Effect of Melatonin on Some Hematological Parameters and Immune Status of Broiler Chicks

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Abstract

Melatonin, the pineal gland hormone, has been shown to play a principle role in maintenance of health and well being of man and animals in all stages of life. The aim of the present study was to investigate the effect of melatonin on some hematological parameters and immune status of broiler chicks. For this purpose sixty-four, three weeks-old chicks of Hubbard strain weighing 500-633g were used. Birds were kept on lighting regimen of 12 hours natural light to 12 hours darkness. Chicks were randomly allocated into four equal groups of 16 birds each. The first group was kept as a control group and fed starter ration sprayed with ethanol saline without melatonin from the 4^{th} to 8^{th} weeks of age. The second, third and fourth groups were fed on the starter ration sprayed with ethanol saline containing 20, 30 and 40 mg melatonin / kg ration, respectively from the 4^{th} to 8^{th} weeks of age. Blood samples were obtained from the tibial vein of all birds at the end of the 1^{st} week of treatment and then every week up to the end of the experiment. The obtained blood were used for determination of red blood cells (RBCs) count, hemoglobin (Hb) concentration, packed cell volume (PCV) as well as total and differential leukocytic count. Tissue samples from liver, spleen, thymus and bursa of Fabricious were taken for histopathological examination. The results revealed that melatonin in the three doses produced a significant increase in RBCs count, PCV, Hb concentration, total leukocytic count and lymphocyte percentage which clarifies the effect of melatonin on improving the health and immune status of the chicks under investigation. The histopathological study in the present investigation revealed that melatonin treatment induced a lymphoid hyperplasia in the liver, spleen, and bursa of Fabricious of all treated groups. This lymphoid hyperplasia might be another explanations for the immunostimulant effect of melatonin. In conclusion melatonin was found to improve the health and immune status of broiler chicks.

Keywords: Melatonin, Hematological parameters, Immune status, Broiler chicks

1. Introduction

Melatonin has been reported to play a fundamental role in the biology of all cells **(Reiter and Robinson, 1995).** It was reported to influence a wide range of physiological processes and reach all organs and cells of the body (Lamosova et al., 1997). In our previous studies on broiler chicks, melatonin was found to have a strong antioxidant effect (Ahmed et al., 2005) and to improve productive performance of chicks through improving their metabolism and stimulating tissue uptake of lipids, proteins and carbohydrates (Ahmed et al., 2006).

The effect of melatonin on blood parameters is controversial and what is available in literature is contradictory. Karimungi and Joshi (1996) stated that a single subcutaneous injection of male rats with 25 µg melatonin at 17.00h for 4 weeks lead to a decrease in total erythrocyte count at 12.00 h, whereas a daily double injection of melatonin at 09.00 h and 17.00 h for two weeks resulted in either a significant decrease in total RBCs count when the samples were collected at 06.00 h and 24.00 h or a significant increase in RBCs count, when the samples were collected at 18.00 h. The erythrocyte indices (mean cell volume MCV, mean cell hemoglobin MCH and mean cell hemoglobin concentration MCHC) in both experiments were found to be correlated with RBCs. Karimungi et al. (1996) moreover, added that melatonin treatment either once daily (25 µg) at 17.00 h or twice daily at 9.00 and 17.00 hrs for two weeks induced a decrease in the RBCs count of rats and the erythrocyte indices (MCV, MCH and MCHC) were correlated with the RBCs count. At the same time Durotoye and Rodway (1996) found that subcutaneous implants of melatonin lead to a reduction in RBCs count and packed cell volume (PCV) in ewe, while the MCV was increased. On the contrary Anwar et al. (1998) reported that I/P injection of rats with melatonin at a dose of 100 µg simultaneously with aracytin (cytotoxic drug) for 10 days lead to a significant increase in RBCs count and could counteract the aracytin – induced decrease in RBCs count.

Concerning the effect of melatonin on total and differential leukocytic counts, Skwarlo-Sonta et al. (1992) stated that melatonin had no effect on total leukocytic count or their fractions in chickens injected with sheep RBCs. Kondo et al. (1997) reported that in chicken the diurnal rhythmic patterns of lymphocytes and monocytes which showed 2 peaks of count per day, disappeared during continuous melatonin administration and the diurnal pattern of granulocytes count was also altered. Abozahra et al. (1998) found that administration of melatonin to broiler chicks increased the total leukocytic count as well as the eosinophil and lymphocyte percentage, while decreased the heterophil percentage. Markowska et al. (2001) stated that melatonin alone had no effect on the proliferation of chicks thymocytes, splenocytes and lymphocytes from bursa of Fibricious. However, it could inhibit the proliferation of PHA-activated thymocytes and splenocytes from young chicks.

