Effect of *Aloe vera* leaf pulp extract on *Ehrlich ascites* tumours in mice

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Among the various known therapeutic effects of *Aloe vera* (L.) Burm. fil., a few recent studies have shown that preparations of the plant leaves can prevent or regress the growth of certain tumours. In this study, undertaken with *A. vera* leaf pulp extract against *Ehrlich ascites* tumours in mice, the animals were separated into five groups: I – healthy control, II – tumour control, III – experiment 1 (extract given before tumour inoculation), IV – experiment 2 (extract given with tumour inoculation) and V – experiment 3 (extract given after tumour inoculation). *Ehrlich ascites* tumours (0.33 ml) were injected subcutaneously into groups II–V. *Aloe* extract was injected at 55 mg protein/kg, twice a week for 21 days. Tumour size, thymus and spleen weights were measured, as well as leucocyte count, tumour necrosis factor-α and sialic acid as tumour markers. The best inhibitory effect on tumour growth was obtained with the extract given prophylactically before tumour implantation (experiment 1), although *Aloe* extract also regressed tumour sizes when given simultaneously with (experiment 2), or therapeutically after (experiment 3), tumour implantation. Accordingly, serum sialic acid and tumour necrosis factor-α levels, chosen as tumour markers, which were raised in the tumour control group, were significantly decreased by the prophylactic administration of the extract. The increase in leucocyte count seen in experiment 1 and 2 groups, along with lymphoid hyperplasia observed in spleen and thymus necroscopy, lead us to think that the tumour preventive effect of *Aloe* could be due to its immunomodulatory activity. According to our results, *A. vera* could be proposed as a prophylactic for cancer prevention. *European Journal of Cancer Prevention* 16:151–157 © 2007 Lippincott Williams & Wilkins.

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Introduction

During the past several decades, there has been growing interest in natural products for the cure and prevention of cancer. Despite a number of plants used in folk and traditional medicine (Graham *et al.*, 2000), only a few have been scientifically explored, and some biologically active compounds have been identified (Mukherjee *et al.*, 2001).

Aloe vera (L.) Burm. fil. (= *Aloe barbadensis* Miller; Liliaceae) is a plant native to Africa and is also cultivated in Turkey. *Aloe* species have been known as folk medicine for centuries and their laxative, anti-inflammatory, immunostimulant, antiseptic (Capasso *et al.*, 1998,) wound and burn healing (Heggers *et al.*, 1995; Chithra *et al.*, 1998), antiulcer (Koo, 1994) and antidiabetic (Ajabnoor, 1990; Beppu *et al.*, 1993; Bunyapraphatsara *et al.*, 1996; Okyar *et al.*, 2001) activities have been scientifically proved. Experimental studies have also reported the antimetastatic activity of *Aloe* juice in rats and mice (Gribel and Pashinskii, 1986) and the inhibitory effect of *Aloe* extract on induction of preneoplastic hepatocellular lesions in rats (Tsuda *et al.*, 1993). In a few studies, different *Aloe* extracts have been reported to prevent or regress the growth of certain tumours (Winters *et al.*, 1981; Desai *et al.*, 1996; Corsi *et al.*, 1998; Shimpo *et al.*, 2002). The purpose of the present study was to confirm the anticancer activity of *A. vera* leaf pulp aqueous extract against *Ehrlich ascites* tumours *in vivo*, before and after tumour inoculation, in order to assess its therapeutic or prophylactic activity.

Materials and methods

Plant material

Specimens of *A. vera* (L.) Burm. fil. (in Turkish 'Sarisabir') were collected from Kale (Demre) in Antalya, identified by N. Sütlüpinar (May 1993; a voucher specimen was deposited in the Herbarium of the Faculty of Pharmacy, ISTE No. 65118), planted and cultivated in

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the greenhouse of the Department of Botany, Faculty of Sciences, Istanbul University. Fresh leaves of this cultivated plant were used in this study.

