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Original Article

Topical Cotton Boll Extract as an Adjunct to Standard Pentavalent Antimonial Therapy in Cutaneous Leishmaniasis Caused by *Leishmania tropica*: A Randomized Pilot Clinical Trial

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Abstract

Background: Cutaneous leishmaniasis (CL) is a widespread disease with a serious global public health concern. Although pentavalent antimonial (MAT) drugs remain the standard treatment, their painful administration, incomplete responses, and poor patient adherence highlight the need for alternative or adjunctive therapies. Herbal and Traditional medicines are increasingly investigated as affordable and accessible options. This research aimed to assess how well a topical herbal treatment made from cotton boll extract worked and how safe.

Methods: This randomized, open-label clinical study evaluated the efficacy of a topical herbal formulation derived from cotton boll extract as an adjunct to MAT therapy. This study was conducted in 2025 at the CL Treatment Center in Kerman and included 58 patients with PCR-confirmed *Leishmania tropica* infection. Volunteer participants were randomly assigned to receive either intralesional MAT plus topical cotton boll extract or MAT combined with cryotherapy. Outcomes included time to lesion healing, changes in lesion size and induration.

Results: Results showed a reduction in lesion diameter and induration in both groups throughout the follow-up assessments. By week 12, complete lesion healing was detected in 95.2% of cases in the experimental group and 83.3% in the control arm; there was no meaningful difference between treatments. No significant adverse effects were observed.

Conclusion: Topical cotton boll extract can be considered as a non-invasive adjunctive therapy in the management of CL.

Introduction

Cutaneous leishmaniasis (CL) is a vector-borne disease (1). More than 20 species of *Leishmania* have been identified as pathogenic to humans (2). Sand flies serve as its biological vectors (1). In the Middle East, Asia and North Africa, the majority of CL cases are caused by two *Leishmania* species: *Leishmania major*, the etiological agent of zoonotic (rural) CL, and *Leishmania tropica*, the etiological agent of anthroponotic (urban) CL (3).

Humans are considered the primary reservoir host for *L. tropica* infections. Following infection by a sand fly, Cutaneous lesions develop after a few weeks and may persist for more than 1 year, that are often difficult to treat, and commonly heal with permanent scarring (1, 4).

In 2020, 89 countries reported endemic transmission of CL. According to WHO, Afghanistan, Iran, Iraq, Colombia and Syria are among the countries most severely affected by CL outbreaks (5). Moreover, armed conflict, political instability, and mass population displacement—particularly following the war in Syria—have led to resurgence and outbreaks of CL in neighboring countries (6-9).

In infections caused by *L. tropica*, treatment of all patients is recommended to reduce the human reservoir and limit disease transmission. The first-line therapy for CL consists of pentavalent antimonial compounds, particularly meglumine antimoniate (MAT, Glucantime®), which is most commonly administered intralesional or intramuscularly. Adjunctive therapies include topical antibiotics, second-line derivatives and liquid nitrogen use as outlined in standard treatment protocols. (10).

However, intralesional injections are often painful, which may result in poor patient adherence, missed follow-up visits, and treatment failure (11). Additional challenge in Iran is the emergence of resistance to pentavalent antimonials, particularly among *Leishmania tropica* isolates. (12-14).

Oral and topical treatment protocols have been proposed, but they are not widely accepted as standard therapy and are rarely used in clinical practice (15).

Many countries have increasingly turned to natural products and plant-derived medicines, particularly those sourced from indigenous flora, to reduce costs and improve accessibility.

The cotton plant (*Gossypium* spp.) has been widely used in traditional medicine globally (16). Different parts of the cotton plant contain a wide range of bioactive compounds, including flavonoids, fatty acids, cyanogenic mixtures, carbohydrates, proteins, and phenolic compounds (17). Gossypol, a bioactive compound found in cottonseed oil, has demonstrated antimicrobial, antifungal, antiparasitic, spermicidal, and abortifacient effects (18). Additionally, caryophyllene, another compound present in the cotton plant, has shown antimicrobial and anti-leishmanial activity (17).

