

MODIFICATION OF RATS ADJUVANT ARTHRITIS BY LAVANDULA STOECHAS PLANT GROWING IN TURKEY

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ÖZET

SIÇAN ADJUVANT ARTRİTİNİN TÜRKİYE'DE YETİŞEN LAVANDULA STOECHAS BITKİSİ TARAFINDAN MODİFİKASYONU

Erkek Wistar siçanlarda 'Complete Freund's adjuvant' (CFA) (10 mg/ml ısı ile denatüre olmuş *Mycobacterium tuberculosis* içermektedir) sağ ayak altına intradermal injekte edilerek adjuvant artriti (AA) oluşturuldu. AA ve karabaş bitkisinin (*Lavandula stoechas*) (100 mg/kg p.o) etkileri araştırıldı ve indometasin ile karşılaştırıldı. CFA sağ ayak hacmini 1. gün (primer reaksiyon), sol ayak hacmini ise (sekonder) 5. günden sonra artırdı. Bunun şiddeti iki hafta sonra arttı ve ön bacak ve kuyruklara (nodül) yayıldı. Bu değişikler ile birlikte IgG ve nötrofilde artış, lenfosit ve RBC'de ise azalma gözlandı. *L. stoechas* primer reaksiyonları artırdı, sekonderleri şiddetlendirdi (kulak ve kuyrukda nodüller) ve generalize etti (burun, göz ve penisi içerdi). Öte yandan *L. stoechas*, CFA'nın neden olduğu lenfosit azalmasını inhibe ederken, RBC'de gözlenen azalmayı daha da artırdı, ama IgG'yi etkilemedi. Bu sonuçlara göre AA'da *L. stoechas* bitkisinin kolaylaştırıcı etkisi antikor üremeyen lenfositin artmasını içermektedir. *L. stoechas* bitkisinin lenfositte artış istenildiği durumlarda yararlı olabileceği sanılmaktadır.

Anahtar Kelimeler: *L. stoechas*, adjuvant artriti, primer ve sekonder reaksiyonlar

SUMMARY

In this study, we investigated the effect of *Lavandula stoechas* on rats' adjuvant arthritis (AA). AA was induced by intradermal inoculation of complete freund's adjuvant (CFA) containing 10 mg/ml heat-killed *Mycobacterium tuberculosis* into the plantar surface of right hind paw. CFA increased volume of the injected paw (primary reaction) and the left paw (secondary reaction) from the day 5. At the second week, the secondary reactions were intensified and extended to the fore limbs and tail (nodules). These changes were accompanied by elevation of IgG and polymorphnuclear leukocytes (neutrophils) with reduction in lymphocyte and RBCs. *L. stoechas* at 100 mg/kg administered p.o. to AA rats, increased the primary reaction, intensified (nodules in ear and tail) and generalized (extended to nose, eye and penis)

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the secondary reactions. Moreover, *L.stoechas* inhibited AA-induced reduction in lymphocytes, further increased the reduction in RBCs, but it did not alter the changes in IgG. The effects of *L.stoechas* were compared with indomethacin. These results revealed the facilitation by *L.stoechas* of changes induced by AA in rats. This effect could be mediated by an action on non-antibody producing lymphocytes. *L. stoechas* could be beneficial in conditions where an increase in the lymphocytes is required.

Key words: *L. stoechas*, adjuvant arthritis, primary and secondary reactions

INTRODUCTION

Lavandula stoechas L. (Labiaceae) is widely distributed in the mediterranean countries including Turkey. The plant is known as *Karabash* and its extract is used by the folk for various purposes as sanative and anticancer (1) and hypoglycemic agent (2).

One of the conditions for which the medicinal plants are prescribed is human rheumatoid arthritis, (RA) (3). Arthritis can be induced in rats by inoculation of Complete Freund's Adjuvant (CFA) containing heat-killed and dried *Mycobacterium tuberculosis*. This type of arthritis is known as adjuvant arthritis (AA) and bears some resemblance to RA. Although AA is said to be a chronic inflammatory reaction (4), plethora of data indicate that AA also involves an immunological reaction (5, 6) with a possible neurogenic component (7).

In a series of experiments on effects of natural products on the haematological parameters, we found that *L. stoechas* increased the lymphocytes of rats (unpublished). The present study was hence carried out to investigate the effect of *L. stoechas* on AA of rats chosen as a model of pathological conditions because it is known to involve the inflammatory and/or immunological responses.

