

SOME PHARMACOLOGICAL STUDIES ON LAVANDULA STOECHAS PLANT GROWING IN TURKEY

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SUMMARY

The flowers and leaves of *Lavandula stoechas* were extracted with 50% ethanol. The extract at 0.001-0.1 mg increased the spontaneous motility of isolated rabbit jejunum, while at 0.3-5.0 mg decreased it. This effect was antagonized by papaverine and was only slightly reduced by atropine, indicating that the effect on intestine could be a direct one and not related to the muscarinic cholinergic receptors. The extract at 0.4-6.0 mg displayed a negative inotropic and chronotropic effect on the isolated rabbit heart. This effect was not changed by dobutamine or digoxin, suggesting an effect not related to β -receptors or $\text{Na}^+ \text{K}^+$ ATPase in the heart. In a series of chronic experiments on rats, the extract (administered at 100 mg/kg p.o for 14 days) increased the lymphocytes and monocytes, whereas decreased neutrophils. No effect was exerted on the body weight.

Key Words: *Lavandula stoechas*, rabbit, isolated intestine and heart, blood

ÖZET

LAVANDULA STOECHAS BITKİSİ İLE İLGİLİ BAZI FARMAKOLOJİK ÇALIŞMALAR

Lavandula Stoechas (Karabaş) bitkisi ülkemizde yaygın bir şekilde bulunur. Bitkinin içinde pinenler, glikozidler, saponinler, cineol ve camphene gibi tıbbi olarak aktif birçok madde vardır. Halk arasında Karabaş bitkisinin sedatif, analjezik, antiseptik, hipoglisemik, spazmolitik ve kardiyotonik etkilerinin olduğunu inanılır. Çalışmamızda, bitkinin çiçek ve yapraklarının ekstresi %50'lik etanol kullanılarak hazırlandı. Ekstrenin 0.001-0.1 mg'ının izole tavşan jejunumunun spontan motilitesini artırmasına karşın, 0.3-5.0 mg'ı motilitenin azalmasına neden oldu. Bu etki papaverin tarafından antagonize edildi, atropin tarafından ise hafifçe azaltıldı. Ekstrenin 0.4-6.0 mg'ı izole tavşan kalbinde negatif inotrop ve kronotrop etkilerin ortaya çıkmasına neden oldu. Bu etkinin dobutamin ve digoksin tarafından değiştirilmemesi, β -reseptörleri ve $\text{Na}^+ \text{K}^+$ ATPaz'dan bağımsız olduğunu göstermektedir. Kronik deneylerde ekstre, 100 mg/kg dozunda oral olarak 14 gün boyunca sıçanlara uygulandı. Hematolojik incelemelerde, total lökosit ve eozinofil değerlerinde anlamlı bir değişiklik bulunmadı; ancak lenfosit değerlerinde ($50.00 \pm 4.14 \rightarrow$

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68.83 ± 4.01 , $p < 0.01$) ve monosit değerlerinde artış ($3.5 \pm 1.06 \rightarrow 5.33 \pm 0.67$, $p < 0.001$), nötrofil değerlerinde ise azalma ($45.5 \pm 4.43 \rightarrow 24.00 \pm 3.95$, $p < 0.01$) olduğu gözlandı. Eritrosit sayısında ise belirgin olmayan bir düşüş görüldü.

Negatif inotrop etki hariç bu çalışmadan elde edilen diğer sonuçlar, Karabaş bitkisinin, özellikle yüksek konsantrasyonlarda, bir antispazmodik ve tonik olarak klinik önemini doğrulamaktadır.

Anahtar Kelimeler: *Lavandula stoechas*, tavşan, izole bağırsak ve kalp, kan

INTRODUCTION

Lavandula stoechas L. (Labiaceae) is widely distributed in the mediterranean countries especially in Turkey where it is known as Karabash. The aerial parts (flowers and leaves) contain triterpinoids (1), pinen derivatives (2), glycosides, saponins, borneol-rich volatile oil and cineol (3) besides camphene (4).

The plant is used by the folk for treating a variety of diseases including cancer. It is reported to be active as analgesic, antiseptic, and useful in cramps and lung ailments (4). It is also effective in eczema (3) and possesses a hypoglyemic activity (5).

The present study was carried out to investigate the effects of the flowers and leaves of *L.stoechas* on some isolated organs of rabbits, and haematological picture of rats.