Melatonin is also known to influence various immune parameters. Haldar et al. (2001) found that injection of squirrels with exogenous melatonin (25 mg/ 100 g B wt/day) at 1730 h significantly increased all the immune parameters, while pinealectomy decreased them significantly. The authors suggested that melatonin is immune-enhancing for tropical squirrel and plays an important role in the maintenance of immunity. Moreover, Rai and Haldar (2003) reported that daily subcutaneous injection of melatonin (25 microg/100 g body mass) at 17.30-18.00 h to adult squirrels for 60 consecutive days significantly increased the lymphocyte count of blood and bone marrow and blastogenic response/percent stimulation ratio of spleen and thymus. The previous authors further added that in pinealectomized spuirrels, decreased total leukocyte count and percent lymphocyte count in peripheral blood and bone marrow, along with the decreased cell density in spleen and thymus was observed histologically. However, melatonin treatment of pinealectomized squirrels resulted in restoration of immune parameters in line with a normal control level. On the other hand, Srinivasan et al. (2005) added that melatonin has the potential therapeutic value to enhance immune function in aged individuals and in patients in an immunocompromised state. In the same respect Cardinali et al. (2008) reported that melatonin stimulates the production of progenitor cells for granulocytes and macrophages. It also stimulates the production of natural killer cells and CD4+ cells and inhibits CD8+ cells. The production and release of various cytokines from natural killer cells and T helper lymphocytes are enhanced by melatonin.

2. Material and methods

2.1 Birds

Sixty-four three weeks old chicks of Hubbard strain (obtained from El-Shemoa Company, Alexandria) weighing 500-633g were used. Chicks were fed on a starter ration during the whole experimental period, which was obtained from Cairo Company for poultry. The ration contains crude protein not less than 21%, crude fat not less than 2.12% and crude fiber not more than 3%. All birds were kept on lighting regimen of 12 hours natural light to 12 hours darkness. Medication and vaccination were carried out according to the recommendations obtained from El-Shomoa Company.

2.2 Melatonin

Melatonin was dissolved in ethanol 95% (1g melatonin / 5 ml ethanol) then stored in refrigerator at 4 ˚C. The dose of melatonin was calculated by dilution of 0.10, 0.15 and 0.20 ml of dissolved melatonin with 16 ml of normal physiological saline to obtain 20, 30 and 40 mg / Kg ration, respectively. The diluted melatonin was sprayed daily on the ration of each corresponding group at 4 p.m. then left to dry in air overnight to minimize the effect of ethanol and then introduced to chicks at 4 p.m. in the next day. The dilution, spraying and drying were repeated daily allover the experimental period.

2.3 Experimental design

Birds were randomly allocated into four equal groups of 16 birds each. They were treated as follows: The first group was kept as a control group and fed on the starter ration sprayed with ethanol saline without melatonin from the 4^{th} to 8^{th} weeks of age. The second, third and fourth groups were fed on the starter ration sprayed with ethanol saline containing 20, 30 and 40 mg melatonin / kg ration, respectively from the 4^{th} to 8^{th} weeks of age.

2.4 Sampling

Blood samples were obtained on EDTA from the tibial vein every week and used for hematological examination. At the end of the 6^{th}, 7^{th}, and 8^{th} weeks of age three birds from each group were slaughtered, respectively, and tissue samples from liver, spleen, thymus, and bursa of Fabricious were taken and fixed in formalin saline 10% for histopathological examination.

2.5 Hematological parameters

Total red blood cells (RBCs) were counted under the microscope by using improved double Neubaur haemocytometer (Natt and Herrick, 1952), the microhematocrite method (Schalm et al., 1975) was used in determination of packed cell volume (PCV), haemoglobin was measured by the cyanomethemoglobin method (Pilaski, 1972) using reagent obtained from Diamond Diagnostics, El Gomhoria Company, Egypt, the total leukocytic count was carried out by the Natt and Herrick's method (1952) using double Neubour hemocytometer and the differential leukocytic count was determined according to the method of Lucas and Jamroz (1961).

2.6 Statistical analysis

All data presented as mean ± standard error (SE) and were subjected to two way analysis of variance (ANOVA) test according to Snedecor and Cochran (1980). Treatments means were compared by the least significant difference test (LSD) at 0.05 and 0.01 levels of probability.

3. Results

3.1 Effect of Melatonin Treatment On Blood Picture

3.1.1 Erythrocyte count (RBCs count)

It appears from table (1) that treatment of broiler chicks with melatonin (20, 30 and 40 mg /kg feed, respectively) for 5 successive weeks induced a significant increase ($P<0.01$) in their RBCs count versus control during most of the experimental period. The overall mean of the RBCs count was significantly higher in all treated groups versus control ($P<0.01$). No significant differences in RBCs count were observed among treated groups except at the end of the first week where the 3^{rd} and 4^{th} groups showed significantly higher values than that of the 2^{nd} group.