MATERIALS AND METHODS

Animals

Male Wistar rats (DETAM, Istanbul), weighing 150-200 g were used. The rats were housed 5 per cage (42x 26x 15 cm) for at least one week before the experiments and were fed Purina lab chow and water *ad libitum* throughout the experiment. The room temperature was maintained at 22±3 °C with 50±5% humidity and 12 hours light:dark cycle (lights on from 07 am -19 pm).

Extraction of Plant Materials

Samples of *L. stoechas* were collected from the Mediterranean coast of Turkey. The samples (5 g, each of the flowers and leaves) were extracted with 50% ethanol. The extraction was performed in 3 successive stages, each with 200 ml of 50% ethanol for 6 hours using a magnetic stirrer with a slight heating (40 °C). This procedure was found

to be sufficient for complete extraction (i.e., exhaustion) of the plant material. The samples were filtered and the solvent was evaporated under reduced pressure. The extract was then concentrated (green-brown) and was found to constitute 33% of the total dry plant weight. It contains glycosides and saponins (1), triterpinoids (8), pinen derivatives (9) and camphene (10). The extract is used by the folk at concentrations up to 5% and the dose used in the present study was chosen accordingly.

Induction of Adjuvant Arthritis

Under light ether anesthesia, the rats were inoculated intradermally in the plantar surface of right hind paw with 0.05 ml of CFA, containing a suspension of heat-killed *Mycobacterium tuberculosis* in paraffin (10 mg/ml). CFA was a generous gift from department of Pharmacology, Faculty of Medicine, Ankara University. These rats were referred to as adjuvant arthritic (AA) rats. The day of inoculation was regarded as day 0. Four groups of rats were used in this study (10 rats in each group). One group received only 0.05 ml paraffin and served as control for the second group that received CFA and normal saline on day 0. This group in turn served as control for groups 3 and 4 that received CFA with either *L. stoechas* extract (100 mg/kg) or indomethacin (2 mg/kg) on day 0, respectively. Administrations were performed daily for 3 weeks at constant volume rates of 0.1 ml/100 g of rats weight. After that, the rats were housed 2 per cage. Food was placed on the floor of the cage to enable the rats getting access to food, because the pain that accompanies AA renders the rats immobile and unable to use their hind limbs and obtain the food from the cage's cover mesh.

The degree of the inflammatory reaction was evaluated by daily inspection and measuring the volume of right (primary reaction) and left hind paws (secondary reaction). The measurements were done before and after inoculation on day 0, and then on days 5, 7, 9, 11, 14 and 21. The volumes of the paws were measured plethysmometrically and expressed as increase in paw volume (ml) from the basal pre-injection values.

The severity of secondary reactions (including left hind paw) was assessed by a numerical grading system using a modified scoring technique (7). Each paw was scored from 0-3 according to degree of swelling: no (0), mild (1), moderate (2) and sever swelling (3). The nodules on ears and tail were scored 0-1 for the former and 0-3 for the latter (according to the extent of involvement of tail). The inflamed nose and prepuce were also scored 0-1 for each one. The maximum scores possible was therefore 15. Lesions in the right hind paw were considered as primary type, similar in all groups injected with CFA and were excluded from the adopted scoring system.

At the end of the experiments (day 21), blood was drawn from the heart for haematological examinations. The immunoglobulines (IgG, IgM and IgA) were determined in serum using single radial immunodiffusion method, NOR-partigen (Behring). The results are expressed as mean g/l.

Table I. Effect of oral administration of *L. stoechas* (100 mg/kg) and indomethacin (2 mg/kg) on the volume of right (R) and left (L) hind paws of rats with adjuvant arthritis (AA).

		Days					
		5	7	9	11	14	21
Paraffin	R	0.40±0.07	0.40±0.07	0.34±0.07	0.34±0.05	0.28±0.07	0.38±0.04
+saline	L	-	0.02±0.00	0.04±0.02	0.06±0.02	0.02±0.00	0.02±0.01
AA	R	1.80±0.19*	1.46±0.15*	1.32±0.17*	1.14±0.15*	1.14±0.14*	1.08±0.33*
+saline	L	0.06±0.02*	0.14±0.05	0.10±0.03	0.08±0.01	0.08±0.01*	0.08±0.01*
AA	R	2.38±0.16*	1.76±0.12*	1.76±0.16*	1.76±0.20*	1.74±0.18*	0.20±0.03**
+L.stoechas	L	0.14±0.03*	0.14±0.03	0.18±0.01*	0.16±0.01*	0.14±0.03*	0.32±0.07**
AA	R	0.62±0.15**	0.40±0.04**	0.35±0.05**	0.40±0.04**	0.30±0.03**	0.20±0.03**
+indomet.	L	0.02±0.00	0	0	0	0	0

Values are mean±S.E. of increase in paw volume (ml). The basal volume of the paws of normal rats averaged 1.18±0.02 ml. Statistically significant differences AA+saline vs paraffin+saline; *p<0.01. AA + *L. stoechas* and AA+indomethacin vs AA+saline; *p<0.05, **p<0.01.