Sharifi et al. investigated the anti-leishmanial effects of the crude cotton boll extract and its fractions experimentally. Their results demonstrated a significant inhibitory effect in intra-macrophage amastigote cell cultures compared with MAT. This study evaluated the impact of the crude extract and 12 chromatographically separated fractions on the growth of *L. major* amastigotes and promastigotes, as well as cytotoxicity against macrophages. The crude cotton boll extract showed significantly greater efficacy than MAT and fractions 4 and 5, while exerting lower cytotoxicity against host macrophages (19). In a subsequent animal study conducted by Sharifi et al., 60 laboratory mice were experimentally infected with *L. major* in the left hind footpad and randomly assigned to nine groups. Over a 28-day treatment period, the

effects of 35% crude cotton boll extract, its fractions 4 and 5, and MAT were evaluated. The study demonstrated a significant therapeutic effect of Iranian cotton boll extract (*Gossypium hirsutum*), showing lesion healing comparable to MAT and a marked reduction in parasite load in the spleen tissue of treated mice (20).

Given the prominent role of cotton in Persian Medicine for wound healing, and considering that in some areas of southeastern Iran, cotton bolls have traditionally been used to treat leishmaniasis-related skin lesions, the present study aimed to evaluate the therapeutic efficacy of a herbal formulation derived from the crude cotton boll extract as a treatment regimen for patients with CL.

Methods

Ethical considerations and design

This randomized, open-label clinical trial was conducted at the Cutaneous Leishmaniasis Treatment Center in Kerman County, Iran.

Ethical approval was obtained from the Ethics Committee of Iran University of Medical Sciences (Approval Code: IR.IUMS.REC.1402.643). The trial was prospectively registered in the Iranian Registry of Clinical Trials (IRCT20230903059337N2) before participant enrollment.

Patients presenting to the Treatment Center were invited to participate by the research team. The trial aims, procedures, potential aids, and risks were thoroughly explained to all candidates. A short educational session regarding the program was prearranged during the patients' initial visit, delivered in person to describe the physical appearance of the lesion, probable risk factors, therapeutic approach, likely relapses, and various surveillance measures related to CL.

Written informed consent from the patients was obtained after detailed information was provided. The patients were permitted to ask questions. All cases received regular diagnosis and therapy at no cost, and no additional interventions were required.

Sample Size

Due to a decline in patient referrals during the study period, the initially calculated sample size—based on the mean lesion diameter derived from animal studies, an effect size of 0.5, a type I error (alpha error) of 0.05, and a statistical power of 0.80 using G*Power software—was not feasible to achieve (20, 21). Consequently, the trial was conducted as a pilot clinical study, with a minimum of 12 participants per group (22). Overall, 58 eligible patients were enrolled over 20 months from Nov 2023 to Jul 2025.

Inclusion Criteria

The following criteria were considered to include the participants: Age between 8 and 60 yr, Fewer than five cutaneous lesions, Lesion diameter less than 5 cm, Disease duration of less than three months, no use of approved anti-leishmanial drugs within the preceding four months, willingness to participate, and provision of written informed consent.

Exclusion and Dropout Criteria

Participants were excluded due to the following reasons: Pregnancy or breastfeeding, Any acute or chronic systemic disease or history of allergic reactions, Lesions located on joints or sensitive anatomical areas, presence of regional lymphadenopathy, previous intramuscular administration of MAT, absence from treatment sessions for more than two consecutive weeks, incomplete or incorrect use of the topical formulation, Development of acute reactions or adverse drug events, PCR confirmation of *Leishmania major*. Participants who showed any exclusion criteria during the study were withdrawn based on the physician's clinical judgment.

Laboratory Confirmation

Samples obtained from patients' lesions were sent to the Leishmaniasis Research cen-

ter for molecular confirmation and species identification of *Leishmania* spp. DNA was extracted from the smear preparations using a standard phenol–chloroform extraction protocol or a commercial extraction kit protocol, following the manufacturer's directions. Species identification was performed by PCR extension of the internal transcribed spacer 1 (ITS1) section, tracked by analysis of amplicon size on agarose gel electrophoresis.