MATERIALS AND METHODS

Animals: Male albino rabbits weighing 2.0-2.5 kg and Wistar rats weighing 180-200 g were used. The animals were housed (rabbits four in 72 X 46 X 36 cm and rats five in 42 X 26 X 15 cm cages) for at least one week before any experiment. Both housing and experiments were performed under standardized conditions of temperature (22 ± 2 °C), humidity ($50 \pm 5\%$) and 12:12 light:dark cycle. Food and water were supplied ad libitum except the rabbits were starved the night before the experiments.

Extraction of plant samples: The flowers (purple) and leaves (dark green) of *L.stoechas* were collected from the mediterranean coast of Turkey. Samples of 5 g. each were extracted by 50% ethanol. The extraction was done by a magnetic stirrer in three successive stages each with 200 ml of 50 % ethanol for six hours. This procedure was sufficient for complete extraction (i.e.exhaustion) of the samples. The samples were filtered and the solvent was evaporated under reduced pressure. The extract was then concentrated yielding a green-brown extract amounted to 33.1 ± 0.5 % of the dry plant weight.

Effects on isolated rabbit heart and intestine: The rabbits were anesthetised with sodium pentobarbital (35 mg/kg, i.v.) and were injected with heparine (500 units/kg, i.v.), then ventilated with room air using a rodent ventilator (Ugo-Basile) set at 80

strokes per minute. The heart was exposed and an aortic cannula (connected to perfusion solution) was introduced to the heart in situ. Then the heart was removed rapidly and attached via the aortic cannula to a modified Langendorff perfusion apparatus (6). The perfusion solution, Krebs-Henseleit (37°C , PH = 7.4 and perfusion pressure = 55 mmHg), contained (mM): NaCl 118.0, KCl 4.7, CaCl₂ 2.52, MgSO₄ 1.66, NaHCO₃ 24.88, KH₂PO₄ 1.18, Sodium Pyruvate 2.0 and glucose 5.55. The solution was aerated by the carbogen (95 vol.% O₂ and 5 vol. % CO₂).

The force of cardiac contractility (left ventricular isovolumetric pressure) was measured by the balloon method. Briefly, a latex balloon (diameter=10 mm, length=14 mm and volume=0.4 ml) was introduced in to the left ventricle through the pulmonary vein and connected via a pressure transducer (Bentley TRANTEC-Physiological pressure transducer, Irvine, California, USA) to a 2-channel recorder (Gemini 7070-UGO Basile). The heart rate and ECG were recorded by electrodes connected to the right auricle and a wick electrode was used as a counter electrode at the apex. The aortic cannula was used as a reference (zero) electrode. The electrode were connected to a Logos 8821 electrocardiograph. The injections were made through a stopcock placed above the aortic canula. The volume of the space between the stopcock and the aortic cannula was 4 ml. The values of the drugs concentrations were corrected accordingly and were expressed as the final concentrations.

The motility of pieces of 2-3 cm jejunum were recorded isotonically (7) by bathing the specimens in Tyrode-standard solution (37°C , PH = 6.8) contained (mM): NaCl 136.9, KCl 2.68, CaCl₂ 1.80, MgCl₂ 1.05, NaHCO₃ 11.90, NaH₂PO₄ 0.42 and glucose 5.55. One end of the jejunum was fixed in tyrode solution in the inner organ chamber (20 ml) and the other end was connected to the 2-channel recorder via an isotonic transducer (UGO Basile Comerio-(va)-Italy). A preload of 2 g was applied to the transducer. The extract was added to the inner organ chamber and the final concentrations were considered.

Haematological studies: The extract (100 mg/kg p.o.) was administered for 14 days. Blood samples were taken directly from the heart before and at the end of the experiments, then sacrificed by deep ether anesthesia and histopathological examinations of heart, lung, liver, spleen and kidney were carried out.

Statistical analysis: Data for the haematological studies were analysed by Mann-Whiyness U-test using Wearnes-Boldline series Computer.

Drugs: The following drugs were used; Atropine sulphate (Galen drugs-Turkey), Digoxin (Sandoz), Dobutamine (Dobutrex-Eli Lilly), Indomethacine (Nobel, Turkey) and Papaverine Hcl (Haver, Turkey).