Liver, spleen and both hind limbs were rapidly removed and fixed in 10% formalin solution. The samples were then dehydrated (bones were decalcified) and sectioned into 5 µm slices and stained with hematoxyline and eosine.

Statistical analysis

Changes in the paw volume, the scores attributed to the secondary reactions and the haematological parameters were evaluated separately for each experimental paradigm using *Mann Whitney U-test* and *Wilcoxon signed ranks test* for inter- and intra-group changes respectively. Values for paraffin+saline were compared with AA+saline to determine the significance of the effect of AA. Values for AA+saline were compared with AA+*L. stoechas* and AA+ indomethacin to determine the effect of either *L. stoechas* or indomethacin.

RESULTS

Effects on right and left hind paw volume

The volume of either hind paws (right or left) of normal rats (before the experiments) averaged 1.18±0.02 ml (n=40 rats).

Table I shows that paraffin produced a slight, non significant, increase (0.40±0.07 ml compared with preinjection value on day 0) in the volume of right hind paw on the day 5.

Injection of CFA induced an intensive inflammatory reaction at the injected right paw. This is known as the primary reaction. It is characterized by an increment of 1.80±0.19 ml on the day 5. The effect remained significant (p<0.01) till the day 21.

Table II. Effect of oral administration of *L.stoechas* (100 mg/kg) and indomethacin (2 mg/kg) on severity of the secondary lesions in rats with adjuvant arthritis (AA).

	2 Weeks		3 Weeks	
	Mean of scores (range)	%Animals with 15 scores	Mean of scores (range)	%Animals with 15 scores
Paraffin+saline	-	-	-	-
AA+saline	2.80±0.58 ^a (2-5)	-	3.60±0.51 ^a (2-5)	-
AA+ <i>L.stoechas</i>	10.20±1.96** (7-15)	40	10.80±1.71** (7-15)	40
AA+indomethacin	0.80±0.37** (1-2)	-	1.40±0.24** (1-2)	-

Values are mean ± S.E. with range of scores in parentheses. Statistically significant differences: AA + saline vs paraffin + saline; ^ap<0.01. AA + *L.stoechas* and AA + Indomethacin vs AA + saline :**p<0.01.

CFA-induced lesion was not confined to the site of injection but involved the whole paw. The rats also showed increased irritability on handling.

Administration of *L. stoechas* (100 mg/kg) increased further CFA-induced swelling and inflammatory reaction. The increase in paw volume was marked on the day 5, amounted to 2.38±0.16 (p<0.05) and became marked on the day 21 (1.68±0.13, p<0.01). On the other hand, oral administration of indomethacin (2 mg/kg) reduced the intensity of primary reaction and the volume of paw, from the day 5 to almost the level of normal rats received paraffin (0.62±0.15 ml, p<0.01), with an effect increased by its daily administration. The volume of the paw was reduced by 0.20±0.03 ml (p<0.01) on the day 21.

Table I also shows that paraffin increased the volume of left hind paw after the first week but only by 0.02-0.06 ml. The volume was increased up to the day 11 (0.06±0.02 ml), and declined thereafter. However, in AA rats the volume was increased on the day 5 (0.06±0.02, p<0.01), then became nonsignificant till the day 14 and then the volume was increased further on the day 21 (0.08±0.01, p<0.01). On the other hand, *L.stoechas* increased the volume of the left hind paw of AA rats by 0.14±0.03 ml on the day 5 (p<0.05) and its effect was increased on the day 21 (0.32±0.07 ml, p<0.01). Indomethacin prevented the increment in the volume of left hind paw after the first week.