3.1.2 Packed cell volume

Table (2) shows that melatonin treatment significantly ($P<0.01$) increased the packed cell volume (PCV) versus control in all treated groups after 3 weeks of treatment up to the end of the experiment. Moreover the overall mean of the treated groups was significantly higher than that of the control group ($P<0.01$). No significant differences in PCV were observed among the treated groups.

3.1.3 Hemoglobin concentration

Treatment of broiler chicks with melatonin induced an increase in hemoglobin (Hb) concentration (table 3) in all treated groups. This increase was statistically significant versus control after three weeks except 20 mg treated group at 4^{th} week of treatment ($P<0.01$). The overall mean of Hb concentration was significantly higher ($P<0.01$) in all treated groups than that of the control group. No significant differences were found among the treated groups at any of the experimental periods.

3.1.4 Total leukocytic count

Table (4) shows the effect of melatonin treatment on the total leukocytic count of broiler chicks. Melatonin at a dose of 30 and 40 mg/kg feed significantly increased the total leukocytic count ($P<0.01$) versus control after one

week of treatment. However, after two weeks of treatment the total leukocytic count was significantly higher ($P<0.01$) in the three treated groups versus control and this increase extended to the end of the experiment. The overall mean of the total leukocytic count in all treated groups was significantly higher than that of the control group ($P<0.01$). There were no significant differences in the total leukocytic count among the treated groups during the whole experimental period except for the first week were the total leukocytic count in the 30 mg treated group was significantly higher than that of the 20 mg treated group.

3.1.5 Differential leukocytic count

Treatment of broiler chicks with the three different doses of melatonin for 5 successive weeks produced a significant decrease ($P<0.01$) in heterophil percent in all treated groups versus control at the end of the 1st and 2nd weeks of treatment. The overall mean of the heterophil percent was significantly lower in all treated groups versus control. No significant differences in the heterophil percent were observed among the treated groups at any of the experimental periods (table 5). On the other hand melatonin treatment increased the percentage of lymphocytes (table 6). This increase was statistically significant versus control after the end of the 1st and 2nd weeks of treatment in all treated groups. The overall mean of the lymphocyte percent was significantly higher than that of the control ($P<0.01$) during the whole experimental periods. There were no significant differences between control and treated groups in the percentage of eosinophils (table 7) and monocytes (table 8).

3.2 Histopathological Study

Liver, spleen and bursa of fabricious of all treated chicks showed a lymphoid hyperplasia. No histopathological lesion could be seen in all tissue examined (figures 1 to 8).

4. Discussion

It is clear from the present study that the red blood cell (RBCs) count, packed cell volume (PCV) and hemoglobin (Hb) concentration were significantly increased in melatonin treated groups. No available literature could be found regarding the effect of melatonin on these parameters in chickens. However, some studies were performed in mammals. Anwar et al. (1998) found that melatonin treatment in rats numerically increased RBCs, Hb and PCV. On the other hand Durotoye and Rodway (1996) proved that subcutaneous implants of melatonin in ewes reduced RBCs count and PCV. It appeared from the study of Karimungi and Joshi (1996) and Karimungi et al. (1996) that the effect of melatonin treatment on RBCs count depends on the time of melatonin injection and the time of blood collection. They reported that a single injection of melatonin at 17.00 h for 4 weeks decreased the RBCs, PCV and Hb when samples were collected at 12.00 h, while twice daily injections of melatonin at 9.00 h and 17.00 h increased RBCs, PCV and Hb at 18.00 h. Reverse results were obtained when blood samples were collected at 6.00 h and 24.00 h. Moreover, The increase in RBCs count, PCV and Hb concentration obtained in the present study may be attributed either to its direct stimulatory effect on bone marrow, which was previously reported by Anwar et al. (1998) or indirectly through stimulation of some cytokins, which was found to have a powerful stimulatory effect on bone marrow cells proliferation (Lissoni et al., 1993).

