. Dilatation of the Bowman's space along with mesangial cell proliferation were the salient features seen in the kidneys.

Group II

The lesion seen in kidney of Group II were mild in comparison with Group I. Mild to moderate degree of congestion, along with moderate degree of vacuolar degenerative changes in the tubular epithelial cells were the prominent lesions observed. The intensity of lesions in the kidney showed a tendency to increase as the duration of treatment prolonged.

4.9 Stress Scores

Higher stress scores were recorded for the following parameters viz., body weight (Table 3), adrenal weight (Table 16), bursa weight (Table 19), thymus weight (Table 22), spleen weight (Table 25), liver weight (Table 28), differential leukocyte count (Table 10) as the duration of treatment increased. Stress scores were particularly high in Group I for the lymphoid organs like bursa, thymus and spleen. Some parameters like pancreas weight and weight of thyroid did not record any stress scores.

Fig.1 Adrenals from birds of Group I, II and III – 28th day

Fig.2 Bursa from birds of Group I, II and III – 28th day

Fig.3 Thymus from birds of Group I, II and III – 28th day

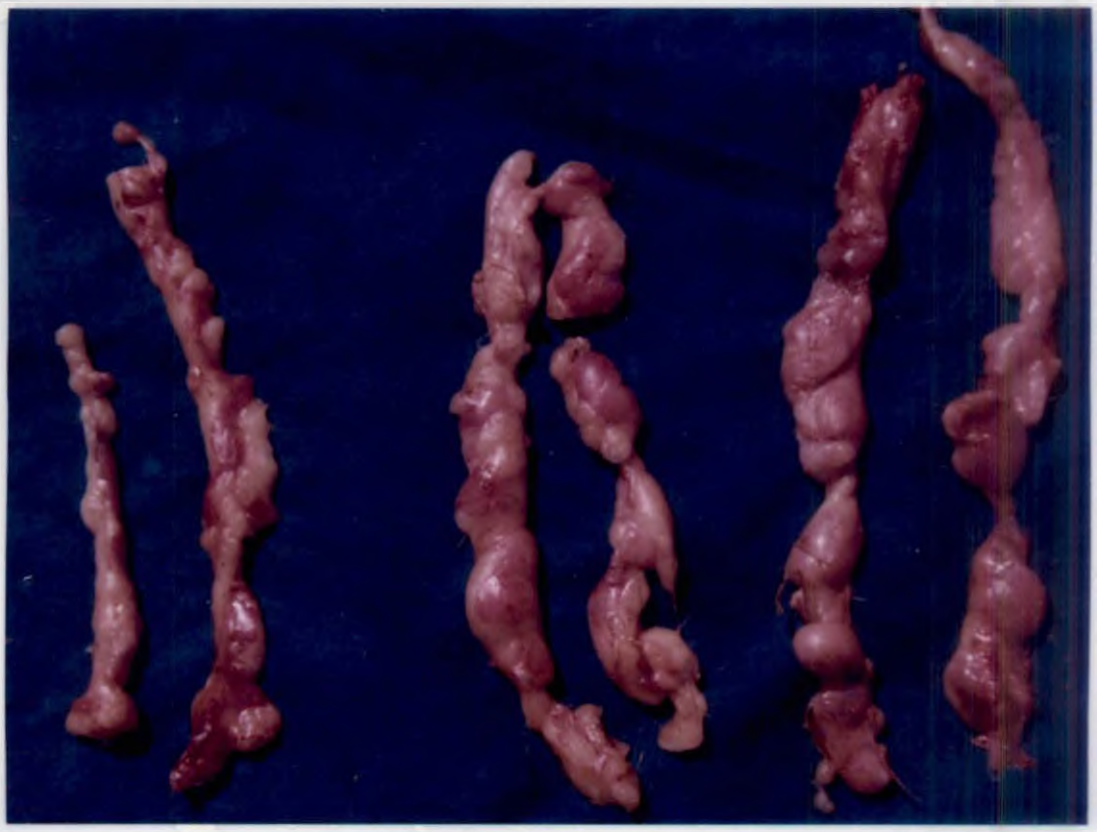
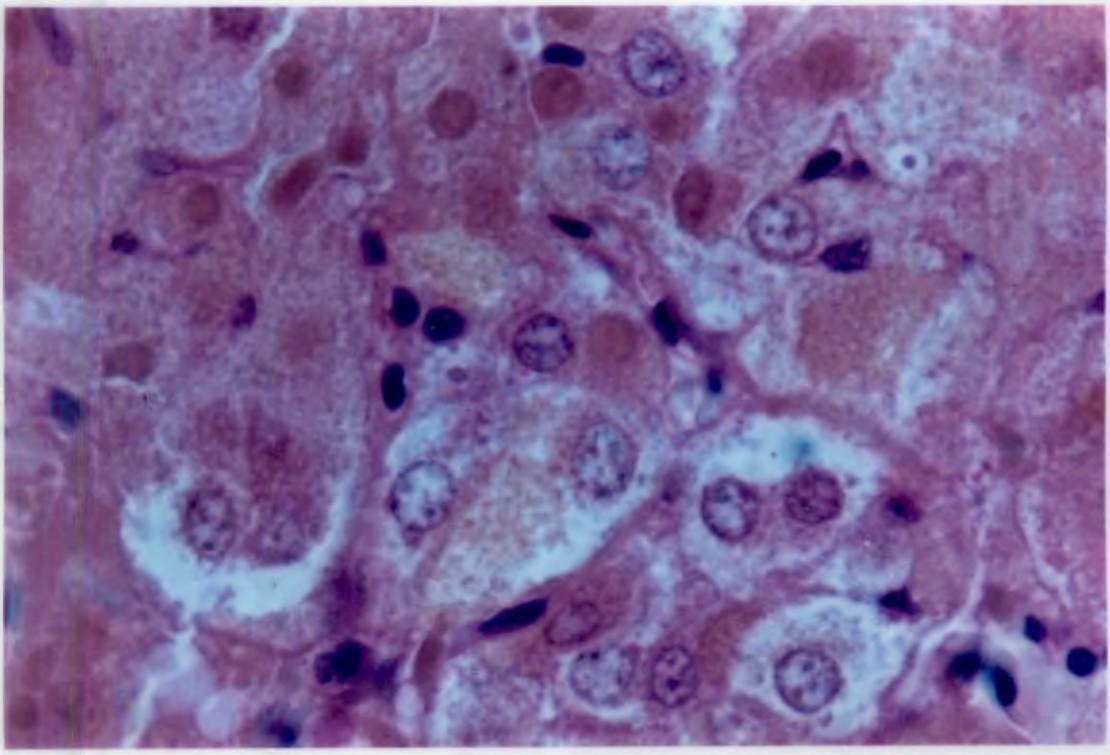


Fig.4 Adrenal: Increased proportion of brownish stained epinephrine producing cells. Yellowish stained norepinephrine cells – Group I : 21st day Woods stain 1000X

Fig.5 Adrenal: Cytoplasmic vacuolar changes of the medullary cells – Group I: 28th day H&E 400X



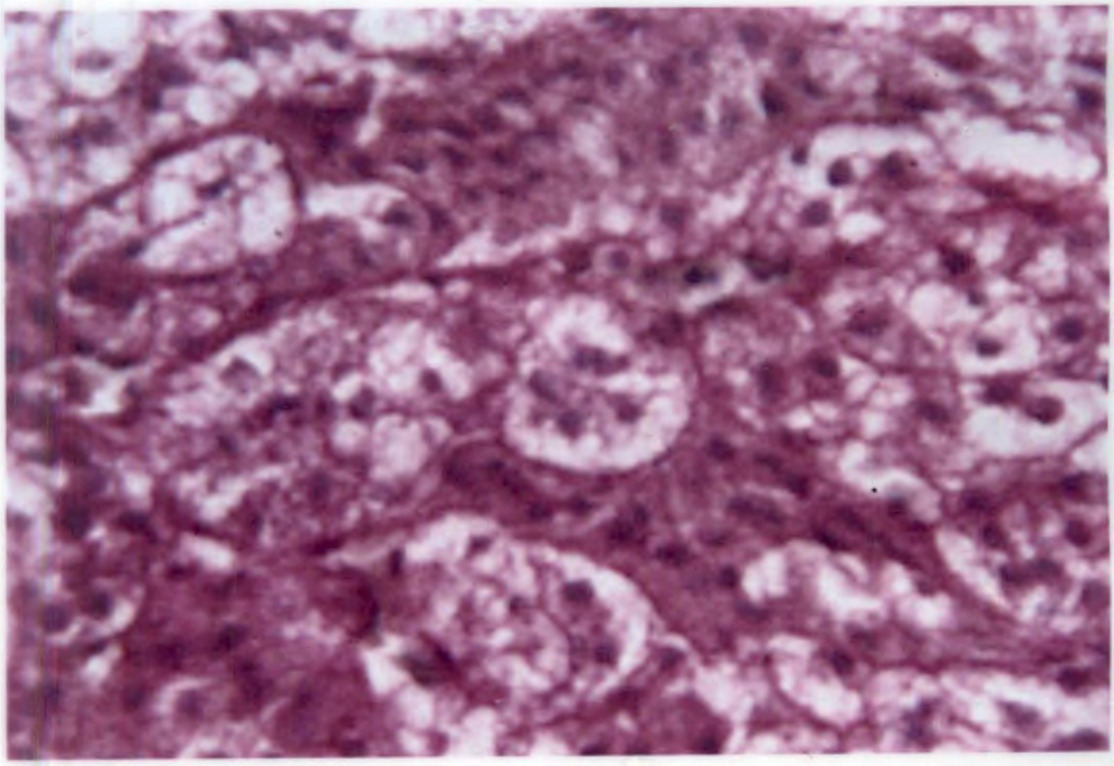
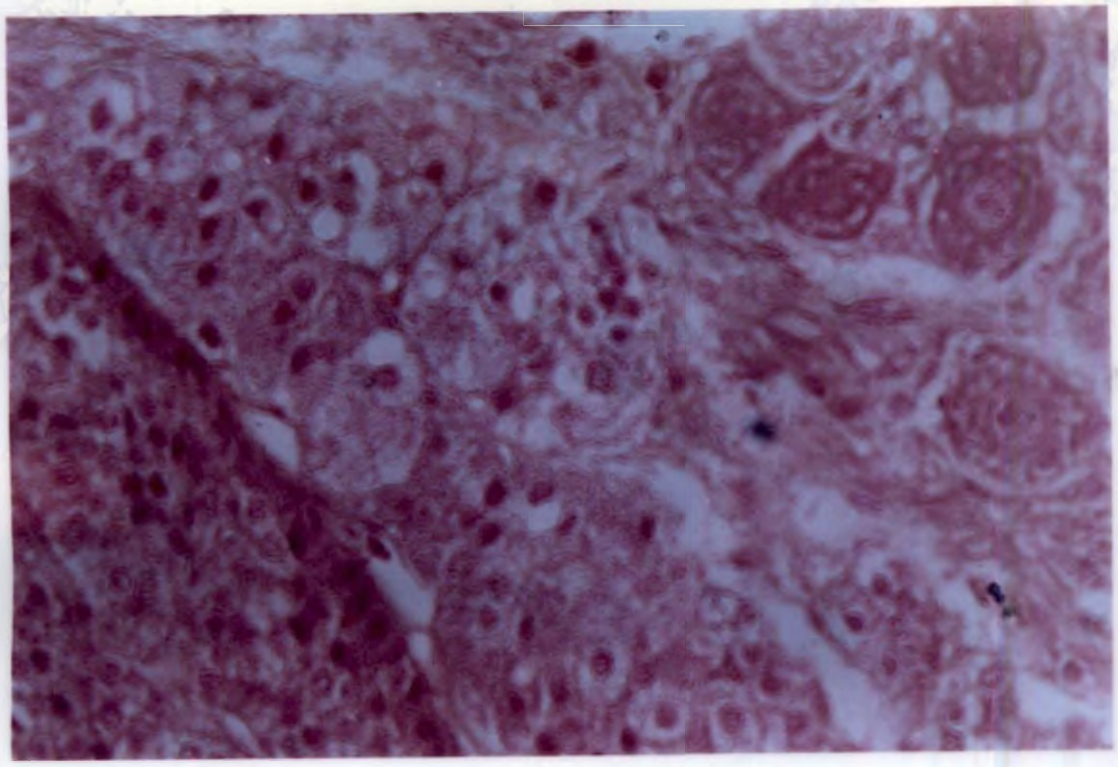


Fig.6 Adrenal: Vacuolation of the ganglion cells – Group I : 35th day H&E 400X

Fig.7 Adrenal: Vacuolation of the cortical and medullary cells – Group I : 42nd day H&E 400X