Effects on the Secondary Reactions Scores (Table II)

Two weeks after the induction of AA, the primary reaction was accompanied by development of secondary reactions. These included erythema and swelling in the left hind paw and both fore paws besides nodules in the tail (score 1 only). The mean scores were 2.80±0.58 with a range of 2 to 5 (p<0.01). At the third week, the scores were

Table III. Changes of body weight (gm) of rats with adjuvant arthritis (AA) received orally either saline or *L. stoechas* (100mg/kg) or indomethacin (2 mg/kg).

	pre	Days								
		1	3	5	7	9	11	14	21	
Paraffin +saline	185.00	186.71	191.89	197.94	197.94	200.53	201.03	217.73	226.04	
	±	±	±	±	±	±	±	±	±	
	10.28	9.89	10.89	11.82	12.37	13.03	13.42	3.98	6.50	
AA +Saline	183.00	184.00	176.00	178.00	176.00	183.00	181.00	172.00 ^a	166.00 ^a	
	±	±	±	±	±	±	±	±	±	
	9.88	7.50	8.72	8.46	9.54	9.95	10.42	10.79	10.30	
AA +L.Stoechas	189.17	176.83	176.67	176.67	175.83	175.83	175.00	170.00	164.67	
	±	±	±	±	±	±	±	±	±	
	10.2	9.85	7.92	8.72	10.44	9.13	9.13	7.75	6.79	
AA +Indomet.	185.00	180.80	178.12	178.12	178.12	180.50	183.04	190.24*	196.14*	
	±	±	±	±	±	±	±	±	±	
	2.50	4.00	3.80	3.80	3.80	4.20	4.50	14.60	11.80	

Values are mean ± SE. Statistically significant differences: AA + saline vs paraffin + saline; ^ap<0.01. AA + indomethacin vs AA + saline; *p<0.05.

increased to 3.60 ± 0.51 , but with the same preceding range and level of significance. None of the AA rats received saline showed complete development and generalization of the secondary reactions or maximum scores of 15. Moreover, only 20% of rats exhibited nodules on the tail and with score 1 only.

L.stoechas increased the incidence and intensity of the secondary reactions. In addition to the inflammatory reactions observed in the fore paws, nodular reactions were also observed in the ears (score 1) and the tail (score 1-3). The inflammatory reaction also involved the nose and penis. The total scores were increased to 10.20 ± 1.96 at the second week and to 10.80 ± 1.71 at the third week with the same level of significance ($p<0.01$). Moreover, the secondary reactions were fully developed in 40% of rats that received *L.stoechas*. Indomethacin significantly reduced the scores of the secondary lesions to 0.80 ± 0.37 at the second week and to 1.40 ± 0.24 at the third week ($p<0.01$). The range of the scores remained between 1 and 2.

Effect on the body weight (Table III)

Development of the secondary reactions at the second week was accompanied by a significant ($p<0.01$) reduction of the body weight of AA rats. Moreover, *L.stoechas* while intensified the secondary reactions reduced further the body weight. Although it appears slightly different from that of AA+saline group, the reduction in the body weight of AA rats by *L. stoechas* was significant at a greater level ($p<0.004$) on comparison with paraffin+saline. However no significant difference was obtained between saline- and *L. stoechas*-treated AA rats. On the other hand, in indomethacin-treated AA rats, reduction of the body weight was less than saline- or *L.stoechas*-treated AA rats. At the second and third week, the body weight averaged 190.24 ± 14.60 gm and 196.14 ± 11.80 gm respectively. This is significantly ($p<0.01$) less than the value for

Table IV. Effect of oral administration of *L. stoechas* (100 mg/kg) and indomethacin (2 mg/kg) on immunoglobulines of rats with adjuvant arthritis (AA).

	Immunoglobulines (g/L)		
	IgG	IgM	IgA
Paraffin+saline	53.37±2.80	-	-
AA+saline	67.33±2.44 ^a	-	-
AA+ <i>L.stoechas</i>	67.31±2.66	-	-
AA+indomethacin	59.81±2.88 ^b	-	-

Values are mean±S.E. Statistically significant differences: AA+saline vs paraffin+saline; ^ap<0.01. AA+indomethacin vs AA+saline; ^bp<0.03.

paraffin+saline group but still greater than the value for AA rats that received either saline or *L.stoechas* (p<0.05).

Haematological effects

Effect on the immunoglobulines.

Table IV shows that IgG was the only immunoglobulin identified in the rats used in this study. IgG was increased significantly (p<0.01) in AA-rats: 67.33±2.44 g/l compared with 53.37±2.80 g/l in normal rats injected with paraffin and received saline. *L.stoechas* did not alter IgG. Indomethacin decreased IgG to 59.81±2.88 g/l (p<0.03) in AA rats.

Effect on blood picture (Table V).