In this study melatonin treatment induced a significant increase in total leukocytic count and lymphocyte percentage. These results are consistent with the previous reports which indicated that administration of melatonin increased the total leukocytic count and lymphocyte percentage in broiler chicks (Abozahra et al., 1998), rats (Anwar et al., 1998), immature chicks (Bernan et al. (2002) and squirrels (Rai et al., 2009). In a previous study Rai and Haldar (2003) reported that daily subcutaneous injection of melatonin at 1730 to 1800 h significantly increased the lymphocyte count of blood in adult male squirrels, while pinealectomy decreased total leukocyte count and percent lymphocyte count in peripheral blood and bone marrow. The precise mechanism responsible for this melatonin-induced increase in total leukocytic count and lymphocyte percentage is not clear. However, several mechanisms may be involved in this respect. The first possible mechanism may be the direct action of melatonin either on bone marrow (Kuci et al., 1987) or on lymphatic tissue (Maestroni and Conti, 1993) to accelerate leukocytogenesis (Conti et al., 1992). The second possible mechanism may be an indirect action through reduction of corticosterone hormone, which was reflected by the elevation of the leukocytic count (Anwar et al., 1998). The third possible mechanism may be through protection of bone marrow from damage by free radicals due to its antioxidant effect (Ahmed et al.,2005). The present investigation showed that melatonin treatment significantly decreased the heterophil percentage. This result is matched with those reported by Abozahra et al. (1998) and Anwar et al. (1998). They found that melatonin significantly decreased the heterophil percentage in chicks and rats, respectively. On the other hand, Skwarlo-Sonta et al. (1992) claimed that melatonin treatment had no effect on total leukocytic count or their fractions in chickens or mice. These contradictory results may be due to the differences in the time of drug administration and/or the time of sample collection. Karimungi and Joshi (1996)

reported that the effect of melatonin on total and differential leukocytic count was reversed in rats when the time of its administration and the time of sample collection were changed.

The Histopathological study in the present investigation revealed that melatonin treatment induced a lymphoid hyperplasia in the liver, spleen, and bursa of Fabricious of all treated groups. This lymphoid hyperplasia might be one of the explanations of the immunostimulant effect of melatonin which was reported by many investigators.Yu et al. (1991) found melatonin binding sites on the spleen of birds and mammals and suggested a direct action of melatonin on the immune system. Liu and Pang (1992) added that the bursa of Fabricious of chicken was a target organ of melatonin. Moreover, Poon and Pang (1994) could detect melatonin receptors in the avian primary and secondary lymphoid system. Furthermore, Calvo et al. (1995) found specific melatonin binding sites on thymus, spleen and bursa of Fabrecious of different rodents and birds and suggested that melatonin may play a physiological role in regulation of lymphocyte and immune function. Also Moore and Siopes (2002) found that melatonin administration accelerated the development of cell mediated and humoral immune response in turkeys through increasing the bursal weight. Melatonin treatment of pineaectomized squirrels resulted in restoration of immune parameters in line with normal control level (Haldar et al., 2001 and Rai and Haldar, 2003). Arlet and Hewison (2004) stated that melatonin acts as immunostimulant in squirrel when tested in vivo as well as in vitro. Moreover, Srinivasan et al. (2005) added that melatonin has the potential therapeutic value to enhance immune functional in aged individuals and in patients in an immunocompromised state.. Sharma and Haldar (2006) attributed the mechanism by which melatonin exerts its immune-potentiating action to be partially due to its action in reducing the free radical load. In the same respect Cardivali et al. (2008) reported that melatonin stimulates the production of progenitor cells for granulocytes and macrophages. It also stimulates the production of natural killer cells and CD4+ cells and inhibits CD8+ cells. The production and release of various cytokines from natural killer cells and T helper lymphocytes are enhanced by melatonin. Rai et al. (2009) reported an age related decline of immune system (almost all the parameters) associated with the decline in melatonin level. The previous authors added that melatonin was able to compromise the parachlorophnylalanin-induced suppression of spleen mass and thereby immunity. However, Skwarlo-Sonta (2002) stated that effects exerted by melatonin on immune parameters are different and depend on several factors, including dose and way of melatonin application, species, sex, age of animal, its immune system maturation, way of immune system activation and parameter examined as well as the season, circadian rhythm of both immunity and pineal gland function, stressfull conditions and accompanying experimental procedure.

5. Conclusion

In conclusion melatonin was found to improve the health and immune status of broiler chicks which may be of value in improving poultry industry.

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Table 1. Effect of melatonin treatment on red blood cells (RBCs) count (million/mm^3) in broiler chicks

Duration of treatment (week)	Control 1st group	Melatonin (mg/Kg feed) 20 2nd group	30 3rd group	40 4th group
1	2.06±0.07^{ab}	1.93±0.18^{cd}	2.53±0.13^{ac}	2.61±0.14^{bd}
2	2.03±0.10^{abc}	2.69±0.09^{a}	2.64±0.13^{b}	2.90±0.14^{c}
3	2.28±0.13^{abc}	3.17±0.14^{a}	3.08±0.07^{b}	3.02±0.09^{c}
4	1.86±0.13^{abc}	2.92±0.06^{a}	2.94±0.10^{b}	2.95±0.08^{c}
5	2.07±0.07^{abc}	3.05±0.04^{a}	3.08±0.11^{b}	3.02±0.07^{c}
Overall Mean	2.06±0.05^{abc}	2.76±0.08^{a}	2.89±0.06^{b}	2.90±0.05^{c}