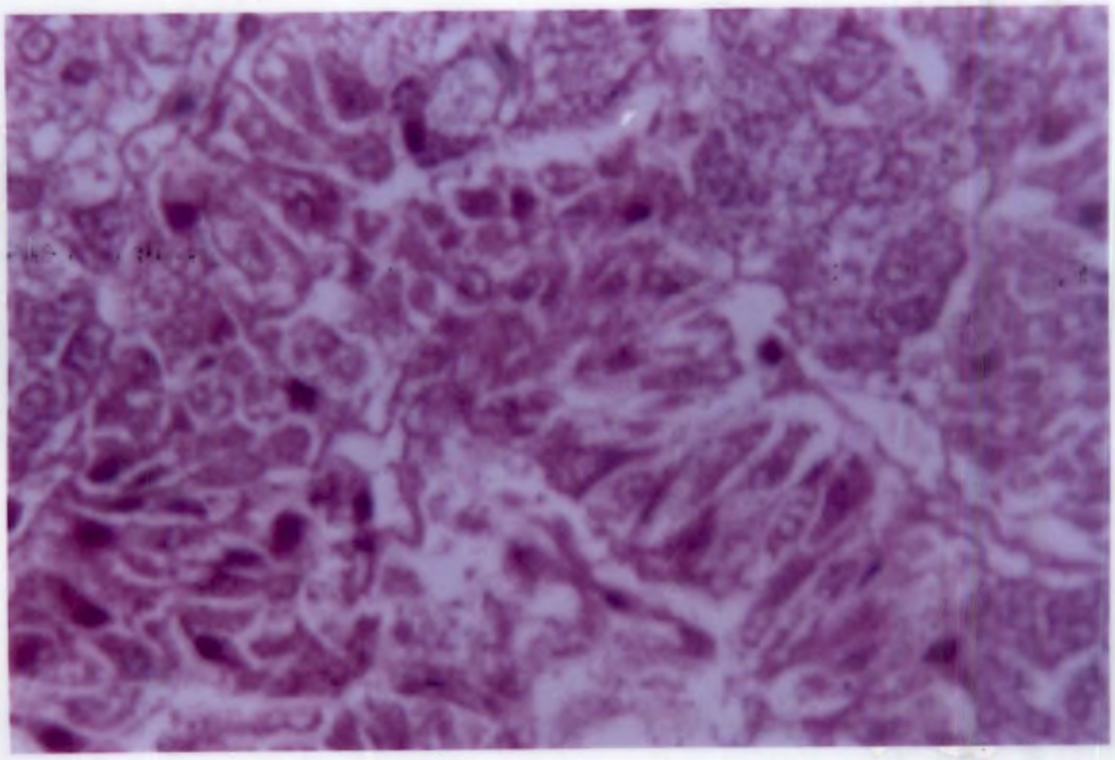
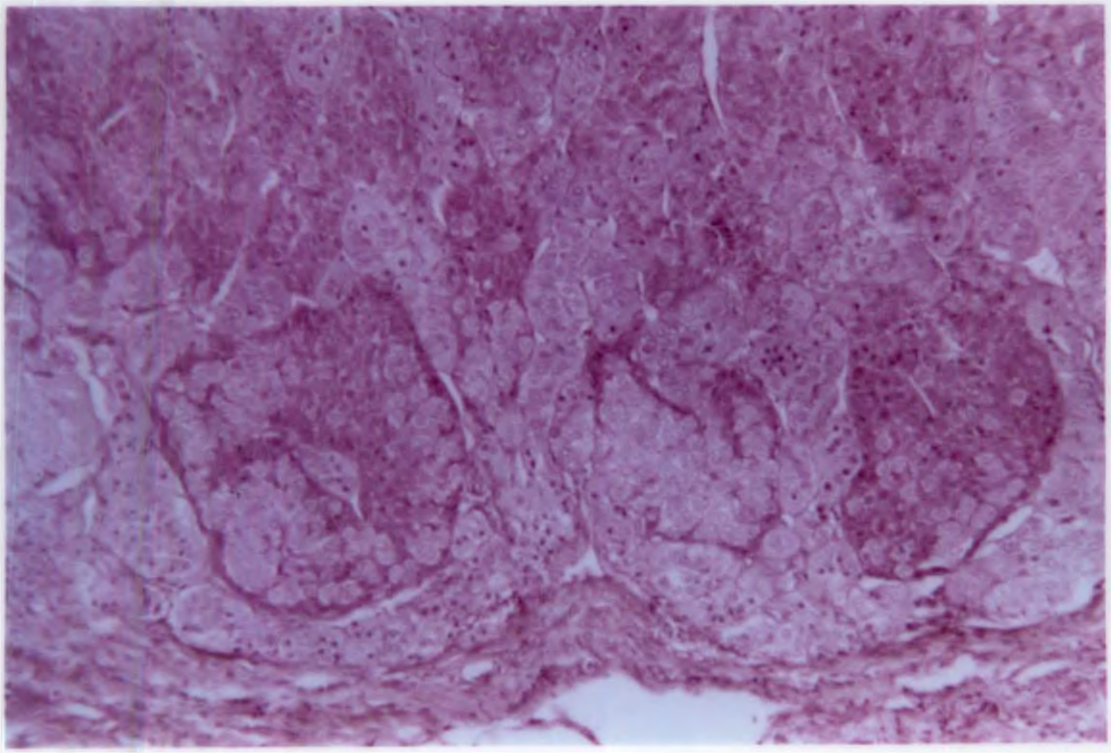


Fig.8 Adrenal: Hyperplastic changes of the cortical cells with tendency to form aggregates – Group II : 21st day Wood's fixation, H&E 250X

Fig.9 Adrenal: Spherical clusters comprising of cortical and medullary cells – Group II : 28th day H&E 250X



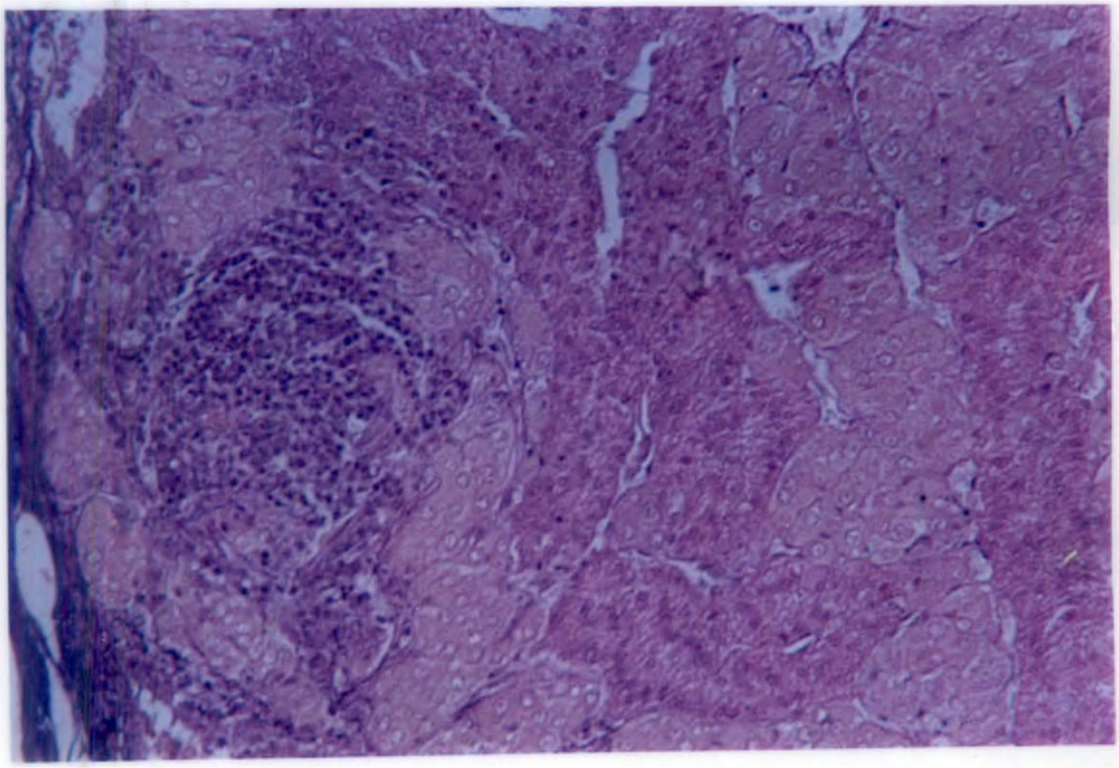
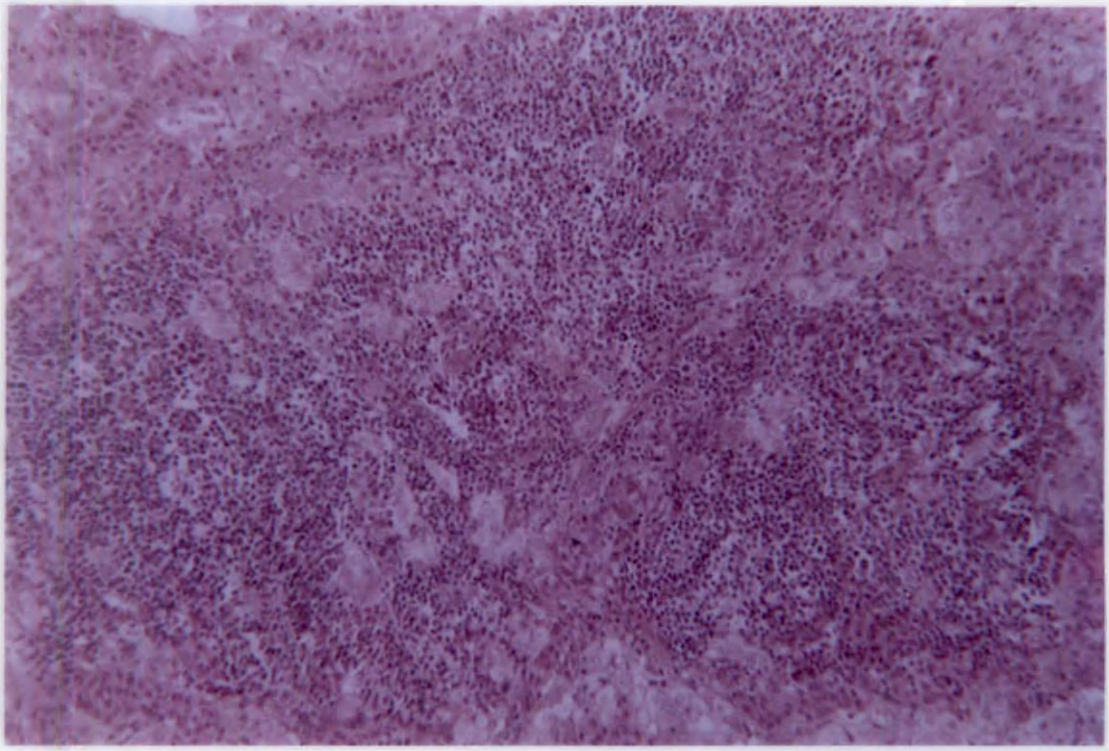


Fig.10 Adrenal: Cyst formation in the peripheral cell clusters -- Group II: 35th day H&E 250X

Fig.11 Adrenal: Rapidly multiplying cortical cells – Group II : 42nd day H&E 160X