Preparation of *A. vera* leaf pulp extract

A. vera leaves (six big leaves) were washed and cut from the middle, and the gel was separated by scratching with a spoon. The leaves without the gel (pulp) were cut into small pieces (514 g) and homogenized with phosphate-buffered saline (pH 7.0, 600 ml) by means of a Waring blender. The extract was kept at 4°C overnight and filtered through cloth, and the filtrate was centrifuged at 45 700 g for 30 min at 2°C in a refrigerated centrifuge (Cryofuge 20-3 Heraeus-Christ; Hanau, Germany). The green pellet was discarded and the clear yellow supernatant was taken and lyophilized (Labconco apparatus; Kansous City, Missouri, USA). Twenty-nine grams of *Aloe* leaf pulp extract was thus obtained. *Aloe* leaf pulp extract (7.5%) was prepared by dissolving the powder in phosphate-buffered saline and mixing thoroughly via a magnetic stirrer.

Tumour cells

The *Ehrlich ascites* tumour cell line was obtained from Istanbul University, Cerrahpasa Faculty of Medicine, Experimental Research Center. Approximately 0.33 ml containing 1.2×10^6 tumour cells was injected subcutaneously into mice.

Animal groups

Male CD-1 mice, weighing 28–30 g, were used. The animals were maintained in separate cages at 18–20°C and 55% humidity conditions and fed with special pellets; water was allowed *ad libitum*.

The animals were separated into five groups, each consisting of 20 mice:

1. healthy control (subcutaneous 0.2 ml serum physiological);
2. tumour control (subcutaneous 0.33 ml *Ehrlich ascites* tumour cells);
3. experiment 1 (extract given prophylactically before tumour inoculation);
4. experiment 2 (extract given simultaneously with tumour inoculation);
5. experiment 3 (extract given therapeutically after tumour development).

Administration of the samples

Aloe extract was injected subcutaneously at a dose of 5 ml/kg (= 55 mg protein/kg), twice a week, until the end of the experiment, for all groups. The animals were also weighed twice a week before the administration of the extract. *Aloe* extract was administered in three different modalities: simultaneously with tumour cells, after tumour development and prophylactically.

Experiment 1 (extract given before tumour inoculation): in order to assess the prophylactic power of *Aloe* extract, the tumour cells were inoculated 10 days after *Aloe* extract injection. On the day of tumour injection, seven mice were killed in order to observe the effect of the extract on tissues before tumour formation. Tumour cells were inoculated into the remaining animals.

Experiment 2 (extract given simultaneously with tumour inoculation): *Aloe* extract was injected 2 h after tumour inoculation. In order to see the effect of the extract on tissues and tumours, 10 days later seven mice were killed and tissue samples were taken.

Experiment 3 (extract given after tumour development): in order to assess its therapeutic power, *Aloe* extract was injected 10 days after tumour inoculation. On the day of *Aloe* extract injection, seven mice were killed in order to observe the effect of the tumour on tissues. *Aloe* extract was administered to the remaining animals.

Mice were weighed at days 0, 7, 14 and 21 of the experiment. Tumour size of tumour-bearing animals was measured by a digital compass twice a week and at the end of the experiment, under anaesthesia.

On the 21st day after inoculation of tumour cells, for all groups, blood samples of the animals were withdrawn by cardiac puncture under ether anaesthesia, tumour diameters were measured, and thymus and spleen tissues were taken for pathological examination.

Total and leucocyte formula counts were carried out immediately after blood puncture; sera were then separated and kept frozen for total protein, sialic acid and tumour necrosis factor-α (TNF-α) measurements.

Measurement of thymus and spleen weights

In tumour-induced groups, thymus and spleen weights are given as first (seventh day) and end values (21st day). First values are the mean of the seven mice killed before tumour formation and end values are those of the mice killed on the 21st day at the end of the experiment or from mice that died before the end of the experiment. In the healthy control group without tumour induction, thymus and spleen weights were obtained at the 10th day (first values) and at the 21st day (end values). Thymus and spleen wet weights were measured by a digital analytical balance.

Biochemical assays

Protein measurement

Serum total protein was measured by the biuret reaction (Weichselbaum, 1946).

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Sialic acid measurement
Serum sialic acid was determined by the thiobarbituric acid method of Warren (1959).

Tumour necrosis factor-alpha measurement
Mause TNF-α ELISA kit (KMC 3012) was used for serum TNF-α determination.

Leucocyte count (WBC)
Leucocytes were counted by means of a hemocytometer.

Statistical analysis
Differences between groups were determined by Duncan's multiple range test (Duncan, 1955) and analysis of variance tests using a Number Cruncher Statistical System (NCSS) software (Hintze, 2000).