- Each value is expressed as a mean ± standard error
- LSD of treatment mean = 0.165
- LSD of treatment X time interaction= 0.37
- Means having the same letter in the same raw are significantly different at P<0.0

Table 2. Effect of melatonin treatment on packed cell volume (PCV%) in broiler chicks

Duration of treatment (week)	Control 1st group	Melatonin (mg/Kg feed) 20 2nd group	30 3rd group	40 4th group
1	35.88±0.77	35.43±1.31^{a}	36.68±0.79	38.63±1.05^{a}
2	36.88±0.77^{a}	40.88±0.95^{a}	39.88±0.44	38.38±0.56
3	36.38±1.08^{abc}	40.75±0.71^{a}	40.00±0.60^{b}	40.62±0.62^{c}
4	36.00±0.71^{abc}	40.37±0.96^{a}	41.38±0.96^{b}	40.25±0.70^{c}
5	34.51±0.56^{abc}	41.57±0.92^{a}	42.43±0.84^{b}	41.71±0.94^{c}
Overall Mean	35.94±0.20^{abc}	39.80±0.20^{a}	40.11±0.23^{b}	39.92±0.33^{c}

- Each value is expressed as a mean ± standard error
- LSD of treatment mean = 1.37
- LSD of treatment X time interaction= 3.07
- Means having the same letter in the same raw are significantly different at P<0.01.

Table 3. Effect of melatonin treatment on hemoglobin concentration (g/dl blood) in broiler chicks

Duration of treatment (week)	Control (1st group)	Melatonin (mg/Kg feed) 20 (2nd group)	30 (3rd group)	40 (4th group)
1	9.38±0.17	8.69±0.41	9.38±0.31	10.00±0.21
2	9.68±0.39	10.71±0.30	10.06±0.31	9.80±0.40
3	9.26±0.28^{abc}	10.92±0.19^{a}	10.80±0.21^{b}	11.87±0.64^{c}
4	9.28±0.38^{ab}	10.51±0.36	11.81±0.61^{a}	11.69±0.68^{b}
5	9.84±0.21^{abc}	11.67±0.57^{a}	12.32±0.57 ^{b}	11.66±0.40^{c}
Overall Mean	9.50±0.34^{abc}	10.50±0.49^{a}	10.90±0.41^{b}	11.00±0.44^{c}

- Each value is expressed as a mean ± standard error
- LSD of treatment mean = 0.665
- LSD of treatment X time interaction= 1.50
- Means having the same letter in the same raw are significantly different at P<0.01

Table 4. Effect of melatonin treatment on total leukocytic (WBCs) count (thousand/mm^3) in broiler chicks

Duration of treatment (week)	Control 1st group	Melatonin (mg/Kg feed) 20 2nd group	30 3rd group	40 4th group
1	21.65±1.85^{ab}	26.61±1.31^{c}	34.60±2.38^{ac}	29.79±1.20^{b}
2	23.94±2.10^{abc}	34.88±0.97^{a}	37.92±2.25^{b}	38.76±1.35^{c}
3	22.50±1.40^{abc}	40.60±0.87^{a}	42.96±1.49^{b}	42.97±1.49^{c}
4	23.64±1.16^{abc}	40.25±0.97^{a}	40.57±1.00^{b}	40.15±1.16^{c}
5	20.45±0.40^{abc}	40.79±0.57^{a}	40.28±0.53^{b}	41.91±0.77^{c}
Overall Mean	22.44±0.67^{abc}	36.63±0.97^{ad}	39.26±0.84^{bd}	38.71±0.95^{c}

- Each value is expressed as a mean ± standard error
- LSD of treatment mean = 2.3
- LSD of treatment X time interaction= 5.11
- Means having the same letter in the same raw are significantly different at P<0.01

Table 5. Effect of melatonin treatment on heterophil percent in broiler chicks

Duration of treatment (week)	Control 1st group	Melatonin (mg/Kg feed) 20 2nd group	30 3rd group	40 4th group
1	28.50±0.93^{abc}	19.13±0.72^{a}	18.88±0.83^{b}	19.75±1.00^{c}
2	25.63±1.28^{abc}	18.50±0.68^{a}	17.25±1.36^{b}	18.38±0.96^{c}
3	24.88±1.25^{ab}	18.00±0.85^{a}	21.50±1.21	20.50±0.93^{b}
4	23.25±0.77^{ab}	18.50±0.57^{a}	20.38±1.16	17.25±0.84^{b}
5	21.13±0.18^{a}	17.38±0.77	18.13±0.77	17.13±0.94^{a}
Overall Mean	24.70±0.59^{abc}	18.30±0.32^{a}	19.23±0.52^{b}	18.60±0.45^{c}

- Each value is expressed as a mean ± standard error
- LSD of treatment mean = 1.56
- LSD of treatment X time interaction= 3.8
- Means having the same letter in the same raw are significantly different at P<0.01