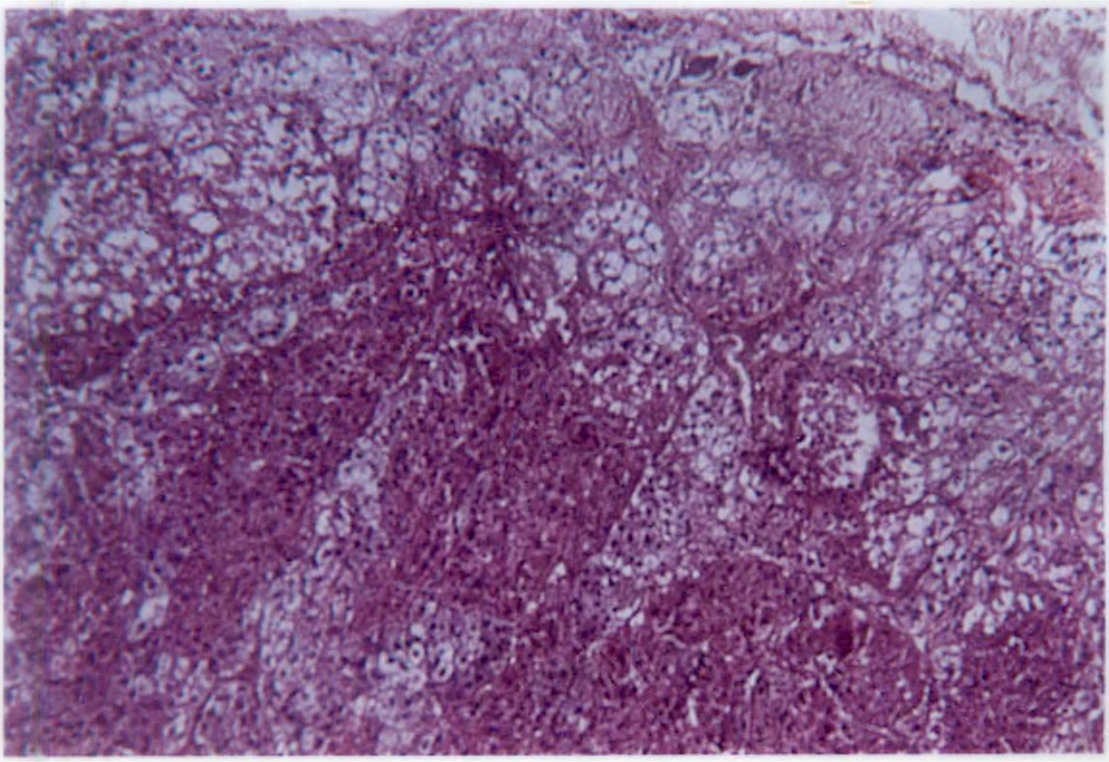
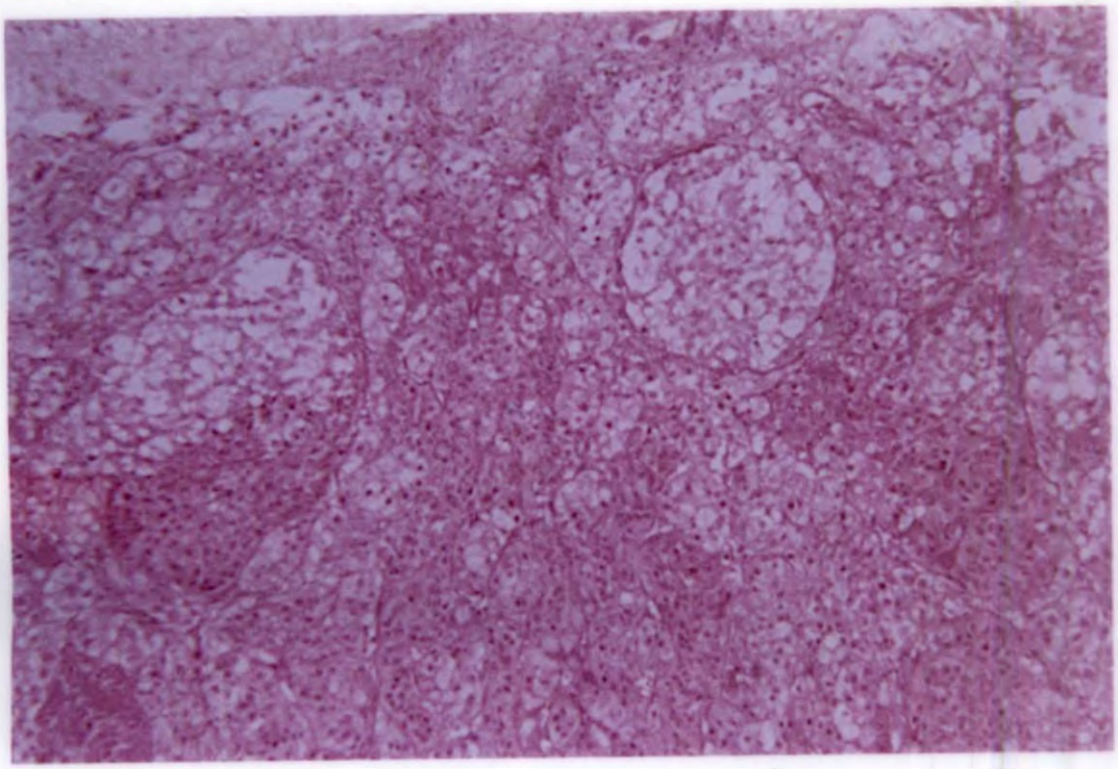


Fig.12 Adrenal: Prominent cyst formation in the peripheral cell clusters– Group II : 42nd day H&E 400X

Fig.13 Adrenal: Predominantly yellowish stained norepinephrine cells – Group II : 42nd day Wood's stain 1000X



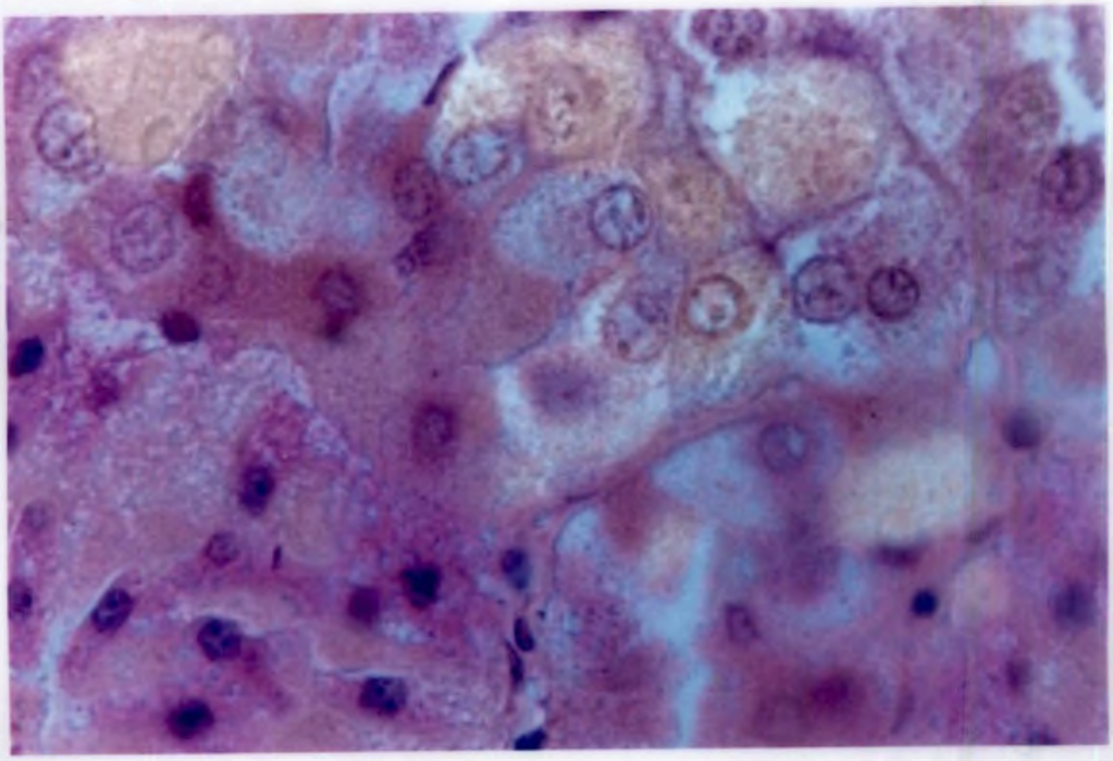
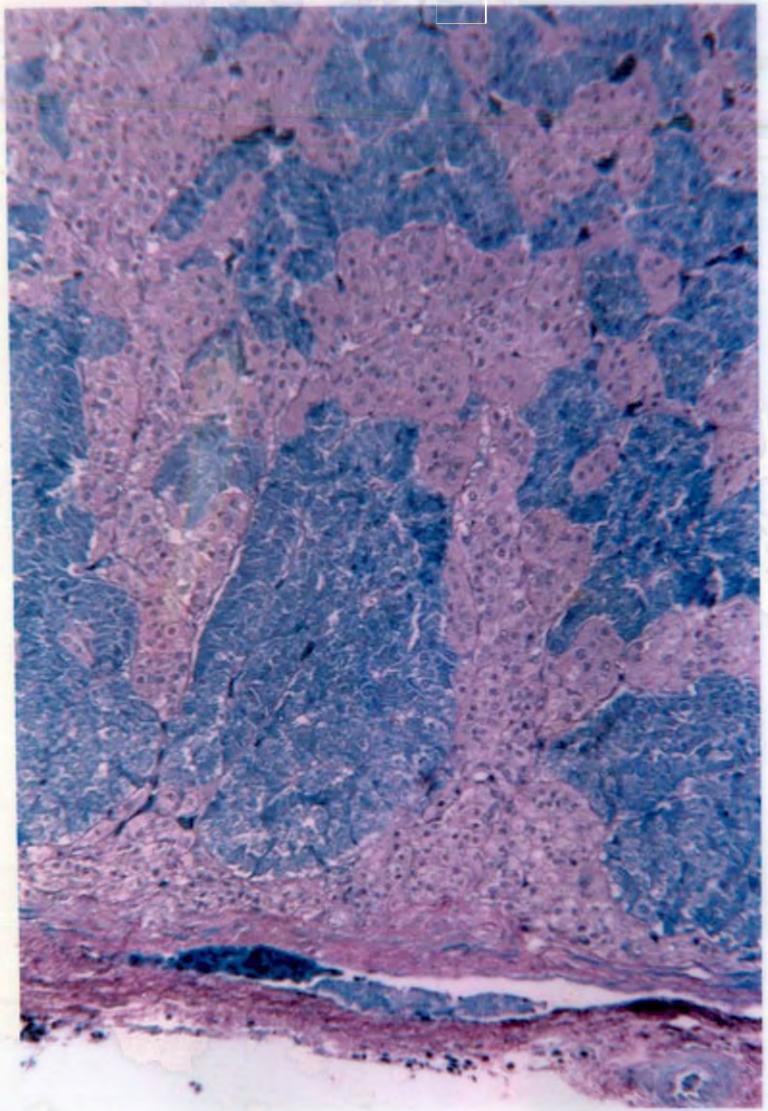


Fig.14 Adrenal: Dark green cortical cells and lighter medullary cells – Group III : 42nd day Van Gieson's fast green 250X

Fig.15 Adrenal: Lightly stained medullary cells at darker cortical cells – Group III : 42nd day Phosphotungstic acid haematoxylin (PTAH) 250X



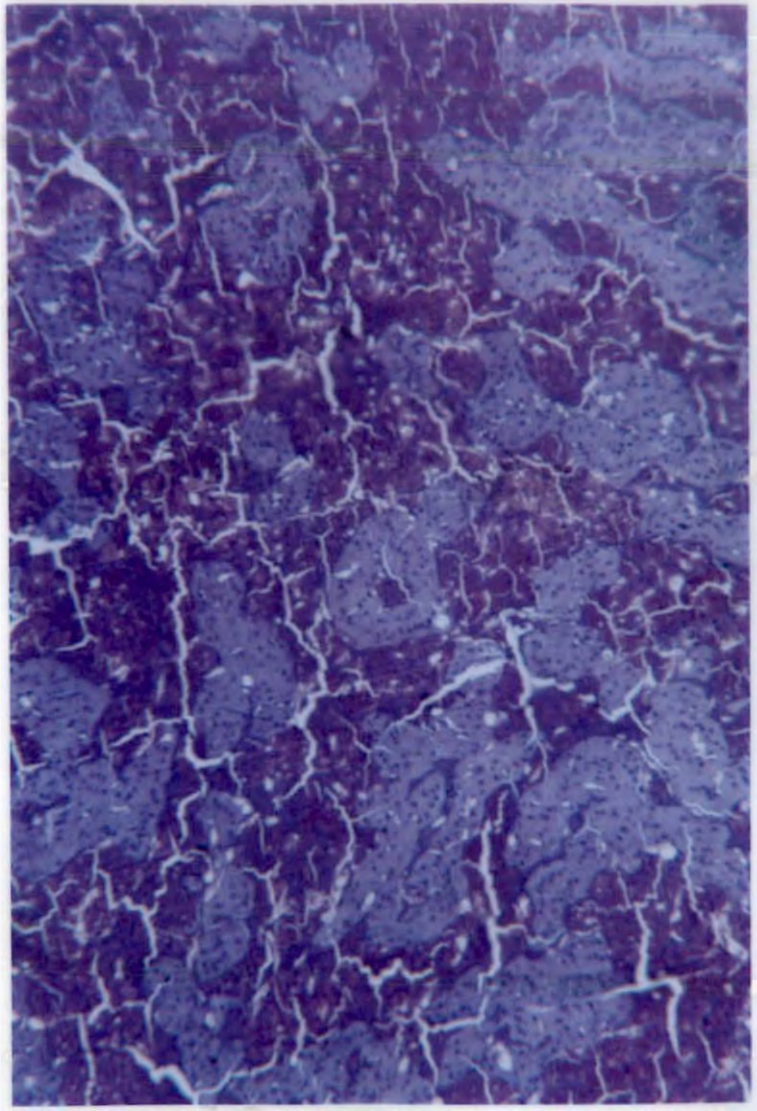
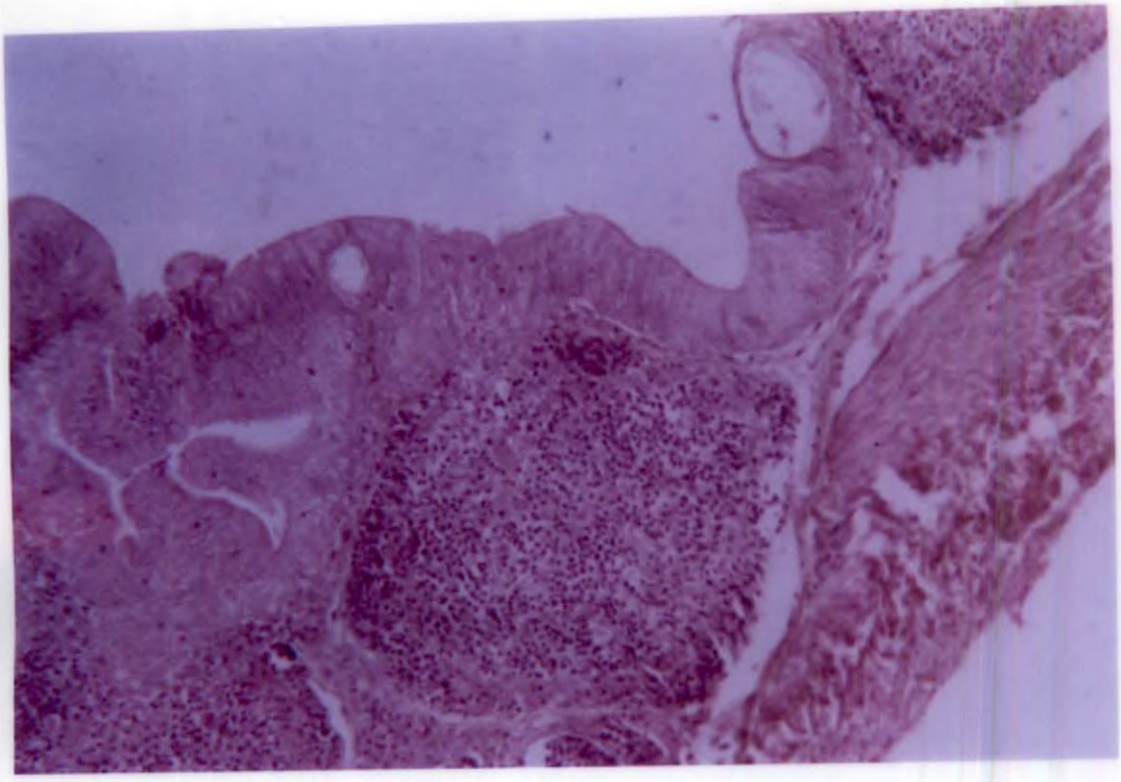