RESULTS

Effects on isolated heart: The extract at concentrations of 0.01-6 mg produced a negative choronotropic (data not shown) and inotropic effect (figure I) with ED50 of 2

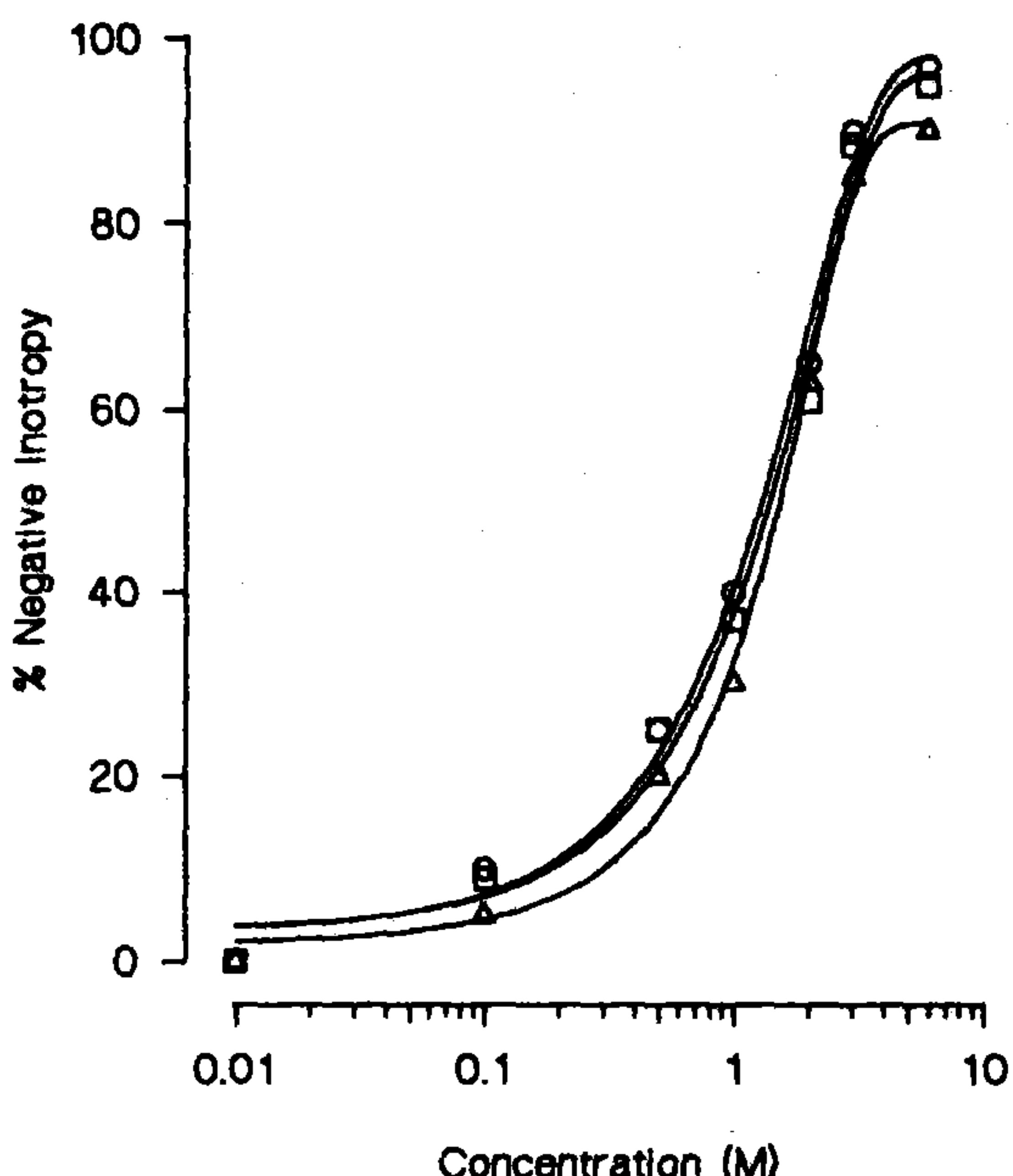


Figure I. Effect of 50 % ethanol extract of *L. stoechas* on the isolated rabbit heart, alone (O) and with dobutamine, 10^{-5} M (Δ) and digoxin, 10^{-6} M (\square). Values are means of at least three separate experiments and expressed as % change of each concentration with the basal pre drug state in case of the extract alone, and with the corresponding digoxin in case of interaction of dobutamine and digoxin.

mg for the isolated heart. A dose response curve was constructed for dobutamine 10^{-5} and 10^{-4} M and digoxin (10^{-7} M to 10^{-6} M). The ED50s of their positive inotropic concentration (1×10^{-5} M for dobutamine, and 1×10^{-6} M for digoxin) were chosen to study their interactions with the extract. Figure I also shows that dobutamine and digoxin did not produce any detectable change in the dose-response curve of the extract.