Randomization

Randomization was conducted using a freely available block-randomization tool (https://www.sealedenvelope.com/simple-randomiser/v1/lists) with block sizes of 6. The allocation sequence was generated by an independent individual and provided to the research team. Patients allocated to receive the intervention or control group at enrollment according to this sequence.

Interventions

Control Group (B): Participants received conventional treatment, consisting of weekly intralesional injections of MAT and liquid nitrogen cryotherapy every two weeks, in accordance with national treatment guideline.

Intervention Group (A): In addition to standard weekly intralesional MAT injections, participants received a topical cotton boll extract solution applied twice daily directly to the lesion. The extract was packaged in 30-mL spray bottles and supplied to the skin lesion.

Treatment duration was determined based on national guideline, the treating physician's assessment, and predefined criteria for complete or partial cure, for a maximum of 12 wk.

Preparation of Cotton Boll Extract

Cotton bolls were collected from Orzuiyeh County (Kerman Province, Iran) and taxonomically authenticated at the Herbarium of Shahid Bahonar University of Kerman (Voucher No. KF131). The same plant material had been used in previous in vitro and in vivo studies (19, 20). The cotton bolls were shade-dried at room temperature, ground into powder, and passed through a 20-mesh sieve. To minimize the risk of microbial contamination, particularly *Clostridium tetani*, the powder was disinfected with 30% hydrogen peroxide spray. Subsequently, 200 g of the powder was macerated in 500 mL of an ethanol–water solvent (80:20) for 72 h. The extract was filtered using a Buchner funnel and Whatman No. 42 filter paper, then packaged in 30-mL spray bottles. To standardize the product, the total phenol content (TPC) of the extract was measured using the Folin-Ciocalteau method and was found to be 5.19±0.57 mg/g of dry extract.

Safety Assessment

Due to the presence of 80% ethanol, the extract was considered microbiologically stable and low risk. Nevertheless, before clinical use, the product underwent safety evaluation at the Comprehensive Food and Cosmetic Laboratory of the Food and Drug Deputy of Kerman University of Medical Sciences, in accordance with Iranian National Standard No. 20111. Approval for topical use was granted (Correspondence No. 3565/2/10). All participants were examined for adverse reactions during treatment. Any adverse event was recorded in the case report form and evaluated by the study team.

Data Collection

The treating physician and clinic registration staff were trained by the principal investigator. Demographic and clinical data were recorded in a Case Report Form (CRF). Clinical evaluations were performed at baseline and at intervals of 4, 6, 8, and 12 wk of treatment. Lesion diameter and induration were measured with a 15-cm digital caliper (INSIZE®, Model 150-1108). The mean of the two largest perpendicular diameters was recorded as the lesion size.

Outcome Measures

The main outcome was time to healing, characterized as complete re epithelialization of the cutaneous lesion and resolution of induration. Healing outcomes were categorized as follows (23, 24):

i. Complete cure: ≥90% reduction in lesion diameter or induration.

ii. Partial cure: 50–90% reduction by week 12.

iii. Non cure: <50% reduction by week 12.

Secondary outcomes included changes in lesion diameter and induration at baseline and at weeks 4, 6, 8, and 12 post-treatment follow-ups.

Participants who developed adverse events, pregnancy, lymphadenopathy, drug resistance, or missed follow-up visits for more than two weeks were withdrawn from the trial.

Statistical Analysis:

Data were entered into Microsoft Excel, and final data verification and statistical analyses were achieved using IBM SPSS software (ver. 22) (IBM Corp., Armonk, NY, USA). Quantitative data were expressed as mean ± standard error, and qualitative data as frequencies and percentages. Independent t-tests were used for normally distributed continuous variables, and the Mann–Whitney U test was used for non-normally distributed continuous variables. Categorical variables were analyzed using the Chi-square or Fisher's exact tests. Time to healing was compared using the log-rank test. A *P*-value of ≤0.05 was considered significant (Fig. 1).