Fig.16 Bursa : Epithelial hyperplasia, cyst formation – Group I: 28th day H&E 250X

Fig.17 Bursa: Subepithelial cyst – Group I : 35th day H&E 400X



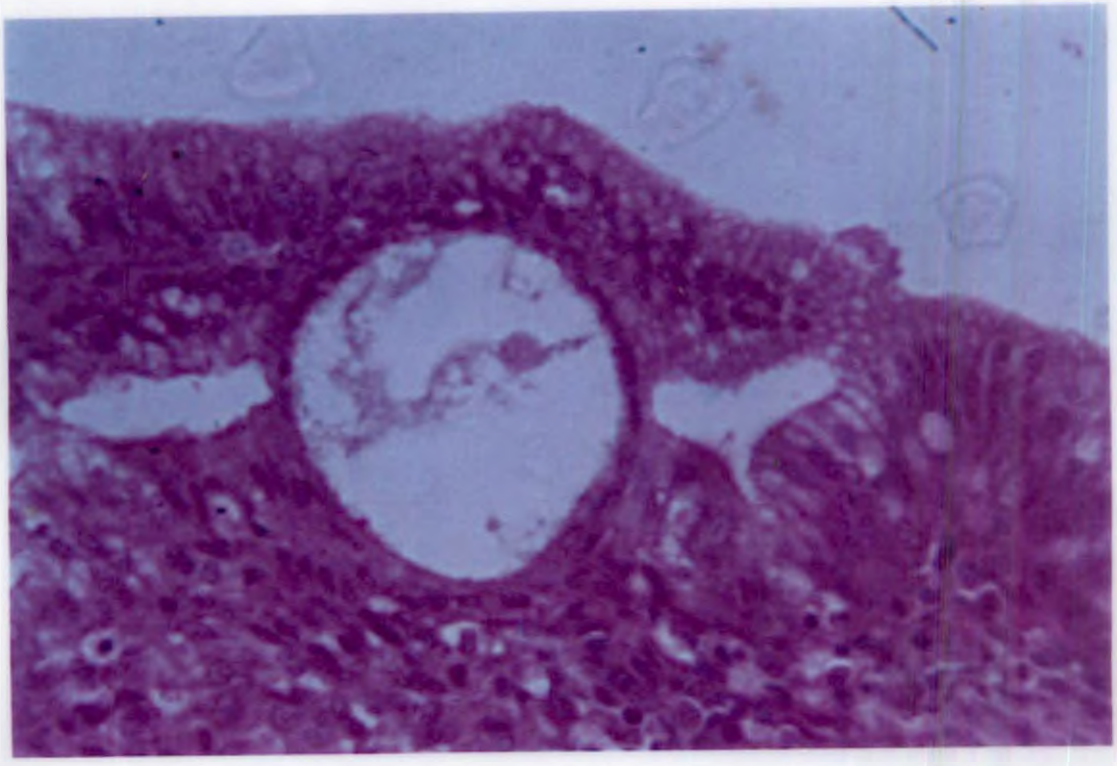
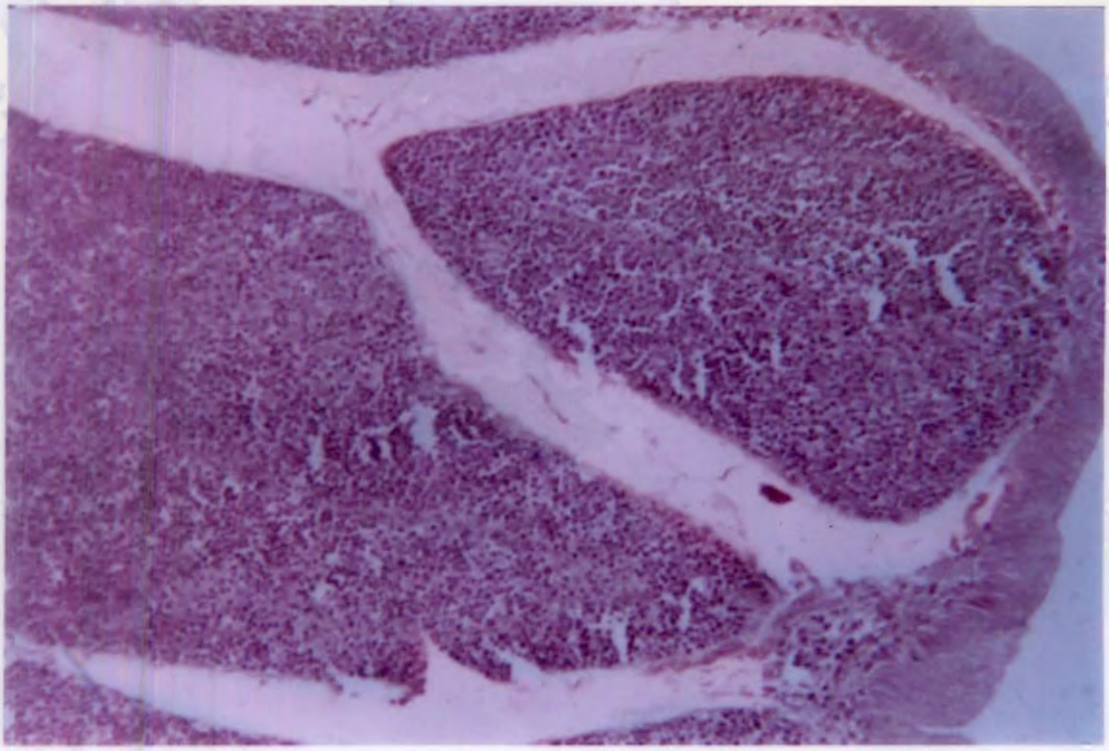


Fig.18 Bursa: Formation of cyst in the follicle – Group I : 42nd day H&E 400X

Fig.19 Bursa: Dilatation of inter follicular space – Group II : 28th day H&E 250X



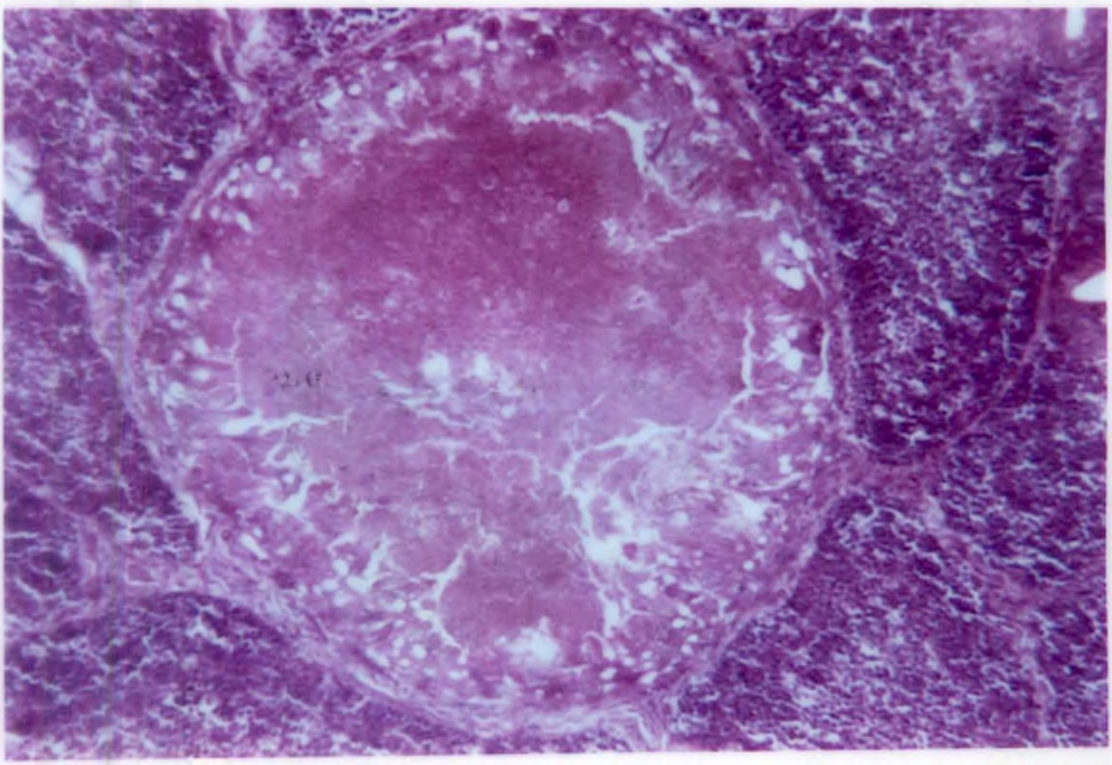
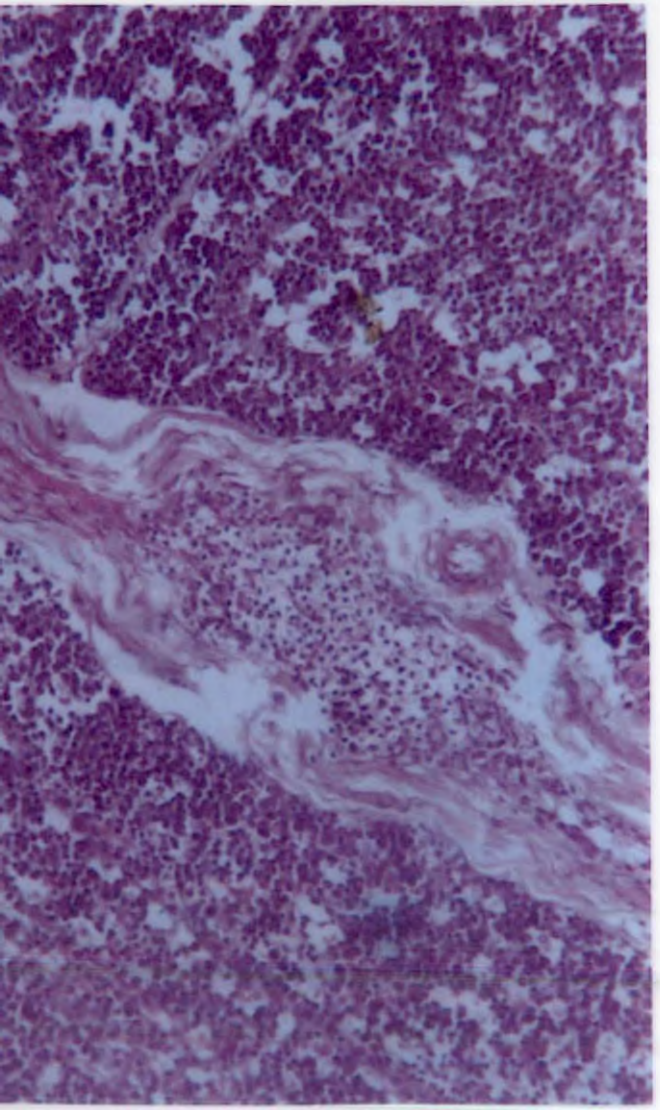


Fig.20 Bursa: Necrotic changes in medulla – Group II : 42nd day H&E 250X

Fig.21 Bursa: Inter follicular fibrosis and cellular infiltration – Group II : 42nd day H&E 400X