Effects on isolated jejunum: Figure II shows that the extract produced a biphasic effect. At small concentrations, 0.001-0.08 mg, the extract increased the spontaneous motility while at larger concentrations, 0.3-4.8 mg, reduced it. In separate experiments, dose response curves for the effect of papaverine (10^{-4} M and 10^{-6} M) and atropine (10^{-7} M and 10^{-6} M) and acetylcholine (10^{-14} - 10^{-8} M) were constructed and the proper ED50 concentrations of each of atropine (1×10^{-6} M) and papaverine (0.4×10^{-4}) produced 50% reduction in the intestinal motility were selected to study their interaction with small concentration of the extract. Figure II shows that papaverine reversed the effect of the extract whereas atropine displayed lesser interaction; it reversed only the effect of the smallest concentration of the extract.

Haematological effects : Figure III shows that the extract at 100 mg/kg p.o. for 14 days produced a significant increase in lymphocytes ($p < 0.01$) and monocytes ($p < 0.003$) while it reduced neutrophils ($p < 0.01$). Eosinophils were reduced non-

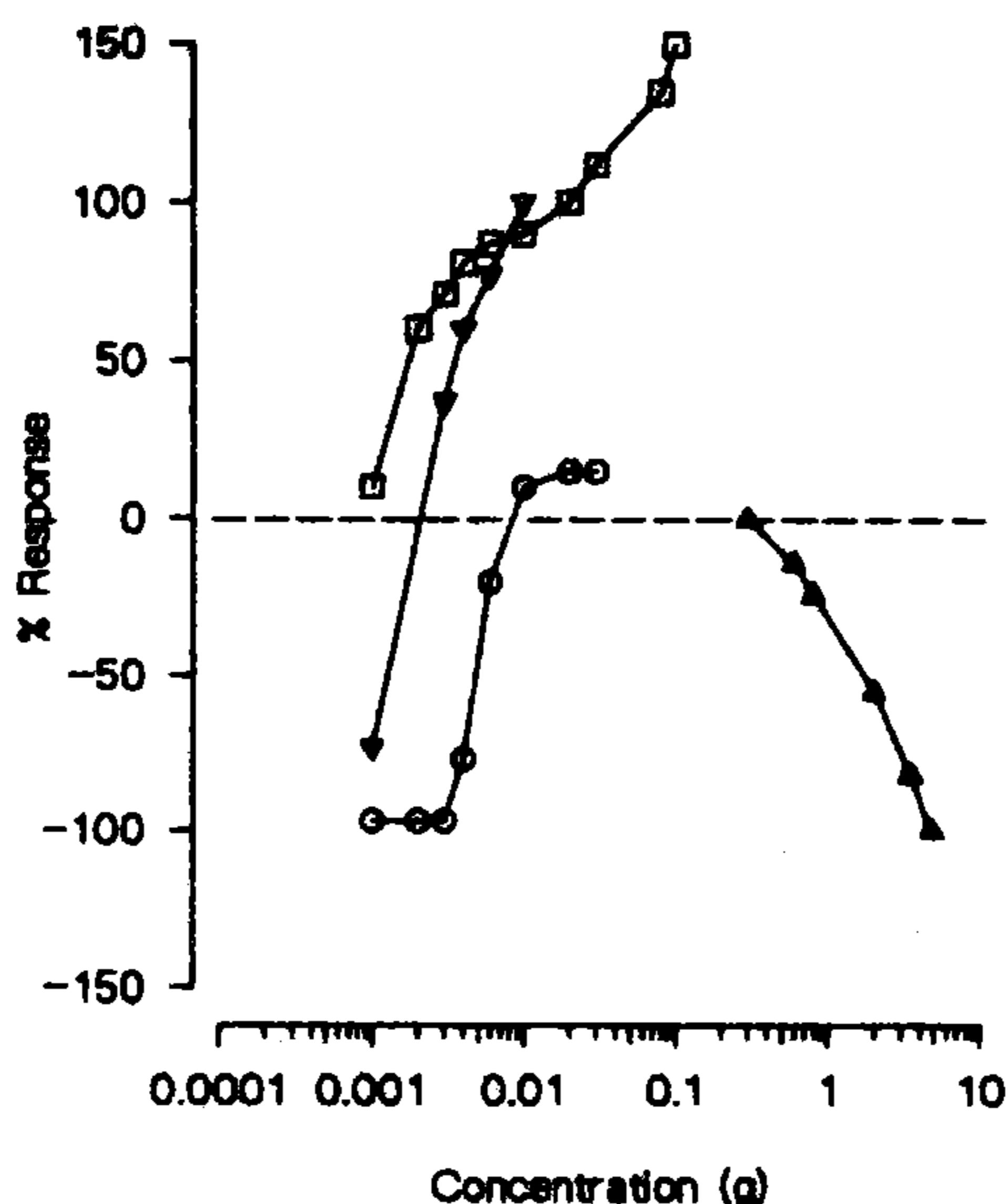