Pathological examinations
Systemic necropsy of tumour-implanted mice was undertaken after cardiac blood puncture. The animals were skinned and tumours formed under the skin tissue were measured by means of a digital compass. During necropsy, besides tumours, organs such as the thymus and spleen were taken. After macroscopic examination, biopsies of appropriate size were taken from all organs and tumours and fixed in 10% formol saline solution. The tissue pieces were kept in this solution for 2 days and then buried in paraffin blocks after routine procedures. Cross-sections taken from these blocks by means of a rotary microtome were stained with haematoxylin–eosin and examined under a light microscope.

Results
Animal weights
At the end of the third week, the live weights of the mice in the tumour control group, probably owing to tumour mass, were significantly higher than those of healthy controls. The live weights of mice in the groups given *Aloe* extract simultaneously with tumour implantation (experiment 2) and therapeutically after the tumour implantation (experiment 3) were similar to those of the mice in the tumour control group. In the groups that received *Aloe* extract prophylactically (experiment 1) because tumour sizes were smaller, the increase in weight was found to be insignificant in comparison with that in the healthy control (data not shown).

Tumour development and tumour size
In experiment 1, tumour development was very slow; in some animals, tumour size reached the first values obtained in the other groups only at the end of the third week. In six animals no tumour was detected until the end of the experiment. On the 7th and 10th days, the tumour sizes of mice in experiment 1 were significantly smaller than those of mice in the tumour control group (Table 1). In experiments 2 and 3, tumours were significantly smaller than in the tumour control group only at the 21st day (Table 1).

Table 1 Tumour sizes (mm) in all groups

Groups	7 days	10 days	21 days
Healthy control	–	–	–
Tumour control	13.44 ± 5.60^{b}	22.09 ± 9.05^{a}	38.5 ± 7.70^{a}
Experiment 1 (tumour after *Aloe*)	3.09 ± 1.75^{c}	5.90 ± 3.81^{b}	17.58 ± 6.82^{c}
Experiment 2 (tumour with *Aloe*)	18.79 ± 6.65^{a}	22.88 ± 7.88^{a}	25.96 ± 11.88^{b}
Experiment 3 (*Aloe* after tumour)	$15.93 \pm 5.80^{a,b}$	24.48 ± 7.85^{a}	$22.12 \pm 8.56^{b,c}$

Different letters (a, b, c, d) show significance between groups in one column. $P < 0.05$ (Duncan test).

Spleen and thymus weights
At the 21st day, no significant difference was found for spleen and thymus weights between the healthy control and tumour control groups. At the 21st day, spleen and thymus weights were higher in the experiment 1 group than in all groups (Table 2).

Sialic acid levels
Sialic acid levels, raised significantly in the tumour control group, were decreased by the administration of *Aloe* extract in all groups (Table 3).

Tumour necrosis factor-alpha levels
TNF-α levels were significantly higher in the tumour control group than in the healthy control group. In accordance with our other findings, at the end of the experiment, TNF-α levels were significantly decreased in experiment 1 whereas no significant difference was observed for experiments 2 and 3 (Table 3).

Leucocyte counts (WBC)
Leucocyte counts were significantly lowered in the tumour control group in comparison with that in the healthy control group. Administration of *Aloe* extract in experiments 1 and 2 increased WBC significantly, whereas no significant difference was observed in experiment 3 compared with the tumour control group (Table 4).

Pathological examination
The results of the pathological findings are summarized in Table 5. In the healthy control group, spleens (Fig. 1) and thymuses were determined to be normal in appearance. The necrosis developed in tumour tissue sections was evaluated according to the size of the necrotic area and graded as + 1, + 2, + 3 (Figs 2–4).

Discussion
In the present study, *A. vera* leaf pulp extracts effectively inhibited tumour growth when given prophylactically

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Table 2 Spleen and thymus weights (g) for all groups