Injection of paraffin was not accompanied by any significant change in the haematological parameters investigated. However, CFA-induced AA was accompanied by a reduction in the number of RBCs from of the pre value 4.84±0.74 x10⁶ to 3.18±0.50 x10⁶ (p<0.05) after 3 weeks. The total WBC count was not changed from the pre-injection value (7750±574.46). The percentage polymorphonuclears (neutrophils) were increased from 43.80±1.21 to 57.80±2.75 (p<0.002). The percentage lymphocytes were decreased from 51.80±2.86 to 38.00±2.89 (p<0.05). No significant changes were obtained for the values of monocytes and eosinophils.

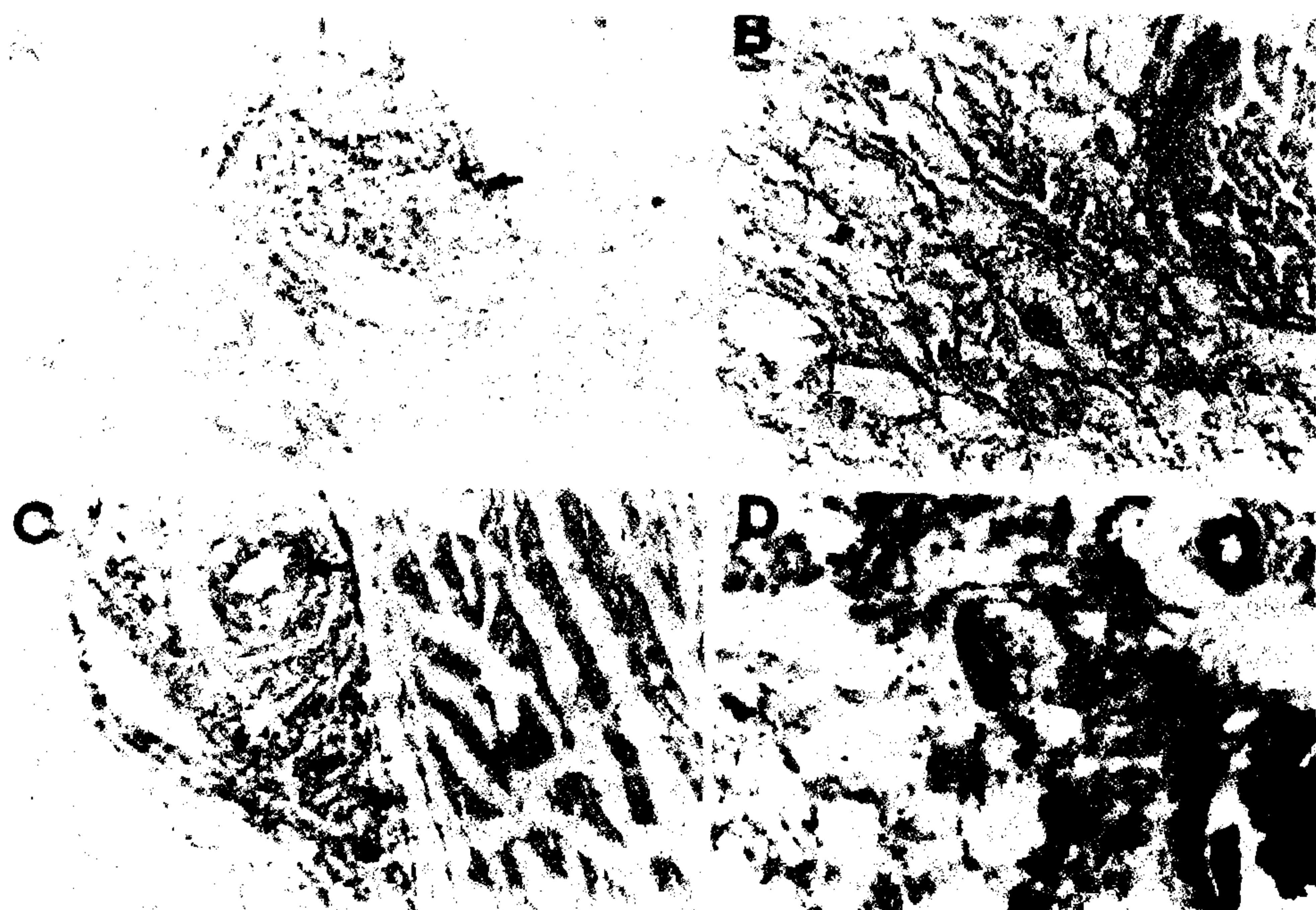
L.stoechas decreased further the number of RBCs in AA rats to 1.84±0.23 x10⁶ (p<0.05). Moreover, *L.stoechas* inhibited AA-induced increase in the polymorphonuclear neutrophils. The percentage value of the latter was 43.20±3.22 for AA+*L.stoechas*. This value was very close to that of paraffin+saline group and significantly less than AA+saline group (p<0.05). *L.stoechas* inhibited CFA-induced decrement in the lymphocytes. It remained almost close to paraffin+saline group (53.00±3.91 %, p<0.05). No other changes were produced by *L.stoechas* in AA rats. Except a slight reduction in the polymorphonuclears, indomethacin at the tested dose did not produce any significant change in the haematological picture.