Figure II. Effect of 50 % ethanol extract of *L. stoechas* at low (□) and high (△) concentrations on the spontaneous motility of isolated rabbit jejunum and interaction of atropine 10^{-4} M (▽) and papaverine 10^{-4} M (○) with *L. stoechas*. Values are means of at least three separate experiments and expressed as % change from the basal pre-extract alone state and in case of interaction experiments with each corresponding concentration of the extract.

significantly. No changes in the total WBC, RBC and hemoglobin were obtained. No gross behavioral changes were observed during the experiment. Some rats showed soft fecal pillets, but no diarrhea was evident.

DISCUSSION

The results of the present study revealed some of the pharmacological activities of *L. stoechas*. Some of the results are in line with the purpose for which the plant is used for by the folk. However, the negative inotropic and chronotropic effects contradict the idea that the plant is cardiotonic (3). Dobutamine is well known as a β -agonist, with more selective effect on β_1 -receptors (9). In this study, dobutamine did not modify the negative inotropic and chronotropic effect of the extract. Moreover, digoxin, which is known to inhibit the membranous Na^+, K^+ -ATPase (10) and increase intracellular Ca^{2+} in the heart (11), did not alter the negative inotropic effect of *L. stoechas*. Accordingly, we suggest that the effect of the extract on the heart did not involve β_1 -receptors or sodium pumps in the heart.