Groups	Spleen		Thymus	
	7 days	21 days	7 days	21 days
Healthy control	0.125 ± 0.031^{b}	0.113 ± 0.015^{b}	0.0254 ± 0.011^{c}	0.0440 ± 0.011^{a}
Tumour control	$0.142 \pm 0.070^{a,b}$	$0.198 \pm 0.011^{a,b}$	0.0450 ± 0.015^{b}	0.0416 ± 0.018^{a}
Experiment 1 (tumour after *Aloe*)	$0.158 \pm 0.039^{a,b}$	0.317 ± 0.010^{a}	$0.0373 \pm 0.018^{b,c}$	0.0508 ± 0.013^{b}
Experiment 2 (tumour with *Aloe*)	0.181 ± 0.050^{a}	$0.198 \pm 0.020^{a,b}$	0.0664 ± 0.024^{a}	0.0472 ± 0.022^{a}
Experiment 3 (*Aloe* after tumour)	$0.162 \pm 0.014^{a,b}$	$0.200 \pm 0.011^{a,b}$	$0.0412 \pm 0.019^{b,c}$	0.0435 ± 0.021^{a}

Different letters (a, b, c, d) show significance between groups in one column. $P<0.05$ (Duncan test).

Table 3 Serum sialic acid and TNF-α levels for all groups

Groups	Sialic acid (mg/dl)		TNF-α (ng/ml)	
	7 days	21 days	7 days	21 days
Healthy control	23.70 ± 4.92^{c}	20.13 ± 8.69^{c}	148.80 ± 16.43^{d}	155.30 ± 18.72^{c}
Tumour control	77.00 ± 29.10^{a}	86.62 ± 24.84^{a}	345.75 ± 22.61^{a}	328.33 ± 12.34^{a}
Experiment 1 (tumour after *Aloe*)	$55.27 \pm 22.96^{a,b}$	56.49 ± 33.72^{b}	225.80 ± 33.19^{c}	212.54 ± 54.11^{b}
Experiment 2 (tumour with *Aloe*)	48.83 ± 16.96^{b}	46.37 ± 18.79^{b}	301.33 ± 22.62^{b}	306.16 ± 34.35^{a}
Experiment 3 (*Aloe* after tumour)	$69.80 \pm 20.98^{a,b}$	64.83 ± 29.31^{b}	$319.25 \pm 21.26^{a,b}$	321.75 ± 25.87^{a}

Different letters (a, b, c, d) show significance between groups in one column. $P<0.05$ (Duncan test).

Table 4 Leucocyte counts for all groups

Groups	Leucocyte count (100/μl)	
	7 days	21 days
Healthy control	$40.40 \pm 4.35^{a,b,c}$	42.20 ± 5.09
Tumour control	24.16 ± 29.79^{c}	29.88 ± 36.16
Experiment 1 (tumour after Aloe)	$59.12 \pm 16.56^{a,b}$	68.90 ± 28.21
Experiment 2 (tumour with Aloe)	64.50 ± 31.18^{a}	106.16 ± 85.13
Experiment 3 (Aloe after tumour)	$38.37 \pm 25.74^{b,c}$	$9.00 \pm$ (one value)*

Different letters (a, b, c, d) show significance between groups in one column. $P<0.05$ (Duncan test).
*Statistical evaluation could not be made in this column. Animal number was insufficient owing to mortality in experiment 3 (21-day value).

before tumour inoculation and also when given with or after tumour implantation, but tumour regression (Table 1) and survival rate in the group that received *A. vera* extract before tumour development was spectacular. Experimental investigation has demonstrated that many naturally occurring agents have anticancer effects. These activities were associated with phenolic compounds such as flavonoids, coumarins, quinones, curcuminoids, etc. (Wang *et al.*, 2005). The antitumour effect of *Aloe* extracts was previously attributed to aloe-emodin (Fahim *et al.*, 1997; Pecere *et al.*, 2000; Fenig *et al.*, 2004) and to acemannans (Peng *et al.*, 1991; Ramamoorthy and Tizard, 1998; Sampedro *et al.*, 2004) or a glycoprotein fraction (Yagi *et al.*, 1997). The anticancer activity of plants is sometimes also attributed to their free radical scavenging effects (Gupta *et al.*, 2004). The prophylactic activity of *Aloe* leaf pulp extract against *Ehrlich ascites* tumours in mice could be due to the antioxidant (Hu *et al.*, 2003) activity of *Aloe* reported in the literature and attributed to different compounds such as aloesins (Yagi *et al.*, 2002) or polysaccharides (Wu *et al.*, 2005).