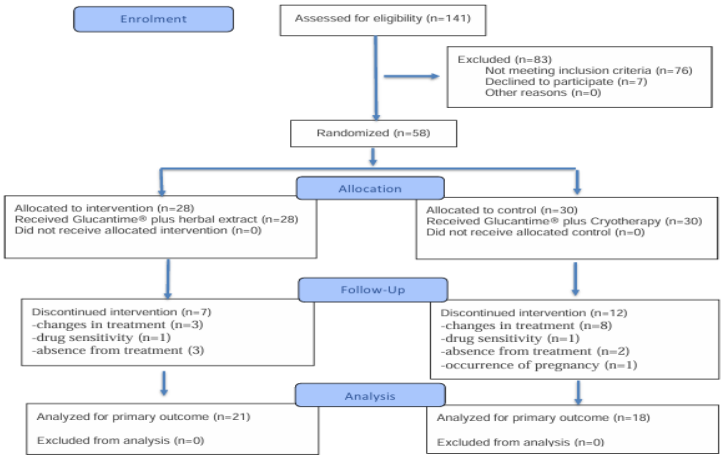

Fig. 1: CONSORT Flow Diagram

Results

Laboratory confirmation

PCR amplification of the ITS1 gene successfully detected *Leishmania* DNA in all examined clinical samples. All samples produced a clear band of approximately 300–350 bp, consistent with the size of the *Leishmania tropica* ITS1 region as reported in previous studies. The amplified fragments from all eight samples appeared at the same position as the positive control (*Leishmania tropica*), confirming the presence of *L. tropica* DNA in all tested samples. Agarose gel electrophoresis of the PCR products demonstrated a single specific band of about 320 bp in lanes 1–8 corresponding to

the clinical samples, together with the positive control band and the DNA ladder. These findings confirmed that all included patients were infected with *L. tropica* (Fig. 2).

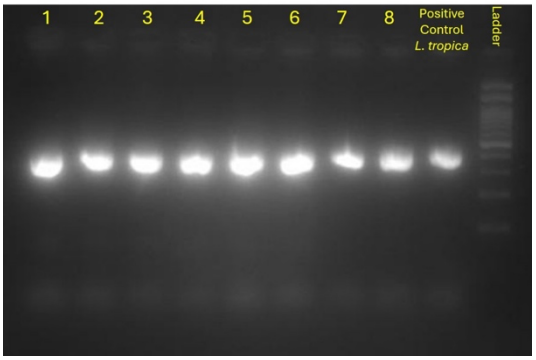

Fig. 2: Agarose gel electrophoresis of PCR-amplified ITS1 gene from *Leishmania tropica*. Lanes 1–8: clinical samples showing a specific band of approximately 320 bp corresponding to the ITS1 region, positive control (*Leishmania tropica*), and Ladder

Participant characteristics

The mean age (± standard deviation) of participants was 23.00 ± 18.37 yr in the intervention group (Group A) and 36.78 ± 20.04 yr in the control group (Group B), showing a significant difference between groups (*P*=0.029). Overall, 28.2% of participants were male, and 71.8% were female. The distribution of demographic characteristics, including sex, education level, and occupation, did not differ significantly between the two groups, except for job status (*P*=0.032) (Table 1).

Table 1: Baseline characteristics of the clinical trial participants

Group		Intervention(Group A)		Control(Group B)		*P*-value
		N	%	N	%	
Sex	Male	7	33.3	4	22.2	0.497
	Female	14	66.7	14	77.8	
Education	Illiterate	0	0	1	5.6	0.054
	Student	12	57.1	3	16.7	
	Under diploma	1	4.8	3	16.7	
	Diploma	6	28.6	5	27.8	
	Bachelor	2	9.5	6	33.3	
Job	Unemployed	1	4.8	1	5.6	0.032
	Housewife	6	28.6	6	33.3	
	Student	12	57.1	3	16.7	
	Employed	2	9.5	8	44.4	

Baseline finding

The number of cutaneous lesions ranged from 1 to 4, with a median of 1 lesion per patient. The mean (± standard error) number of lesions was 1.6 ± 0.8 in Group A and 1.7 ± 1.06 in Group B, with no statistically significant difference (*P*=0.926).