Table V. Effect of oral administration of *L.stoechas* (100 mg/kg) on the haematological parameters of rats with adjuvant arthritis (AA).

	pre	After 3 weeks		
		Paraffin+saline	AA+saline	AA+ <i>L.stoechas</i>
Haemoglobin (g/dl)	11.7±0.44	11.96±0.21	10.72±0.45	10.20±0.13
RBC ($\times 10^6$)	4.84±0.74	4.95±0.35	3.18±0.50 ^a	1.84±0.23*
WBC				
Total count	7750±574.46	7700±714.00	7520±816.95	8120±875.82
Polymorphes(%)				
Neutrophils	43.80±1.21	43.23±5.41	57.80±2.75 ^b	43.20±3.22*
Eosinophils	1.60±0.31	1.75±0.45	1.60±0.24	1.60±0.32
Basophils	-	-	-	-
Lymphocytes(%)	51.80±2.86	52.10±2.91	38.00±2.89 ^a	53.00±3.91*
Monocytes (%)	2.80±0.57	2.92±0.51	2.60±0.32	2.20±0.37

Values are mean±S.E. Statistically significant differences: AA+saline vs paraffin+saline; ^a p<0.05, ^b p<0.002. AA+*L.stoechas* vs AA+saline ;*p<0.05.

Fig. I. Light micrographs of representative histopathological changes observed in the synovium of AA rats. A-right hind paw of AA+saline rats: cellular infiltration with vascular changes. B-left hind paw of AA+saline rats. C-right hind paw of AA+*L.stoechas* rats: cellular infiltration, vascular changes with muscular hypertrophy and oedema. D-left hind paw of AA+*L.stoechas* rats: cellular infiltration with vascular changes. Magnification:x400.