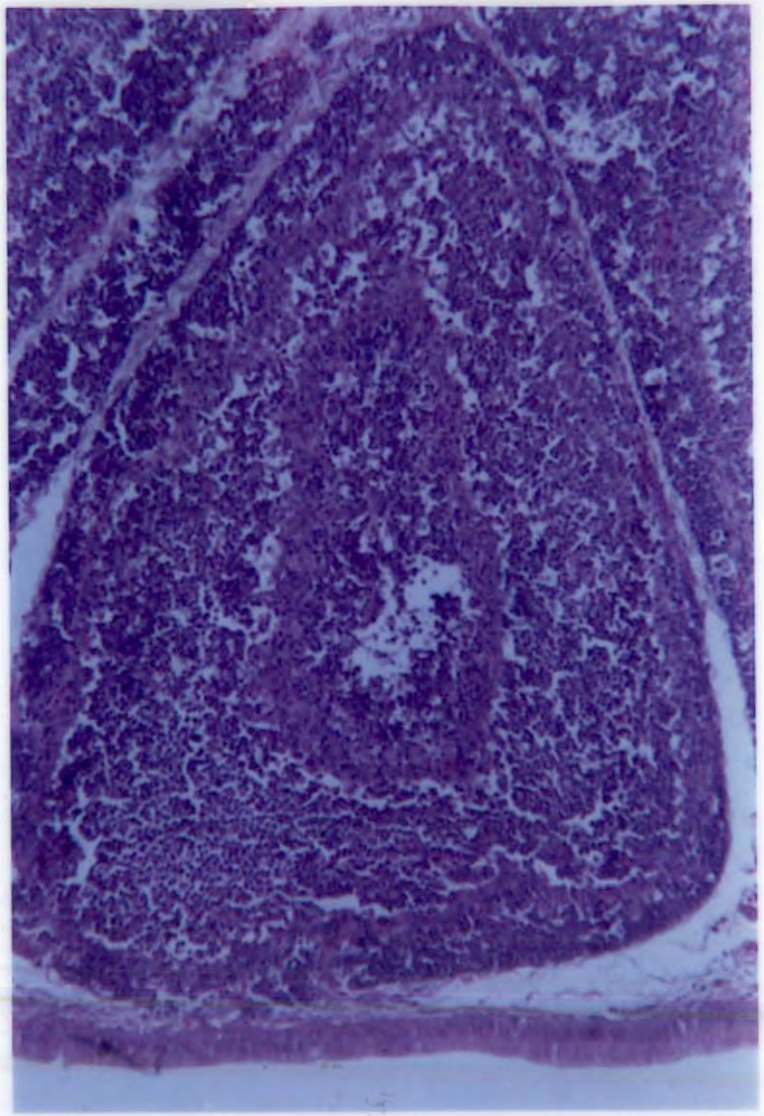
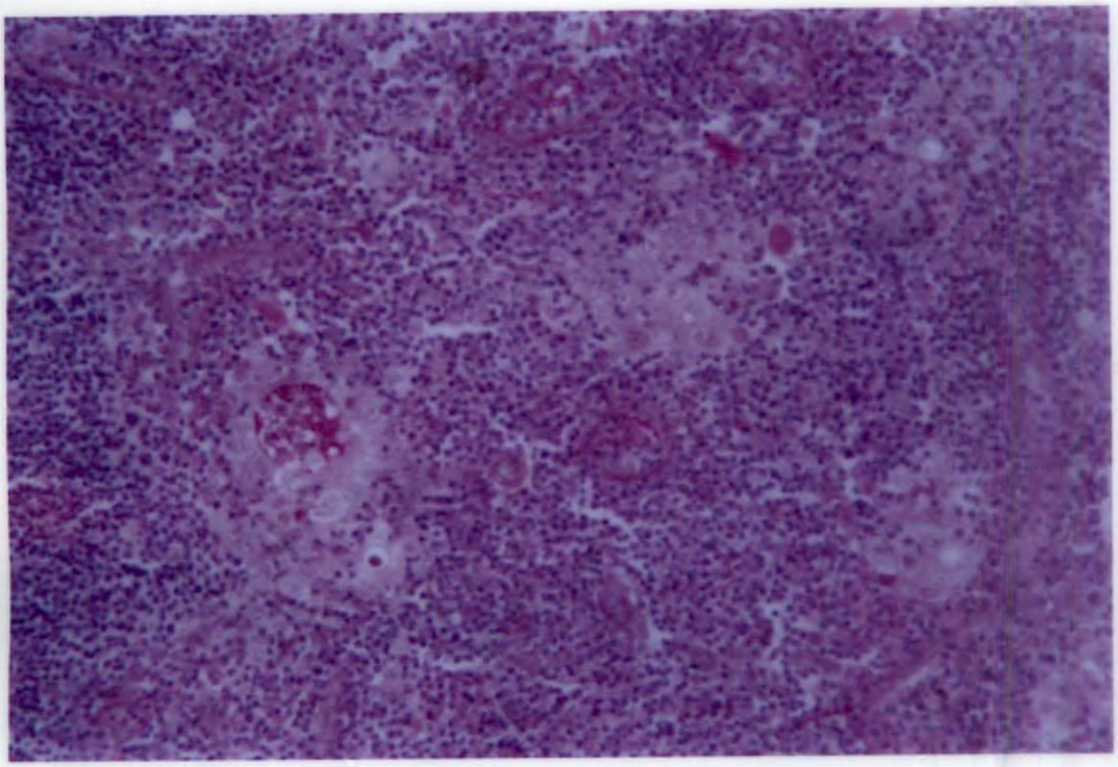


Fig.22 Thymus : Tendency for formation of Hassels corpuscles – Group I : 28th day H&E 250X

Fig.23 Thymus : Homogenous eosinophilic substance in the cells of the medullary region – Group I : 28th day H&E 400X



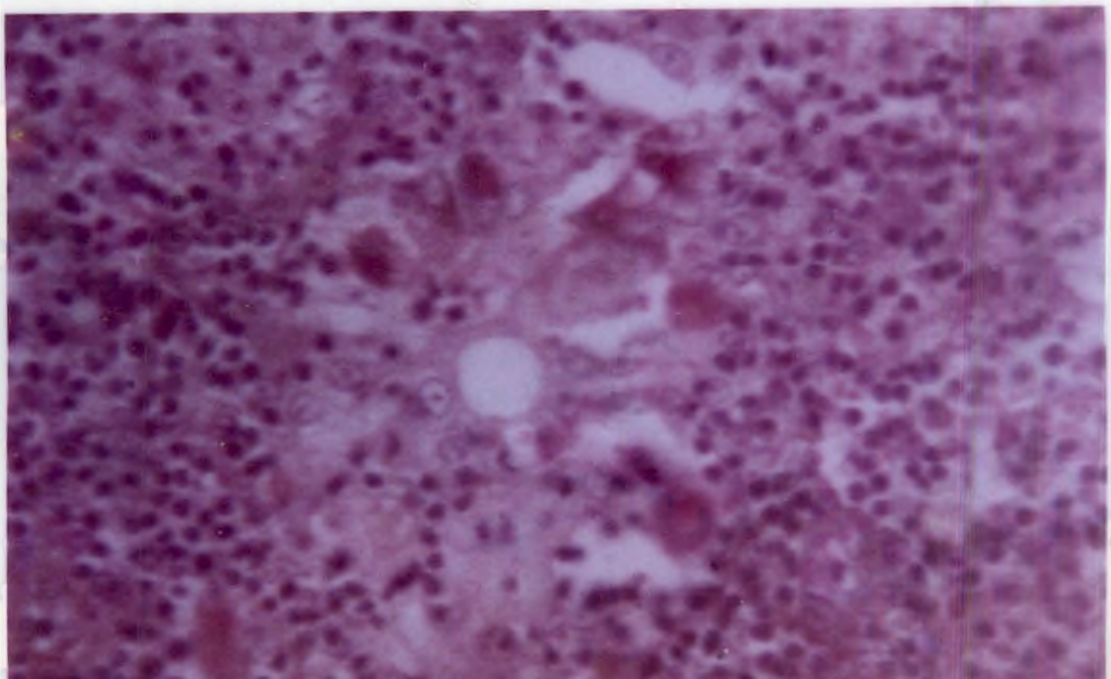
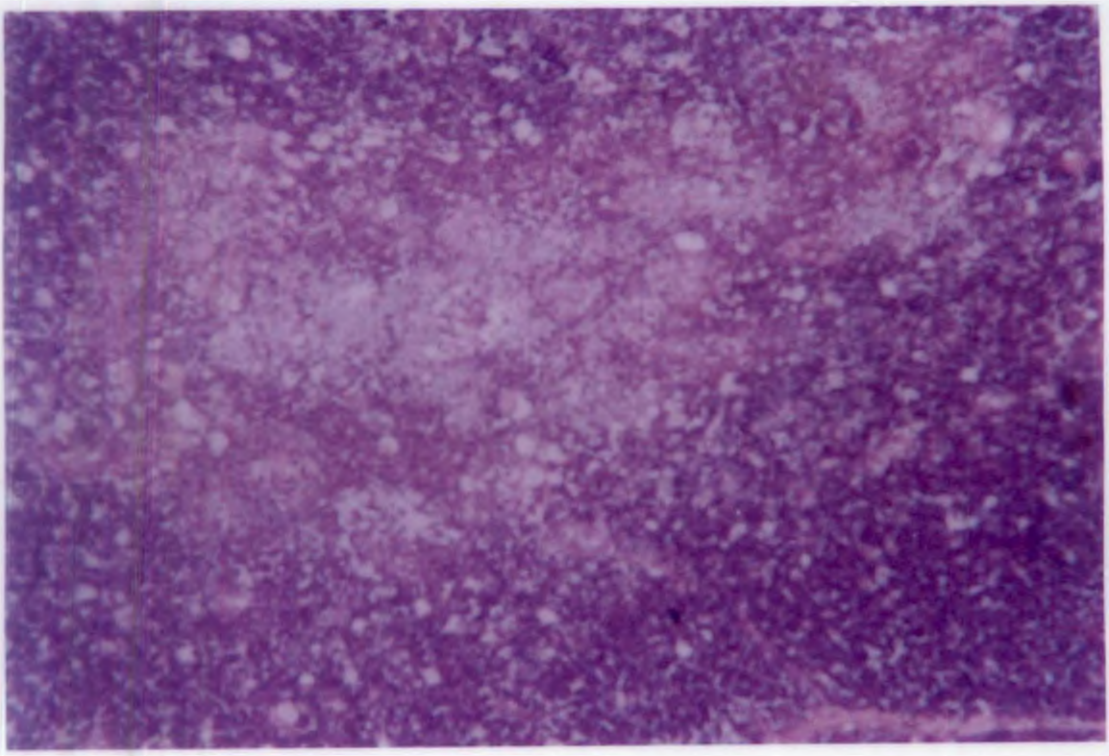


Fig.24 Thymus : Mild diffuse lymphoid depletion in the cortex – Group I : 35th day H&E 160X

Fig.25 Thymus : Moderate degree of lymphoid depletion in the cortex – Group II : 35th day H&E 160X