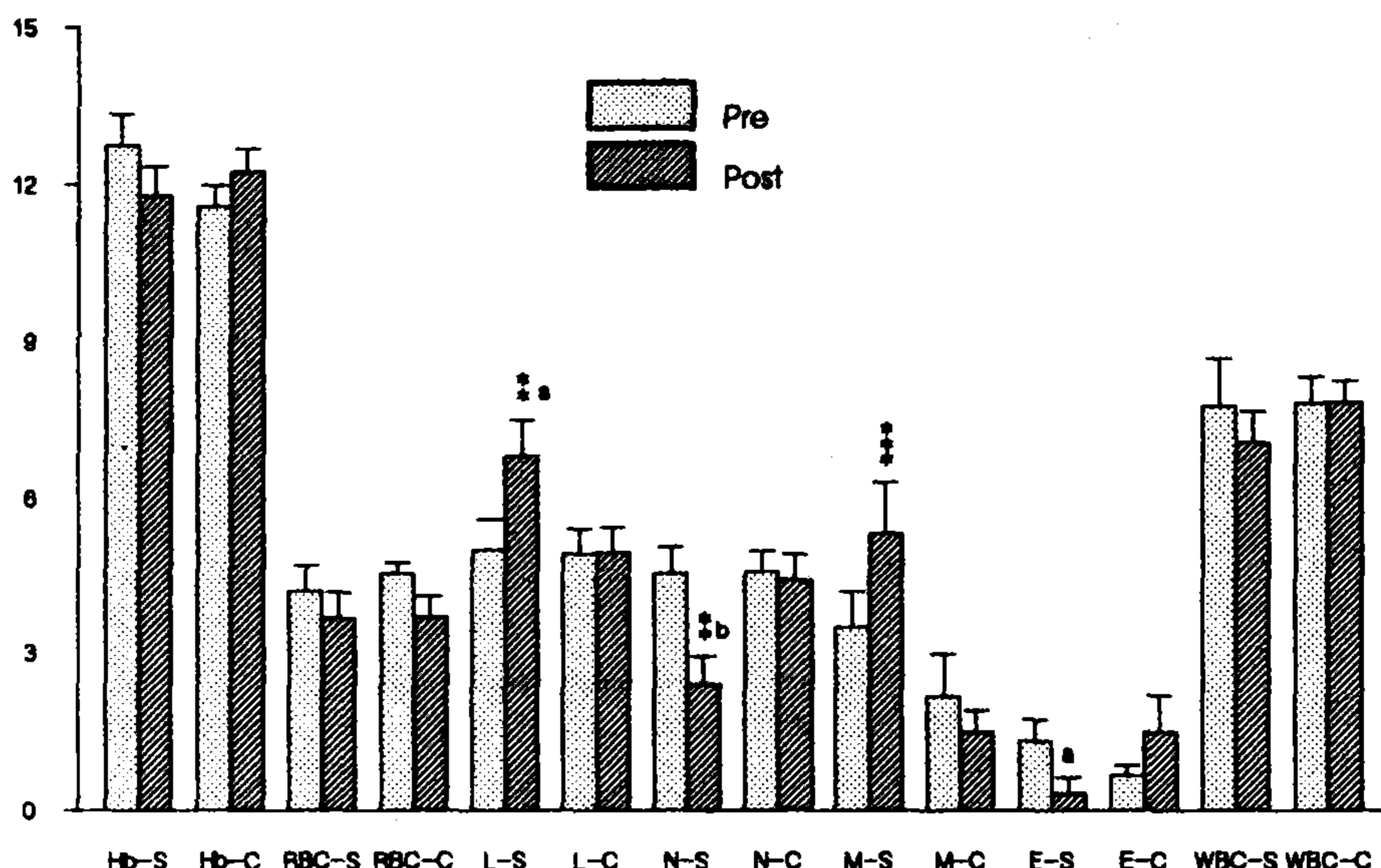


Figure III. Effect of 50 % ethanol extract of *L. stoechas* (100 mg/kg, p.o.) administered for 14 days on blood of rats. Values are mean \pm S.E. of 10 rats in each group. Statistically significant differences (Mann-Whitney U-test): extract vs control: ** $p<0.01$, *** $p<0.003$; pre vs post: * $p<0.001$, $^b p<0.01$. Abbreviations: Hb, hemoglobin; RBC, red blood cells; WBC, total white blood cells; E, eosinophils; L, lymphocytes; M, monocytes; N, neutrophils.

In experiments on the isolated intestine, it was found that atropine (a muscarinic cholinergic blocker) did not produce any significant interaction with the effect of the extract on jejunum. Moreover, papaverine is a generalized smooth muscle relaxant. It acts by inhibiting intracellular phosphodiesterase and increases cytoplasmic cAMP (12). In this study, papaverine produced a prominent antagonism of the effect of small concentrations of the extract. These findings suggest that the increase in the spontaneous intestinal motility involves more possibly a direct effect on the intestine and is not mediated by the muscarinic receptors. The latter effect may explain the softness of the faeces produced by the extract, but no diarrhea or pathological changes were observed in the chronic experiments. It can be suggested that the small concentrations of the extract may be useful for conditions in which increased intestinal motility is required.

The haematological effects are of great interest because the plant is thought by the folk to be useful in some inflammatory conditions and in cancer. In this study, the extract increased lymphocytes and monocytes while reduced neutrophils in normal rats. These results show that *L. stoechas* could be promising for the conditions in which

increased lymphocytes and/or monocytes are required. However, in our studies on effect of the extract on adjuvant arthritis we found that the plant did not change immunoglobulines (unpublished data). Accordingly, the effect of the extract on the blood seems to not include any effect on the immunoglobuline-producing cells. The type of lymphocytes involved in the effect of the extract remain to be clarified by further studies.

In conclusion, *L. stoechas* seems to possess interesting pharmacological effects which render it useful in the treatment of a variety of diseases. The active ingredients in *L. stoechas* and its other effects are going to be determined by further studies.

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