Table 6. Effect of melatonin treatment on lymphocyte percentage in broiler chicks

Duration of treatment (week)	Control 1st group	Melatonin (mg/Kg feed) 20 2nd group	30 3rd group	40 4th group
1	66.00±1.00^{abc}	75.25±0.80^{a}	76.13±1.06^{b}	72.37±1.84^{c}
2	68.87±1.67^{abc}	76.63±1.13^{a}	78.38±1.15^{b}	77.13±1.20^{c}
3	71.75±1.30^{a}	78.13±0.72^{ab}	73.63±1.50^{b}	75.50±0.96
4	72.75±1.31^{a}	76.25±1.00	75.88±1.14	78.50±1.04^{a}
5	74.50±0.78^{a}	77.80±0.96	78.00±0.78	79.00±0.93^{a}
Overall Mean	70.4±0.72^{abc}	76.8±0.43^{a}	76.4±0.56^{b}	76.50±0.65^{c}

- Each value is expressed as a mean ± standard error
- LSD of treatment mean = 1.9, LSD of treatment X time interaction= 4.2
- Means having the same letter in the same raw are significantly different at P<0.01

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Table 7. Effect of melatonin treatment on eosinophil percentage in broiler chicks

Duration of treatment (week)	Control 1st group	Melatonin (mg/Kg feed) 20 2nd group	30 3rd group	40 4th group
1	2.53±0.37	2.13±0.16	2.25±0.30	2.25±0.26
2	2.35±0.37	2.33±0.26	2.38±0.28	2.25±0.15
3	2.41±0.20	2.35±0.17	2.24±0.19	2.33±0.29
4	2.25±0.30	2.13±0.27	2.35±0.40	2.43±0.27
5	2.56±0.30	2.50±0.33	2.31±0.26	2.43±0.15
Overall Mean	2.34±0.12	2.31±0.13	2.29±0.10	2.42±0.13

- Each value is expressed as a mean ± standard error

Table 8. Effect of melatonin treatment on monocyte percentage in broiler chick

Duration of treatment (week)	Control 1st group	Melatonin (mg/Kg feed) 20 2nd group	30 3rd group	40 4th group
1	3.53±0.35	3.53±0.25	3.66±0.39	3.75±0.22
2	3.88±0.28	3.60±0.20	2.88±0.39	3.60±0.27
3	3.50±0.42	3.18±0.28	3.25±0.18	3.10±0.32
4	3.53±0.23	3.39±0.39	3.03±0.35	3.00±0.29
5	3.13±0.23	3.34±0.31	3.06±0.19	3.22±0.23
Overall Mean	3.51±0.19	3.41±0.16	3.18±0.15	3.33±0.16

- Each value is expressed as a mean ± standard error

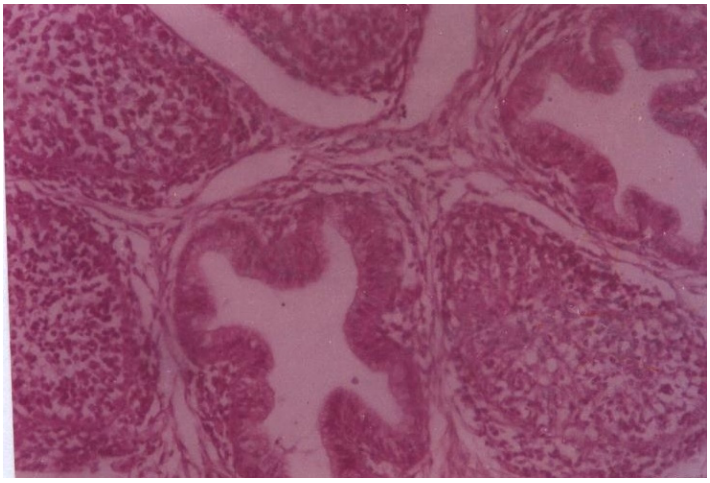

Figure 1. Bursa of Fabricius of control chicks, 3 weeks after treatment showing physiological involution, lymphoid depletation and cyst formation (H&E X10)

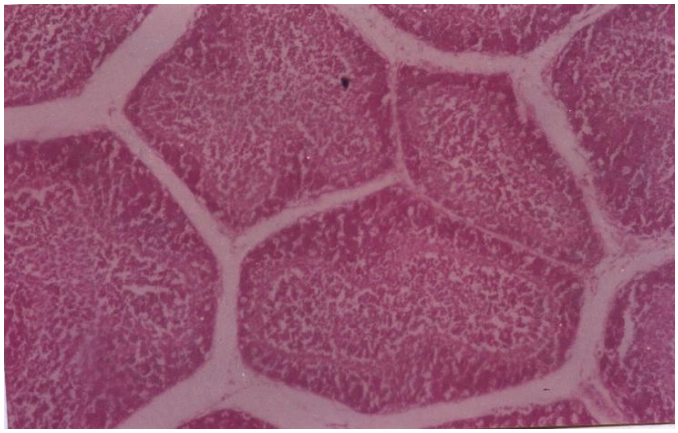

Figure 2. Bursa of Fabricius of 20 mg melatonin treated group, 1 week after treatment (H&E X10)