The increase in leucocyte (WBC) count is one of the parameters chosen for immunomodulatory activity (Melchart *et al.*, 1995; Raphael and Kuttan, 2003). An immunopotentiator could also increase spleen and thymus weights (Zheng *et al.*, 2005). The fact that leucocyte number was significantly increased in the experiment 1 and 2 groups (Table 4), and the weight increase in the thymus (Table 2) along with lymphoid hyperplasia observed in the spleen (Fig. 3 and Table 5) and cortical hyperplasia in the thymus (Fig. 4 and Table 5) tissues could be a sign for the immunomodulatory effect of *Aloe* extract reported in the literature (Hart *et al.*, 1988; Qiu *et al.*, 2000).

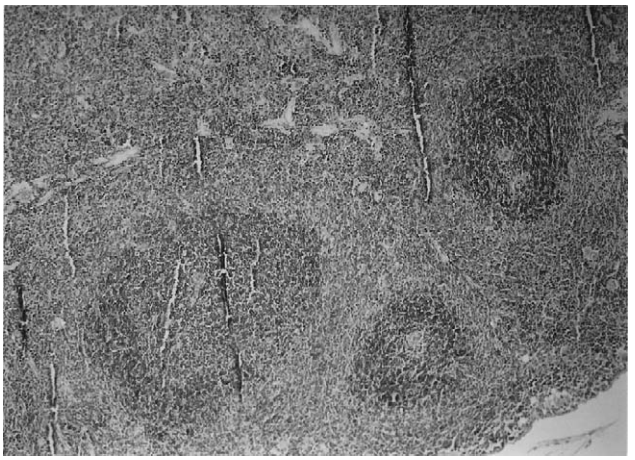
Clinical studies have shown that total sialic acid concentrations in serum were significantly elevated in different types of cancers compared with the values of healthy people and those with nonmalignant diseases (Shamberger, 1984; Turkmen *et al.*, 1997). Sialic acid is now proposed in the literature as a tumour-derived compound marker for neoplasia (Cylwik *et al.*, 2005). The increase in sialic acid as a result of cancer in the tumour control group was lowered in the groups given *Aloe* extracts (Table 3) in accordance with the antitumour effect. Serum TNF-α is also a tumour marker for apoptotic changes (El-Aziz *et al.*, 2005). TNF-α levels were found to be increased in the sera (Zhou *et al.*, 2003) and tissues (Catalano *et al.*, 2003) of tumour-bearing animals. Accordingly, in this study, TNF-α levels were higher in the tumour control group than in the healthy

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Table 5 Results of the pathological examinations on tumoural tissues, spleens and thymuses for all groups compared with the healthy group

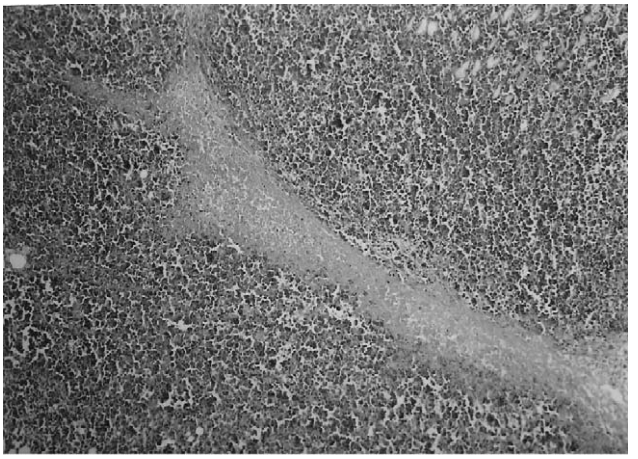
Groups	Tumoural tissue	Spleen	Thymus
Tumour control	Tumoural tissue developed was subcutaneously located. An extensive necrotic area was observed, severity of which was scored as +3 (Fig. 2). Inflammatory cells such as leucocytes and mononuclear cells were determined among the tumoural cells. The extent of invasion of the muscle and fat with tumoural cells was fairly dense.	Mild hyperemia and dilatation were observed in the red pulp due to the infiltration of inflammatory cells, which was generally composed of leucocytes.	The thymuses of all animals had normal appearance, except those of three in which mild cortical hyperplasia was observed.
Experiment 1 (tumour after *Aloe*)	No tumoural development was observed in a total of six animals (numbered between 1 and 6). In the other animals, tumoural cells were observed to have invaded the muscular tissue beneath subcutis. In this tumoural tissue, a necrotic area, which had centrifugal development and with different dimensions, was observed. As the tumoural tissue was graded according to the wideness of the necrosis, this group was scored as +2.	The expansion of the lymphocentral zones in the white pulp was prominent in this group. The number of inflammatory cells was increased in the red pulps. Accordingly, prominent lymphoid hyperplasia was determined to have developed in the spleen (Fig. 3).	Mild cortical hyperplasia was observed in the thymuses and thus the medullary areas were determined to be narrowed (Fig. 4).
Experiment 2 (tumour with *Aloe*)	The tumoural cells progressed into the muscle and the fat tissue and necrosis developed in the centre of the tumoural areas. The degree of necrosis was +2.	Lymphocentrums were evidently enlarged. In addition, there were infiltrations of inflammatory cells in the red pulps. Hyperplastic splenitis was determined to have developed.	Hyperplasia developed in the cortical areas of thymuses and at different dimensions.
Experiment 3 (*Aloe* after tumour)	Tumoural cells were determined to have intensively progressed into the muscle and fat tissue. Tumoural tissue was widespread; necrotic areas were extensive. The degree of necrosis was +3.	Lymphocentrums were slightly enlarged and hyperplastic splenitis, which was characterized by an increase in the inflammatory cells, was developed in the red pulps.	Thymuses were observed to be normal.