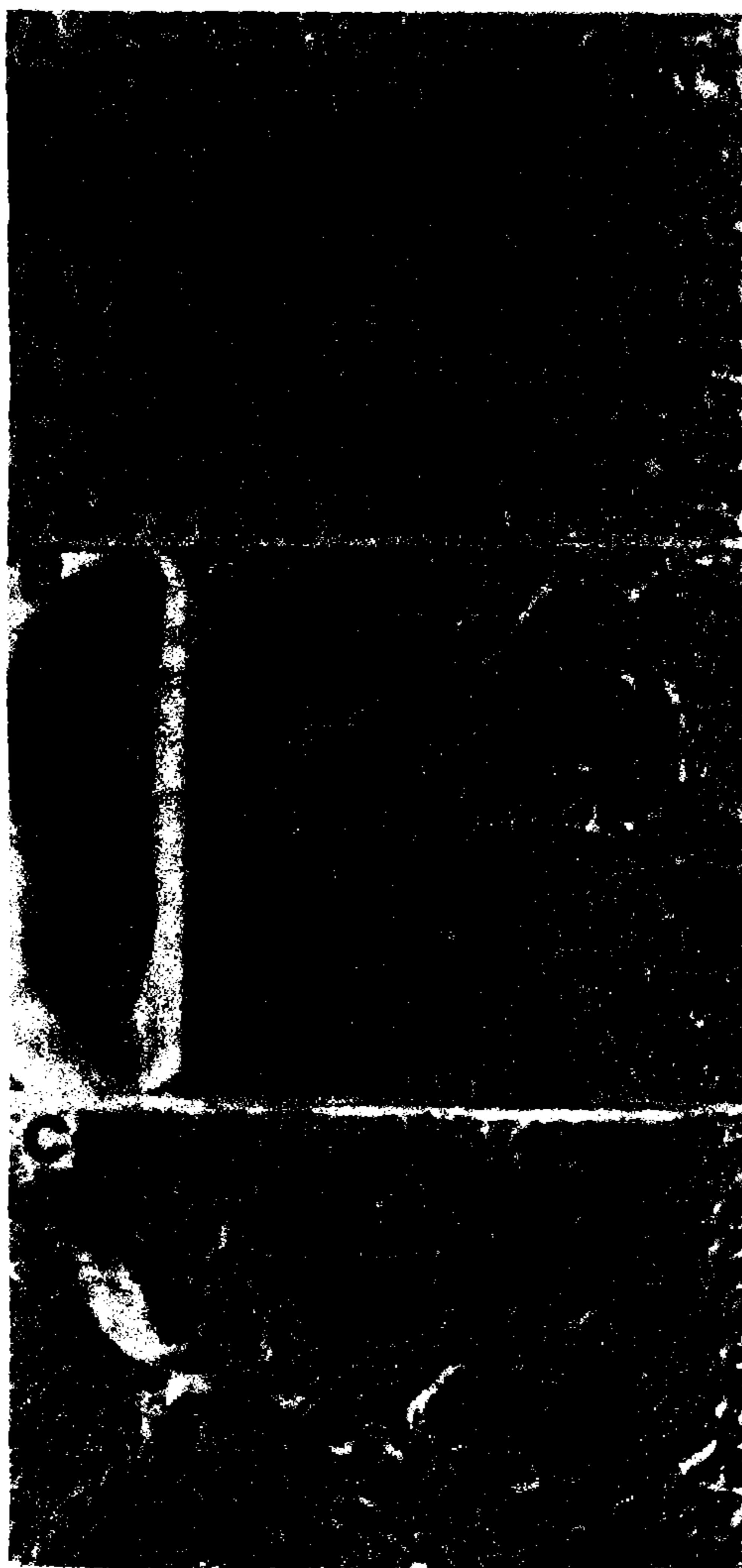


Fig. II. Light micrographs of representative histopathological changes observed in the spleen of AA rats. A- AA+saline: extensive infiltration of the red pulp with mononuclears. B and C are sections from AA rats received *L.stoechas*. B- Coagulative necrotic areas (arrows). The intercept is the enlarged spleen from which the section was performed. Infarcted areas can be seen (arrows). C- hyperplastic areas around the white pulp (arrows) with extensive mononuclear cellular infiltration . Magnification: x 160.

Histopathological examinations

Histopathological sections from AA rats showed reactive and destructive changes in the articular surface with inflammatory reactions in the synovium and the surrounding soft tissues. The representative changes are shown in fig. I. An extensive inflammatory cellular infiltration (lymphocytic, plasmocytic and polymorphnuclears) could be seen in the synovium of right paw especially around the blood vessels (fig. I A). Erosion of the articular cartilage was also evident. No distinctive changes were evident in the left synovium (fig. I B). On the other hand, in the right paw of AA rats that received *L.stoechas*, there was an extensive vascular reaction (proliferation, dilatation and thickened walls) in addition to the changes observed in AA rats received saline. The blood vessels were seen encuffed by inflammatory cellular infiltration with an evidence for inflammatory reactions in their walls. The intravascular erythrocytic content was less abundant in comparison with AA rats received saline. Muscular hypertrophy with edema could also be seen surrounding the right knee (fig. I C). Moreover, in the left paw of AA rats that received *L.stoechas* there were discrete foci of inflammatory mononuclear (lymphocytic and plasmocytic cells) with increased vascularization (fig. I D).

As to the spleen, fig. II A shows that the red pulp of the spleen of AA rats that received saline was infiltrated with a yellow-brown pigment containing macrophages. On the other hand, in the spleen of AA rats received *L.stoechas*, foci of coagulative necrosis with infiltration of macrophages containing yellow-brown pigment could be seen in the red pulp (fig. II B). Moreover, the lymphoid follicles were more distinct and there was an extension of the germinal center (lymphoid hyperplasia) in the spleen of AA rats received *L.stoechas*, (fig. II C).

The liver of all samples examined did not show any significant pathological changes except a slight infiltration with inflammatory mononuclear cells in the periportal area (data not shown).

DISCUSSION

We have investigated the effect of *L. stoechas* on AA of rats. The unique feature of this work is that the extract facilitated AA with an effect could be involving the lymphocytes.

Since its advent, AA of rats gained a wide popularity as an animal model analogous to RA of humans (11). AA is used for both investigating the effects of antirheumatic drugs and mechanisms involved in the development of arthritis.