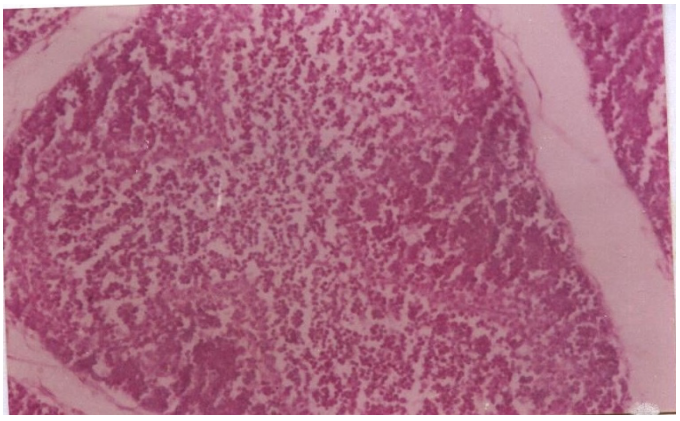

Figure 3. Bursa of Fabricius of 40 mg treated chicks, 3 weeks after treatment (H&E X10)

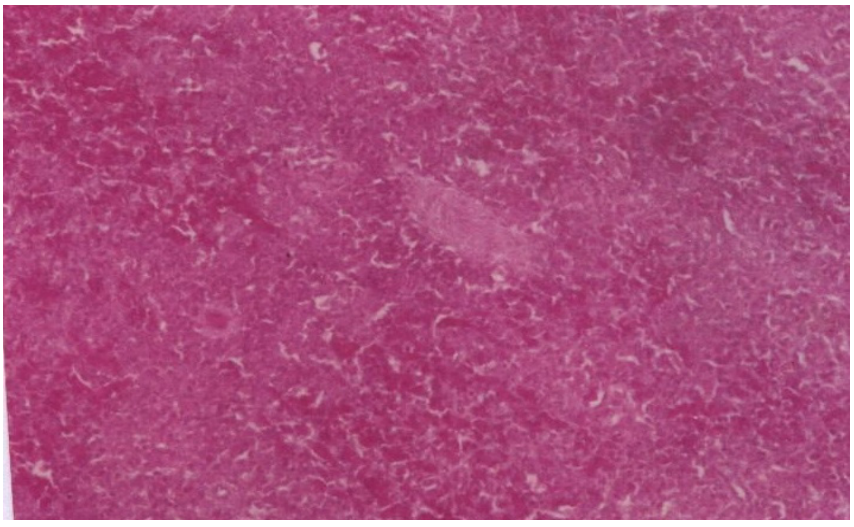

Figure 4. Spleen of control chicks, 3 weeks after treatment (H&E X20). No secondary follicles were observed

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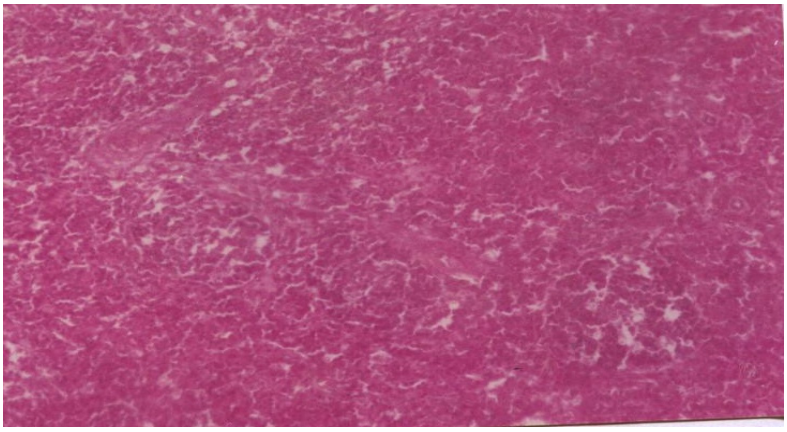

Figure 5. Spleen of 2o mg melatonin treated chicks, 3 weeks after treatment (H&E X20)