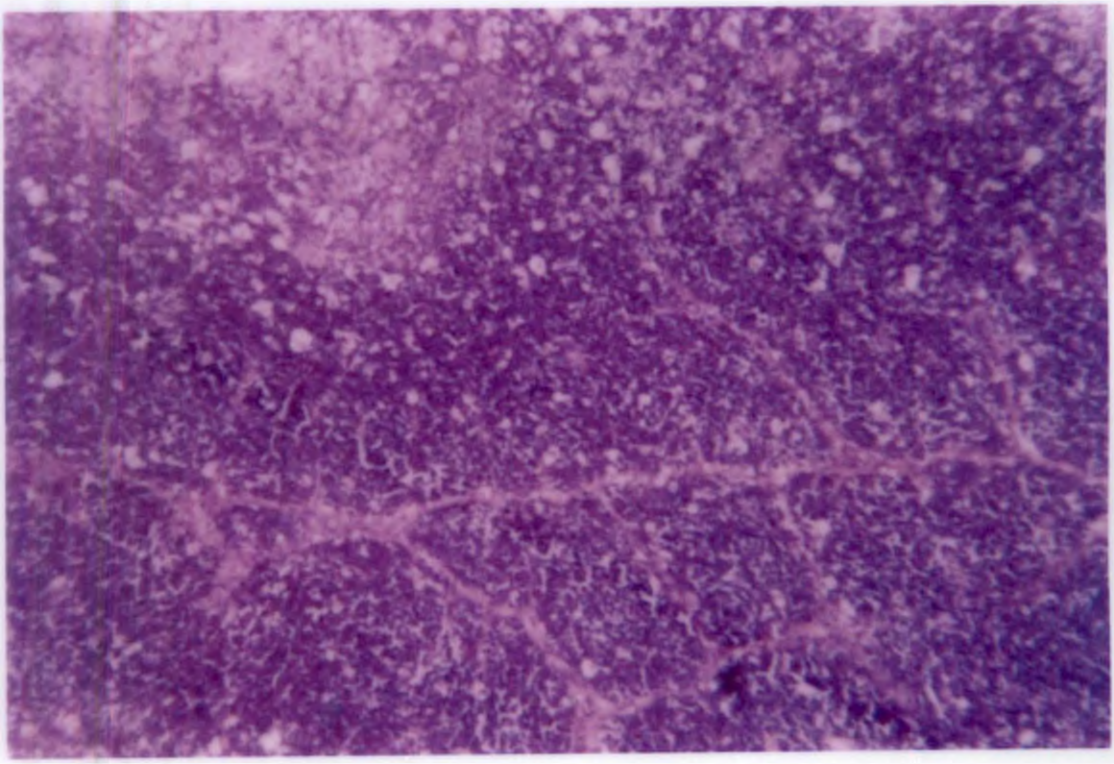
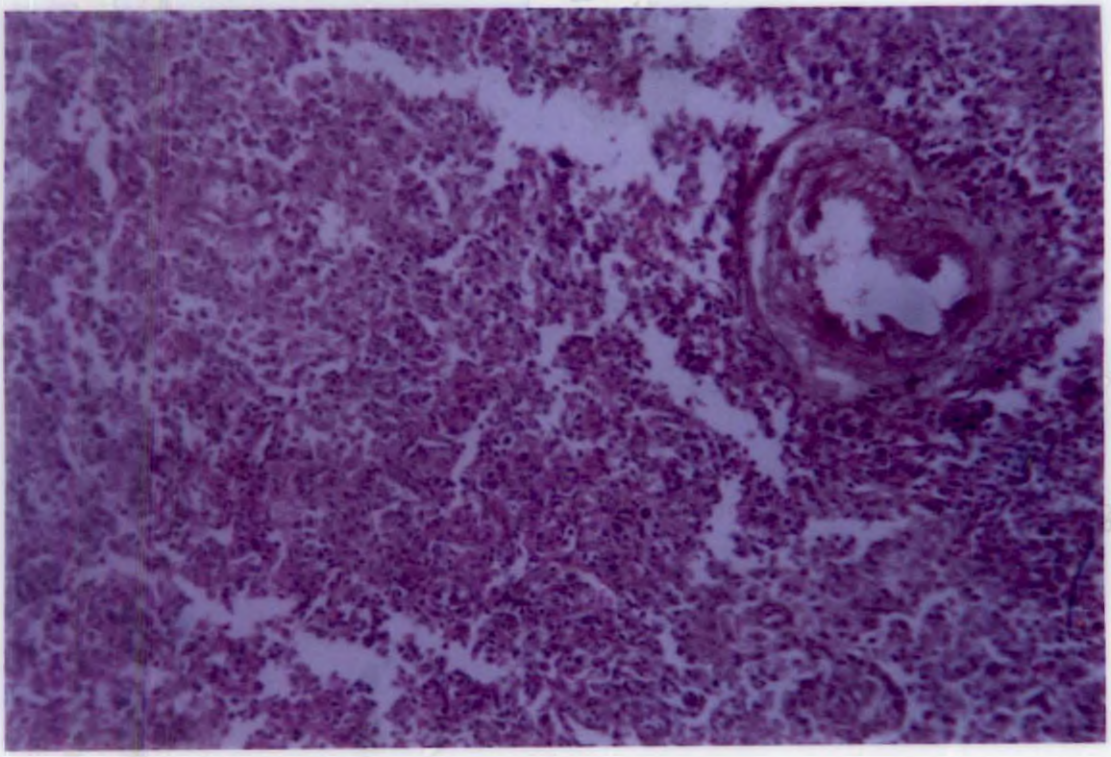


Fig.26 Thymus: Prominent reticular fibres, depletion of lymphocytes – Group II : 35th day H&E 250X

Fig.27 Spleen : Sparsely distributed lymphoid cells – Group I : 28th day H&E 400X



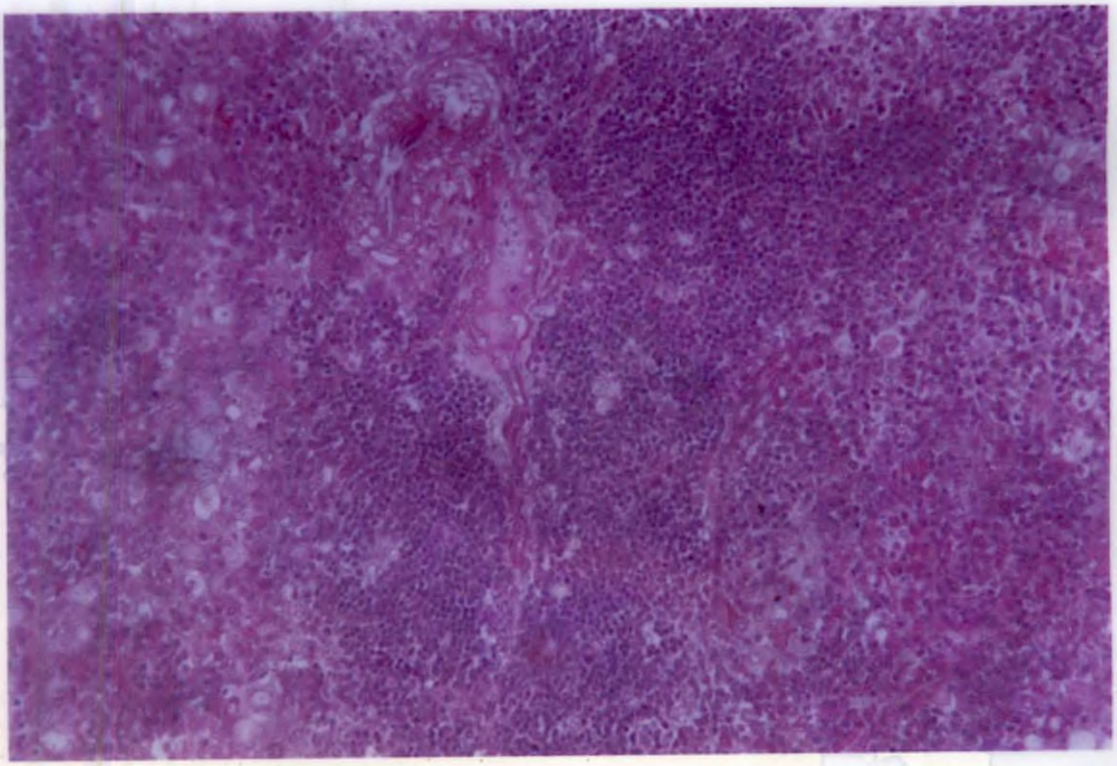
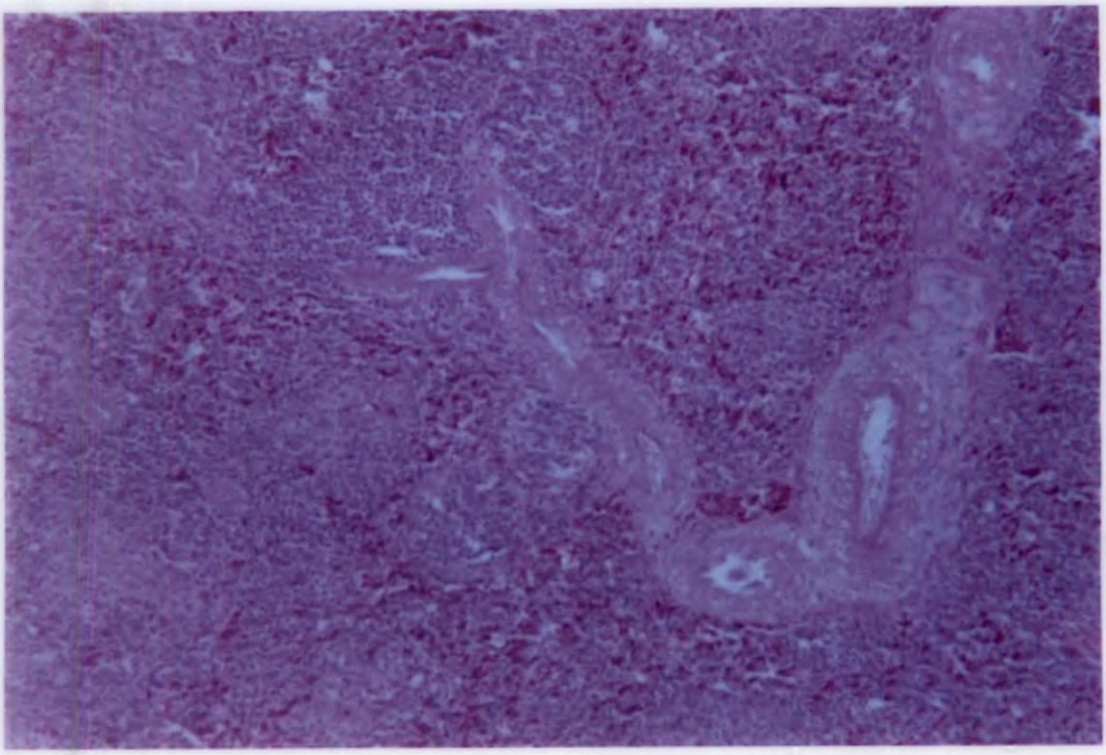
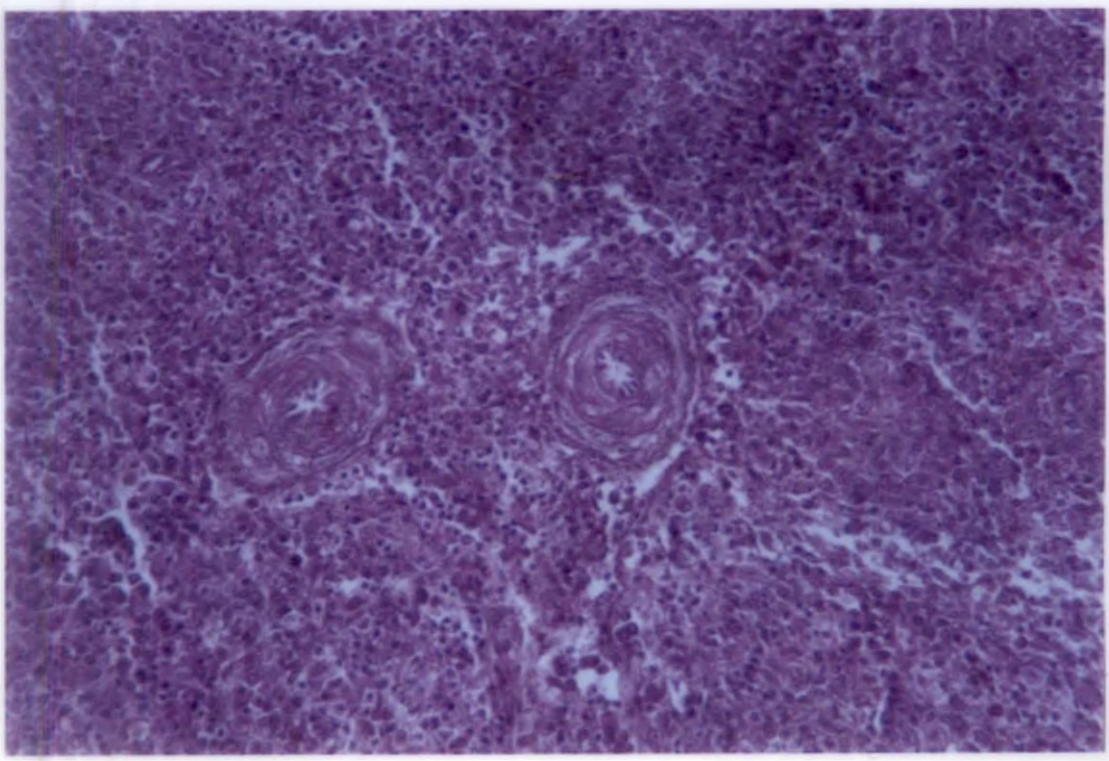


Fig.28 Spleen : Thickening of blood vessels – Group II : 28th day H&E 400X

Fig.29 Spleen : Thickening of blood vessels, moderate degree of lymphoid depletion – Group II : 42nd day H&E 160X





Discussion

5. DISCUSSION

The experiment was designed to study the pathology of induced stress in broiler chicken. The stressors were physical and chemical in nature, which consisted of space restriction and dexamethasone administration respectively. The stress response was evaluated based on the clinical as well as behavioural manifestation, production indices, haemogram, humoral as well as cell mediated immune responses along with the gross and microscopic changes of the various organs.

The birds in both Group I and II showed clinical and behavioural abnormalities of varying degrees. Dexamethasone administered group was lethargic and developed diarrhoea and tracheal rales. The pronounced symptoms observed on the 21st day was attributed to the high dose of dexamethasone (50 ppm). Hence the dose was reduced subsequently. The clinical symptoms noticed on the subsequent days could be consequent to the hepatic damage caused by corticosteroids (Rutgers, 1996; Leib, 1997) as well as its immunosuppressive effect (Adams, 1982).

Birds in the overcrowded group initially showed poor development of feathers. The slower rate of feather development could be due to the increased warmth, as they remained closer to each other. It was observed that, on the 28th day, when the floor space was increased to 0.5 ft^2/bird, birds reacted by showing increased aggression and hyper response to stimuli. This finding is

similar to the alarm reaction to stress as described by Selye (1946) and is in consonance with the observations of Mench *et al.* (1986) and Shea *et al.* (1990). However, as the days passed, birds became dull with ruffled feathers and showed lameness. Martrenchar *et al.* (1997) has also reported a higher incidence of lameness in birds when subjected to overcrowding. These clinical and behavioural symptoms shown by the birds stocked at higher densities could be related to the rapid caking of the litter, which produced increased ammonia in the environment.