The primary and secondary reactions observed in this study are similar to those reported for AA rats (4, 12). These changes in AA rats include alterations in the polymorphonuclears and lymphocytes. The initial (primary) reaction is suggested to be due to irritation (inflammatory response) while the later occurring (secondary) reactions are presumably immunological events (12). In this study, B-cells could be involved in

the development of AA; as IgG was significantly increased in AA rats. The peripheral WBC count was not changed. This result agrees with that reported in RA (13). Moreover, our results showed that AA decreased the lymphocytes and increased polymorphnuclears (neutrophils) but did not change the monocytes. This result is in line with that reported for AA (14). The decreased lymphocytes in AA rats could be ascribed to decreased T-suppressor cells (15), mixed lymphocyte reaction (16) and to increased T-helper/T-suppressor cell reaction following decreased suppressor cells (17). This result could be a consequence to shunt of lymphocytes towards B-lymphocytes synthesis. Moreover, the secondary type reaction has been shown to be mediated by deposition of immune complex, especially in the nodular type reactions (18). The elevated IgG level, infiltration of the red pulp of the spleen by macrophages containing yellow-brown pigment and changes in the walls of the blood vessels may also suggest that AA involves an immunological reaction.

L.stoechas, as a part of its facilitatory effect, increased the primary reaction (the volume of right hind paw of AA rats). More interestingly, *L.stoechas* intensified the secondary reactions after the second week and generalized the reaction in 40% of AA rats.

Several agents used in immunopharmacology are reported to aggravate the secondary reaction in AA rats. This effect is mediated by either increasing T-cells as in case of levamisole (19) or by influencing preferentially the suppressor cells as it was suggested for very small doses of cyclophosphamide (20). *L.stoechas* also seems to influence lymphocytes in a similar way because the effect on B-type was excluded as *L.stoechas* did not alter the level of IgG in AA rats. Moreover, *L.stoechas*, while intensified the secondary reaction, antagonized AA-induced reduction in the lymphocytes. This result may suggest an effect of *L.stoechas* on T-cells and a shift in the balance T-helper/T-suppressor cell to the T-helper lymphocytes side. However, this probability remains to be verified by further studies. Moreover, splenomegaly of rats received *L.stoechas* and the histopathological changes, namely the lymphoid hyperplasia and coagulative necrotic changes in the spleen, besides the reactive changes in the walls of the blood vessels, may explain in part the involvement of the immunological reactions in the facilitation of AA of the rats by *L.stoechas*.

The reduced erythrocytes in this study is suggested to be of chronic disease type because in AA rats serum total iron binding capacity is increased whereas ferritin levels are not changed (our unpublished data). This result could involve immunological events and it is in line with the results reported for AA. It could be ascribed to a variety of factors including sequestration of the erythrocytes by the endothelial slits of the spleen (21). In this regard, an interesting effect was that produced by *L.stoechas* on the spleen. Histopathological examinations revealed sequestration of what could be erythrocytes in the slits of the spleen. The latter was more pronounced in AA rats received *L.stoechas*. This effect and the scarcity of erythrocytes in the blood vessels of AA rats received *L.stoechas* may also explain, at least in part, the further reduction of RBCs in AA rats by *L.stoechas*. However, other effects such as a defect in mobilization

of iron due to an extracorporeal hemolytic factor (22) or complement-induced haemolysis should also be considered (23). Moreover, deficiency of erythropoiesis is also suggested as a factor involved in RA-anemia. Erythropoiesis is influenced by T-lymphocytes-dependent immunological processes (24). This result adds further support to the suggestion that immunological processes contribute to the development of AA-induced anemia (24).

Another change paralleled the secondary reactions was the reduction in the body weight. Although there was no significant change among the groups in the first 11 days, the body weight of AA rats was reduced significantly on the second week, the time when the secondary reactions were evident. Moreover, *L. stoechas* while intensified the secondary reactions also reduced the body weight. This effect could be due to a reduced food consumption caused by pain associated with the secondary reactions in AA rats. We do not think that the reduction in the body weight was caused by inability of the rats to get access to the food, because the food was placed inside the cage and at the floor. On the other hand, the body weight of indomethacin-treated AA rats was greater than AA rats received either saline or *L. stoechas*. This may be ascribed to the reduction in the intensity of the secondary reactions produced by indomethacin.

In conclusion, *L. stoechas* seems facilitating AA by increasing lymphocytes which could be of T-type. *L. stoechas* seems as a promising natural product for cases in which an increase in the lymphocytes is required.

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