Fig. 1



Spleen in the healthy control group. Haematoxylin–eosin × 100.

Fig. 2



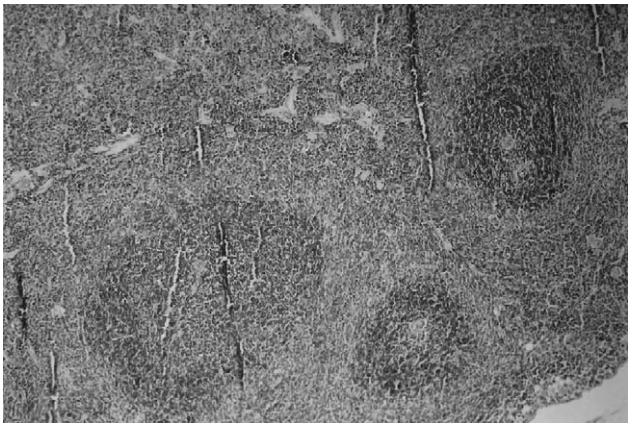
Tumoural tissue in the tumour control group showing necrosis. Haematoxylin–eosin × 100.

group, but significantly lower in the experiment 1 group (Table 3), showing the cancer preventive effect of the extract. In experiment 1, *A. vera* administration before tumour inoculation causes an increase in cellular immunity and as a result the necrosis is limited (Table 5) and tumour size is smaller than in the other groups (Table 1).

Much research is needed to understand the real factor responsible for the biological activity of plant extracts. Isolated pure compounds were found to be less effective than whole plant extracts and, as claimed by Liu (2004), it can be stipulated that, as in all the plant kingdom, the additive and synergistic effect of phytochemicals present in *A. vera* may be responsible for this cancer preventive activity. According to our results, *A. vera* leaf pulp extract could be proposed as a prophylactic for cancer prevention.

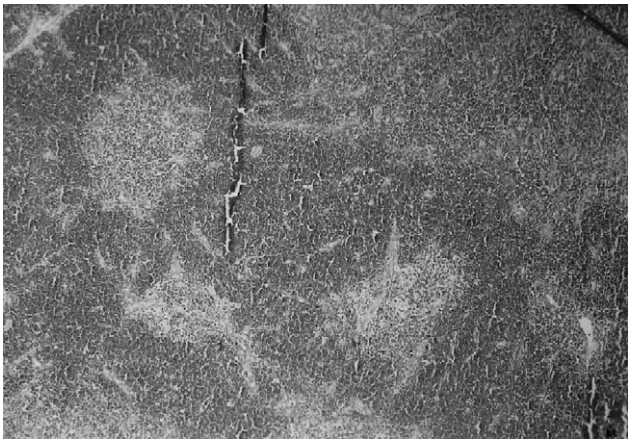
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Fig. 3



Spleen in the experiment 1 group showing lymphoid hyperplasia. Haematoxylin–eosin × 100.

Fig. 4



Thymus in the experiment 1 group showing cortical lymphoid hyperplasia. Haematoxylin–eosin × 100.

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