Body weights of the birds in Groups I and II subjected to different stressors were lower than the control, except on the 21st day, during which the overcrowded group showed higher body weight than the control. Pesti and Howarth (1983) have also reported of such superior performance by three week old chicks reared under 0.25 ft^2/bird than those housed in different densities like 0.18 ft^2/bird, 0.38 ft^2/bird and 0.77 ft^2/bird. They attributed superior growth to higher feed efficiency as the birds were warmer. The observation of lower body weights for the Group II birds during the rest of the period as well as for the Group I birds was in accordance with the observations of Tomhave and Seegar (1945), Heishman *et al.* (1952), Martrencher (1997), Peterson and Siegel (1998), Puvadolpirod and Thaxton (2000a, b, c, d) and Sorenson *et al.* (2000). However, Siegel and Coles (1958) reported no significant difference in the body weight of broilers grown at floor space levels ranging from 0.5 to 1.25 ft^2/bird. This difference could be due to difference in genetic and environmental factors.

The difference in the effect of stressors on the body weight at different weeks could be due to the variation in either the duration of exposure to stressors or the age of the bird or a combined effect (Garren and Shaffner, 1956).

Feed efficiency in Groups I and II were lower than the control group during all the periods except on the 21st day for Group II, where the efficiency was better. This is in accordance with Pesti and Howarth (1983) who reported higher feed efficiency for birds reared in a floor space of 0.25 ft^2/bird during the 2nd and 3rd week. The poor feed conversion efficiency of both the groups during the rest of the periods is in agreement with the findings of Gross *et al.* (1980), Elrom (2000) and Puvadolpirod and Thaxton (2000a, d). Poor feed efficiency was attributed to poor digestibility specifically that of dry matter, carbohydrates and protein, while fat digestibility was unaffected (Puvadolpirod and Thaxton, 2000a, d). Litwack and Singer (1972) showed that corticosteroids altered metabolic levels of various digestive enzymes, especially involved with lipolytic, glycolytic and gluconeogenic reactions. Moreover, increased water intake during stress would probably cause a dilution effect and result in changes in pH and osmolarity (Elrom, 2000). These changes together with greater tendency for fat deposition could have amplified the problem of poor feed efficiency. The greater effect on the Group I could be attributed to the hepatic damage caused by dexamethasone.

The stress induced, had a profound influence on various haematological parameters. TLC indicated leukocytosis. These could be attributed to increased blood levels of corticosteroids released during stress. Leukocytosis due to stress or consequent to injection of adreno-corticotropic hormone (ACTH) has been reported (Shapiro and Schechtman, 1949; Hubble, 1955 and Pierson, 2000). RBC count, VPRC and haemoglobin concentration showed that haemopoiesis was affected in stress. Anaemia was reported as an after effect of stress by Hoffman and Leighton (1985). Hepatic and renal damage observed in birds of Group I could be the reason for severe anaemia observed in this group. The higher VPRC values obtained on the 21st day in Group I could be attributed to haemoconcentration following diarrhoea.

The alterations in the haematological parameters could be due to changes in the feed intake caused by stress. This, along with changes in the levels of hormones, hepatic and renal lesions, could have also contributed to the altered haematological values.

The heterophil counts of dexamethasone treated group and the group subjected to overcrowding was higher than the control. This observation is in accordance with the findings of Gross and Siegel (1983), Gross and Siegel (1993), Vijayan and Rema (1997) and Puvadolpirod and Thaxton (2000a, c). However, Peterson and Siegel (1998) reported no significant change in the percentage of heterophils in birds subjected to increased cage density.

The heterophil count in the overcrowded group, however, showed a decreasing trend towards the end of the experiment. This indicated a biphasic cellular reaction where the initial heterophilia noticed tended to decrease in magnitude as the treatment progressed. The present observations is in agreement with those of Maxwell (1993) and Maxwell and Robertson (1995) which stated that heterophilia tend to disappear as the stress became more life threatening.

The lymphocyte count of the Group I and the Group II were lower than the control group. Similar observations were made by Gross and Siegel (1983), Gray *et al.* (1989), Gross and Seigel (1993), Vijayan and Rema (1997) and Puvadolpriod and Thaxton (2000a, c). The present findings are not in agreement with the findings of Peterson and Siegel (1998) who reported no change in the lymphocyte count due to increased cage density.

Monocyte and eosinophil count of both the treatment groups showed no significant variation from the control group. This observation is in accordance with the findings of Shapiro and Schechtman (1949), Stamler *et al.* (1950), Gross and Siegel (1983), Maxwell *et al.* (1990) and Maxwell (1993).

Increase in the basophil count was observed only on the 42nd and 45th day in Group II. This suggests that stress was severe enough at that point to cause the second phase of biphasic cellular reaction (basophilia) as described by Maxwell (1993) and Maxwell and Robertson (1995). The rest of the period in Group II and in all the periods in Group I showed no significant change in the

basophil count. This is in accordance with the findings of Gross and Siegel (1983).

Heterophil to lymphocyte ratio is considered to be a very reliable index for assessing stress (Gross and Siegel, 1993). Results of the present study showed consistently higher heterophil to lymphocyte ratio throughout the experiment. This finding is in consonance with the observations made by Gross and Siegel (1983), Grey *et al.* (1989), Gross and Siegel (1993), Vijayan and Rema (1997) and Puvadolpirod and Thaxton (2000a, c).

In Group I and II, significant reduction in the T lymphocyte count was observed. Munck and Guyne (1991) attributed the reduction in the circulating lymphocytes to directed lysis or apoptosis by stress induced increase of corticosteroids. Activation of HPA system and release of corticosteroids was found to have a major effect on T cell system, which is manifested by lymphopenia occurring within hours of exposure to severe stress (Cupps and Fauci, 1982 and Griffin, 1989). Sapolsky (1992) opined that delay in maturation of lymphocytes could further exacerbate the problem of reduced number of lymphocytes in the circulation.

Lower HI titres were recorded for Group I than the control group. This indicated the poor immune response mounted by the host. This observation is in agreement with Benjamin (1986). In Group II birds the titre did not show much difference from the control till the 35th day. Subba Rao and Glick (1977) and Regnier *et al.* (1980) have reported that antibody titres need not be always

reduced in response to all the stressors. However in the present study as the treatment prolonged a depression in HI titre was noticed. This indicated that humoral immunity was also altered though only in the latter stages.

The leukocyte migration inhibition property was considerably reduced in both the experimental groups. However, the degree of this reduction was more pronounced in Group I. This indicated the stress-induced loss of primary line of defence and antigen recognition.

The adrenals of the birds from Group I weighed lesser than the control group. However, adrenal body weight ratio was always higher in the treatment group. This observation suggested that adrenal weight to body weight ratio could be a better index for measuring stress than the adrenal weight alone. In Group II birds, both the adrenal weight and adrenal to body weight ratio remained higher. These findings are in agreement with the findings of Garren and Shaffner (1956), Siegel and Siegel (1961), Siegel (1959, 1960) and Freeman *et al.* (1980).

Weight of the bursa and the bursa weight to body weight ratio of Group I was lower than the control throughout the experiment. Lower bursal weights could be attributed to the regression in the size of the bursa due to lymphocytolytic action of the corticosteroids. This finding is in agreement with the observations of Garren and Shaffner (1956), Wolford and Ringer (1962), Frankel (1970), Freeman (1971), Gross *et al.* (1980), Siegel (1995) and

Puvadolpirod and Thaxton (2000a,b) and not in agreement with Vijayan and Lalitha (1997) who reported of bursal enlargement following acute stress.

Birds of the Group II recorded higher weights for the bursa and bursa to body weight ratio than the control group in all the periods of the experiment. This could be attributed to bursal oedema. This observation is in agreement with the observations of bursal enlargement noted by Vijayan and Lalitha (1997). However it was not in accordance with Siegel (1960), Pesti and Howarth (1983), Poon *et al.* (1994), Siegel (1995) and Puvadolpirod and Thaxton (2000 a, b, c).

The bursa weight to body weight ratio gave a higher difference among the groups and hence it is a better index than the bursa weights taken alone.

The mean weight of the thymus and the thymus to body weight ratio of both the treatment groups were lower than the control. However, in Group II, the difference became significant only from the 35th day. This is in agreement with the observations of Siegel and Latiner (1970), Gross *et al.* (1980), Muhmed and Hanson (1980), Poon *et al.* (1994), Siegel (1995) and Puvadolpirod and Thaxton (2000 a, b, c) and is not in agreement with Wang-Shu Bai *et al.* (1999), who reported no change in birds subjected to heat stress. Lower thymus weight could be attributed to the lympholytic action of corticosteroids (Elrom, 2000).

Spleen weights were significantly lower in Group I birds throughout the period of the experiment and from the 28th day in Group II. The relative weights of the spleen were also lower in both the groups. Lowered spleen

weights and relative weights of the spleen were in agreement with the findings of Freeman (1971), Siegel (1980, 1983), Wang-Shu Bai *et al.* (1999), Elrom (2000) and Puvadolpirod and Thaxton (2000a, b, c). However Poon *et al.* (1994) reported no change in the spleen weight and an increase in the relative weights of the spleen in ducks injected with cortisol.

Weight of the liver of Group II birds showed statistically significant reduction than the control on the 21st day. However, during the rest of the periods, birds in the Group II recorded higher weight of the liver than the control group. Weight of the liver from Group I birds were higher all through the period of the experiment. Increased liver weights is in consonance with the observations of Puvadolpirod and Thaxton (2000a, b, c). The relative weights of the liver showed a more pronounced difference and hence, it can be considered as a better indicator of stress.

The weight of the pancreas and the thyroid did not show any significant variation between the groups.

Group I birds showed icterus, enlarged liver with fatty streaks. This indicated liver damage. Corticosteroids cause liver damage (Rutgers, 1996; Leib, 1997). Nephrotic changes could be subsequent to the liver damage. Regression of the lymphoid organs could be attributed to the immunosuppressive action of corticosteroids. Emaciated appearance of carcass with prominent keel bone could be because of modified metabolic status in which muscle protein is catabolised. This observation is in accordance to the

findings of Puvalodpirod and Thaxton (2000a, d). Increased abdominal and visceral fat could be body's response to corticosteroid therapy wherein birds try to store more energy reserves in the form of fat at the expense of protein (Puvadolpirod and Thaxton, 2000a, d).

Birds subjected to overcrowding showed bruises and scratches on the breast muscles and pododermititis and blisters in the footpads which increased significantly with age. This observation was in accordance with Martrenchar *et al.* (1997), Sorensen *et al.* (2000). Slight increase in the abdominal and visceral fat observed could be the result of corticosteroid release that occur in stress.

Histologically the adrenals from the Group I revealed vacuolar degenerative changes of the cells and hyaline changes of the arterial walls leading to necrotic changes in the later stages. The decrease in the adrenal weight, increased adrenal body weight ratio, the histological evidence of degenerative changes and lack of evidence of any secretory activity indicate that the stress induced by dexamethazone affected the adrenal as part of the generalised effect in the system.

The histological observation of the adrenal from the overcrowded group suggested a reacting adrenal to the chronic stress. The hyper-cellularity due to hyperplasia of the cortical cells and the medullary cells along with the cystic changes mainly at the periphery are suggestive of an active reaction of the adrenal gland to the stress. Wood's staining was found very effective in differentiating between epinephrine and nor epinephrine producing cells. Nor

epinephrine producing cells were found to be more predominant which indicated that adrenal produced higher amounts of nor epinephrine as a reaction to overcrowding. Van Gieson's fast green staining and PTAH were found as suitable staining techniques to differentiate avian adrenal cortex and medullary cells.

Grossly, the bursa was hard in consistency. Histologically, the bursa showed depletion along with degenerative changes leading to necrotic changes in the lymphoid cells. The lymphoid cells revealed variation in size and shape. The epithelial cell layers showed cystic changes indicative of increased secretory activity. The histological findings caused by corticosteroid are in consonance with the findings of Wolford and Ringer (1967), Siegel and Latimer (1970), Frankel (1970), Freeman (1971), Gross *et al.* (1980), Muhmed and Hanson (1980), Siegel (1995) and Puvadolpirod and Taxton (2000a, b, c).

Sections of bursa from Group II birds revealed inter follicular and intra follicular oedema. Mild degree of infiltration of leukocytes, moderate fibrosis and occasional haemorrages indicated that there was increased capillary permeability in the bursa. Hypertrophy of the peripheral aldosterone secreting cortical cells in the adrenal could cause an increase in aldosterone secretion leading to oedema. In addition to the effect of aldosterone, other adrenal secretions too could have affected the bursa. Manisha De and Ghosh (1998) observed that epinephrine caused a reduction in the size of the lymphoid follicle. Compactness of the medullary cells was decreased by parenchymal

disorder, forming irregular spaces. Thus bursal oedema could be a result of multiple factors.