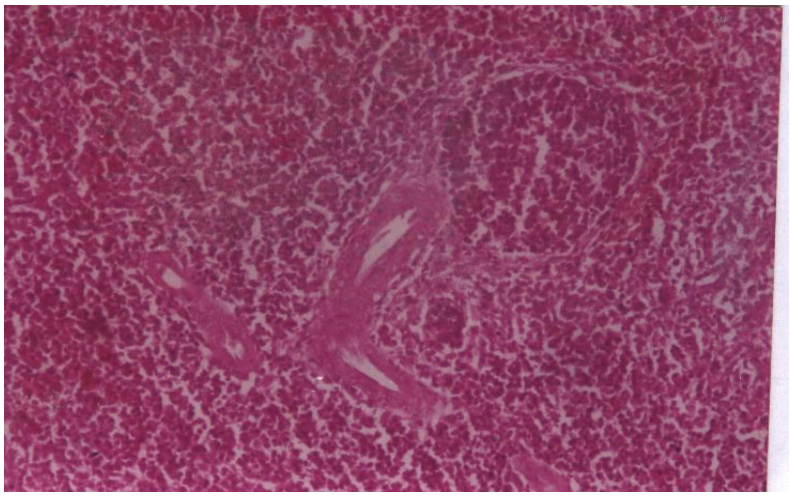

Figure 6. Spleen of 40 mg treated chicks, 2 weeks after treatment (H&E X20)
Notice the presence of secondary follicles in the spleen of melatonin treated chicks

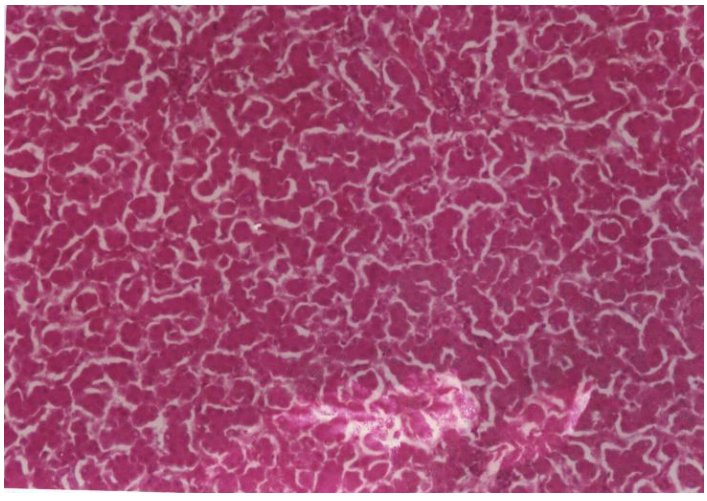

Figure 7. Liver of control chicks, 3 week after treatment (H&E X20)
Notice the absence of lymphatic follicles

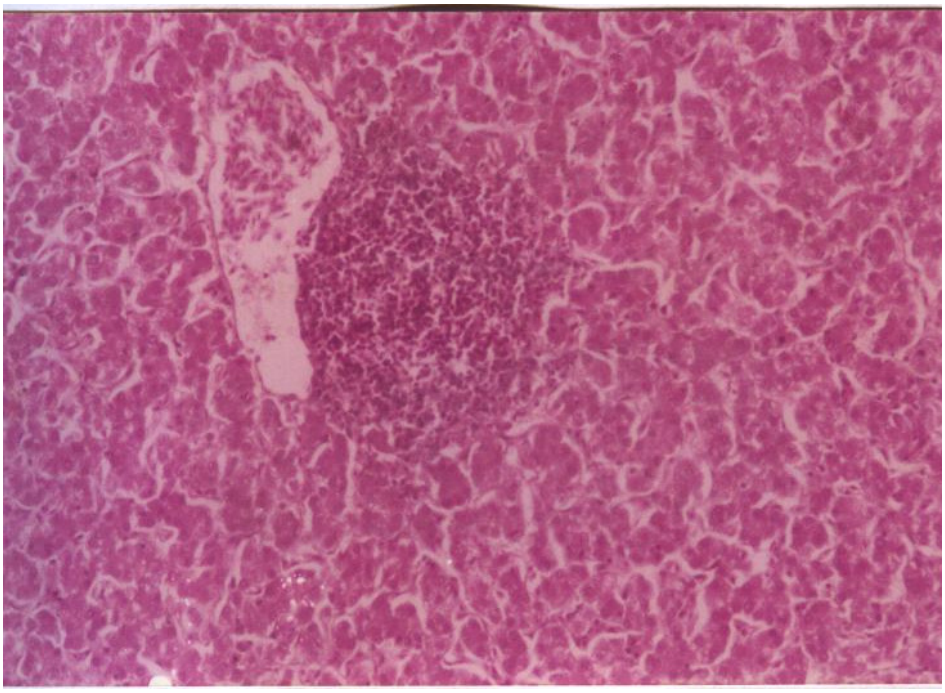

Figure 8. Liver of 20 mg melatonin-treated chicks, 1 week after treatment (H&E X20) showing formation of lymphatic follicles

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