Depletion of lymphocytes from the thymus was observed in both the groups. Tendency to form the Hassals Corpuscles at an early stage was also noticed. Sato and Glick (1970), Pardue and Thaxton (1984) and Siegel (1987) reported that the primary immunological effects of corticosteroids in the chicken was on the T cell population. Depletion of the lymphocytes from the thymus observed in the present study is in agreement with the findings of Mohmed and Hanson (1980), Gross *et al.* (1980), Sepolsky (1992) and Puvadolpirod and Thaxton (2000a, b, c).

Histopathological changes noticed in the spleen revealed lymphoid depletion in both the treatment groups. This is in accordance with those of Siegel (1995), Elrom (2000) and Puvodolpirod and Thaxton (2000 a,b). The spleen of the birds subjected to overcrowding showed degenerative changes in the arteries. Those changes could have been caused by hypertension as a result of secretion of adrenal medullary hormones (Adams, 1982; Elrom, 2000). By this investigation, it was demonstrated that in the stress response, the lymphoid organs were adversely affected resulting in the atrophy of these organs and consequently, there were functional defects in the humoral and the cell mediated immune response. This effect on the immune system will have substantial undesirable effect on the health of chicken.

The sections of the liver of Group I birds revealed vacoular degeneration and necrosis. The sections were negative for fat. This observation is in consonance with that of Rutgers (1996) and Leib (1997) who reported vacuolar degeneration of hepatocytes when treated with cortiosteroids. Lesions in the Group II birds were mild and could be attributed to corticosteroids released in stress. Mild nephrotic lesions observed in both the treatment groups could be the result of an after effect of the hepatic damages (Vegad and Katiyar, 1998).

Dexamethozone, a synthetic corticosteroid could cause a negative feed back on the pituitary but the high dose of corticosteroid given could itself cause direct lympholytic effect. Moreover avian leukocytes can produce ACTH or ACTH like substances (Siegel, 1995; Elrom, 2000). Therefore, a combined lympholytic effect of ACTH and dexamethazone could be the reason for such an extensive damage caused to the lymphoid organs.

Various markers were employed to identify a suitable marker to assess the stress response in chicken. From the result of this investigation, it can be summarised that in practice, it would be better to have a battery of tests like the clinical observations, behavioural changes, haematolgoical parameters, organ weight to body weight ratio along with gross and histopathological observations for identification and assessing stress..

The effect of various stressors on the different parameters studied varied in different time frames. For each parameter observed the adverse effects were

greatest at different time frames of the experiment depending on the type of the stressor.

Higher stress scores were observed for the parameters that showed greater difference from the control. This indicated that stress scores could be used as a tool for identifying the best marker for assessing the stress response. Employing stress scores, the significance of the atrophy of the lymphoid organs like the thymus, bursa and the spleen was delineated. It was shown that this could be a potential marker for assaying the stress response.

The gross and histopathological findings observed in this investigation were almost similar to those described earlier, although, there were certain qualitative and quantitative differences in the pathomorphological changes.

Histopathological changes in the adrenal and the lymphoid organs were found to be aggravated as the exposure to the stress was prolonged. Changes in the adrenal leading to lymphocytolysis could be demonstrated clearly. The advancement of the lesions in the adrenals was also reflected in the lymphoid organs like the bursa and thymus. The functional alterations noted from the HI titre, T-lymphocyte count and LMIT could be attributed to the structural changes observed. Hence, the changes in the adrenal could lead to immunodepression.

It was clarified by histopathological studies that dexamethazone has significant adverse biological effects on the hepato-renal system when administered in slightly higher doses. This observation implies that, in severe

stress response, the damaging effect of dexamethazone by itself will be an inducer of undesirable health response.

By this investigation, the immunological effect of stress response in the chicken was clarified by taking HI titre against New Castle disease as a biological marker. It was demonstrated that stress had slightly reduced the immune response in chicken. This has practical significance in the field situations. Vaccination in a stressful condition will lead to a poor immune response, which may even lead to break down of immunity. It is, therefore, essential to educate the farmers, the need for minimizing or avoiding stress in the management of birds.

Employing immune markers like LMIT and enumeration of T-lymphocytes, it was clarified that stress affected the cell-mediate immunobiological response of chicken significantly.

In addition to inducing stress, overcrowding also caused moderate to severe damage to the skin and muscles. This could lead to a decrease in marketability and profitability of the farmers. It is, therefore, imperative that overcrowding has to be necessarily avoided by the farmers.

Summary

6. SUMMARY

The experiment was designed to study the pathology of induced stress in broiler chicken and to identify suitable markers for recognition of stress. Day old broiler chicks (n=102) were divided randomly into three groups of 34 birds each. Birds of Group I were subjected to chemical stressor by administration of dexamethasone orally at the rate of 50 ppm on the 20th day followed by 25 ppm on days 27, 34, 41 and 44. Group II was subjected to physical stress by stocking at higher density of 0.25 ft^2/bird till 28th day and then at 0.5 ft^2/bird, while Group III served as control. Behavioural and clinical symptoms, production parameters, haemogram, immunological parameters, and pathological changes in the adrenal, bursa, thymus, spleen, liver and kidney were recorded to study the pathology and to identify suitable markers of stress.

Birds of Group I were drowsy and lethargic with mild inflammation of conjunctiva and upper respiratory tract. In Group II the prominent signs were poor feather development, increased pecking and hyper responsiveness to external stimuli in the initial stages followed by dullness and greater incidence of lameness.

Mean body weights of both the experimental Groups, I and II were lower than the control group except for Group II on the 21st day, where higher body weights were recorded.

Feed efficiency of birds in Group II was higher on the 21st day. However, during the rest of the periods in Group II and all through the

experiment in Group I, the feed conversion efficiency was lower than the control group.

Leukocytosis along with lower values of RBC, haemoglobin and VPRC were found in both the treatment groups. Differential leukocyte counts revealed that both the stressors caused heterophilia along with lymphopenia. Basophilia was also observed in birds of Group II towards the end of the experiment. Heterophil to lymphocyte ratio remained higher for both the stressed groups than the control group.

The mean adrenal weights of Group I was lower than the control group however the adrenal weight to body weight ratio remained higher than the control group. Birds of Group II recorded higher mean weights and mean relative weights for the adrenal than the control.

Birds of Group I recorded lower mean weights and mean relative weights for the bursa than the control group, while in Group II the bursal weights and relative bursal weights were higher than the control. The mean weights and mean relative weights for the thymus and spleen were lower for both the stressed groups than the control group. The mean liver weights and relative liver weights for both the stressed groups were higher than the control. The mean weight and the relative weights of pancreas and thyroid did not show any significant variation.

Grossly, the carcasses of birds of Group I were icteric. Increased deposition of fat in the abdominal viscera, along with moderate degree of wasting of the muscles. The keel bone was prominent.

Carcasses of birds of Group II showed increased incidence of bruises as well as scratches in the breast muscles along with pododermatitis and blisters. The bursa was enlarged and soft to touch. The adrenals and the liver were larger in size whereas the thymus and the spleen were smaller when compared to the control group. A moderate increase in the abdominal fat was also observed.

Adrenals from birds sacrificed on the 21st day showed predominantly epinephrine producing medullary cells, which were enlarged and swollen. However by 28th day the numbers of cortical cells increased. Adrenals from the 35th day to the end of the experiment revealed various degrees of degeneration and necrosis of the cortical as well as medullary cells.

Adrenals from Group II revealed hyperplasia and hypertrophy of the cortical and medullary cells which tended to get organized into large spherical clusters at the periphery of the gland. Extensive areas of hyperplasia of cortical cells appearing as dense cell aggregates with hyperchromatic nucleus and scanty cytoplasm was also observed. As the duration of treatment advanced the cell clusters showed tendency for dilatation and cyst formation. Nor epinephrine producing cells were predominant. Van Giesons fast green staining and phosphotungstic acid haematoxylin staining methods were found to be effective staining techniques for differentiating the adrenal medullary and cortical cells.

Bursa from Group I revealed degeneration and necrosis of the lymphocytes along with cyst formation both inside the lymphoid follicle and in the mucosal epithelium. Hyperplasia of the mucosal epithelium observed as foldings of the epithelium was also observed. Bursa from Group II showed intra and inter follicular oedema. Towards the latter stages, degeneration and necrotic changes were observed in the follicles along with interstitial fibrosis and mild cellular infiltration.

Thymus from both the stressed groups showed varying proportion of degeneration and necrosis of the lymphocytes and stromal proliferation. The lesions being more severe in Group I.

Spleen of both the stressed groups showed depletion of lymphocytes with degenerative changes in the arteries. Birds of Group I showing prominent lesions.

Liver from both the stressed groups showed vacuolar degenerative changes, necrosis of the hepatocytes with kupffer cell activation. Liver from birds of Group I was more severely affected.

Parenchymal cells of the kidneys revealed vacuolar degeneration and necrosis of varying degrees in both the treatment groups however the intensity of the lesions were more severe in Group I.

Higher stress scores were obtained for the parameters that were affected more severely.

From results of the present study it was clarified that stress had affected the immunobiological response of chicken significantly and therefore it is essential to avoid or minimise undue stress in birds when chicken are vaccinated against infectious diseases.

The effect of various stressors on the different parameters studied varied in different time frames. For each parameter observed the adverse effects were greatest at different time frames of the experiment depending on the type of the stressor.

The role of adrenal and the correlation between the changes in the adrenal and the immunological as well as other organ systems were delineated.

For assessing stress it would be better to adopt a battery of tests like clinical observation, behavioural changes, alterations in the haemogram, production parameters together with the gross and microscopic changes in the various organ systems, especially the adrenal gland.

Estimation of stress scores could prove useful not just as a good marker for stress but also as a tool to identify suitable markers for each type of stress.

Wood's staining technique, is a suitable technique for differentiating the adrenal medullary epinephrine and nor epinephrine producing cells. Van Gieson's Fast green and phosphotungtic acid haemotoxylin staining methods can be used for effectively differentiating the adrenal cortical and medullary cells.

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PATHOLOGICAL EFFECTS OF INDUCED STRESS ON THE LYMPHOID ORGANS IN BROILER CHICKEN

By

SURAJ. S.

ABSTRACT OF A THESIS

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COLLEGE OF VETERINARY AND ANIMAL SCIENCES

MANNUTHY, THRISSUR - 680651

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ABSTRACT

The experiment was designed to study the pathology of induced stress in broiler chicken and to identify suitable markers for recognition of stress. One hundred and two, day old broiler chicks were divided into three groups of 34 birds each. Birds of Group I was administered dexamethasone orally at the rate of 50 ppm on the 20th day followed by 25 ppm on days 27, 34, 41 and 44. Group II was stocked at higher density of 0.25 ft^2/bird till 28th day and then at 0.5 ft^2/bird, while Group III served as control. Behavioural changes, production parameters, haemogram, immunological parameters, and pathological changes in the organs were recorded to study the pathology and to identify suitable markers of stress.

Birds of Group I were depressed and developed mild infection while Group II showed poor feather development and hyper responsiveness to stimuli initially followed by depression and lameness.

Birds of Group I and II showed lower body weights and feed efficiency except for Group II on the 21st day when higher body weights and better feed efficiency was observed.

Leukocytosis, lower values for RBC, haemoglobin and VPRC along with heterophilia, lymphopenia and higher heterophil to lymphocyte ratio were recorded for both the stressed groups. Basophilia was observed towards the end of the experiment in Group II.

Birds of Group I showed increased tendency to deposit abdominal fat along with wasting of muscles while in Group II bruises as well as scratches in breast muscles and pododermatitis were prominent lesions observed.

The mean weights of the adrenal was lower in Group I however the mean adrenal weight to body weight ratios were higher. Both mean and relative mean adrenal weights were higher for Group II. The mean weights and organ weights to body weight ratio of bursa, thymus, and spleen were lower for both the stressed groups. Mean liver weight and liver weight to body weight ratio were higher for both the stressed groups.

Adrenals from Group I showed increased proportion of epinephrine producing medullary cells on the 21st day but on 28th day the numbers of cortical cells had increased. During the latter stages of the experiment the cortical and medullary cells were seen in various stages of degeneration and necrosis. In Group II hypertrophy and hyperplasia of the cortical and medullary cells which were organised into spherical clusters along with aggregation of cortical cells in the periphery were seen during the initial half of the experiment. Towards the latter stages the cell clusters showed tendency for cyst formation. Bursa from Group I showed degeneration and necrosis of the follicles along with mucosal hyperplasia and cyst formation. In Group II bursal intra follicular and inter follicular oedema followed by degeneration of the lymphocytes were observed. Thymus and spleen showed lymphoid depletion in both the treatment groups. Liver and kidneys of both the stressed groups

showed degenerative and necrotic changes. The intensity of pathological lesions were more in Group I than in Group II.

Stress scores were found to be good marker for identification of stress and can serve as a useful tool to identify suitable markers for stress.

The results of the present study highlights the adverse effects of stress on the immunobiological response. The correlation between the changes in the adrenal and the immunological organs were delineated. It would be better to use a battery of tests like behavioural alterations, haemogram, production indices together with gross and microscopic changes in the various organs for assessing stress response. Stress scores was identified as a useful marker and tool to identify markers of stress. Van Gieson's fast green and phosphotungstic acid haemotoxylin staining methods were identified as suitable staining methods for differentiating adrenal, cortical and medullary cells.