At baseline, the mean (± standard error) induration diameter was 15.86 ± 2.39 mm in Group A and 18.20 ± 3.33 mm in Group B, with no significant difference between groups

(*P*=0.632). Similarly, the mean wound diameter at treatment initiation was 13.02 ± 2.38 mm in Group A and 21.05 ± 4.69 mm in Group B, which did not differ significantly (*P*=0.128).

Follow-up finding

During follow-up assessments at weeks 4, 6, and 8 following treatment, induration size and wound diameter decreased progressively in both groups. No significant differences were observed between Groups at any follow-up time point (Table 2).

Table 2: Follow-up assessments of induration and lesion diameters at weeks 0, 4, 6, and 8

Group		Intervention(A)		Control(B)		P-value
		Diameters(mm)	Std.Error	Diameters(mm)	Std.Error	
Week0	Induration Size	15.86	±2.39	18.20	±3.33	0.632
	Wound Size	13.02	±2.38	21.05	±4.69	0.128
Week4	Induration Size	11.23	±2.49	12.84	±2.41	0.345
	Wound Size	10.85	±2.07	15.56	±3.44	0.414
Week6	Induration Size	5.90	±2.36	8.68	±1.93	0.078
	Wound Size	5.35	±1.53	11.35	±2.62	0.086
Week8	Induration Size	6.11	±2.44	3.05	±1.10	0.571
	Wound Size	3.53	±1.00	3.47	±1.19	0.713
Week12	Induration Size	2.12	±1.40	1.50	±0.95	0.700
	Wound Size	1.10	±0.56	1.71	±1.05	0.830

At week 12, no statistically significant difference was found between groups (*P*=0.830).

No cases of complete cure were observed before week 4 of treatment.

By the end of week 12, 20 cases (95.2%) in the intervention group (A) and 15 patients (83.3%) in the control arm (B) achieved complete cure. Partial cure was observed in one patient in Group A and three patients in Group B, though there was no significant difference (*P*=0.222).

No cases of nonresponse or treatment failure were reported at the end of treatment period. Some pictures of the improvement in the intervention group are shown below (Fig. 3).

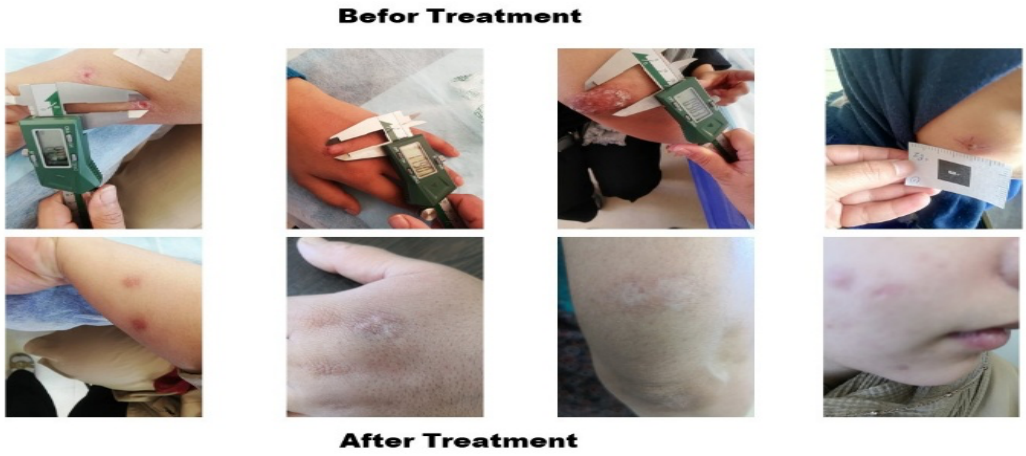

Fig. 3: Pictures of the lesions in patients after treatment

Among patients who attained complete cure, the mean (± standard error) number of intralesional MAT injections was 8.67 ± 0.65 in Group A and 9.17 ± 0.56 in Group B, with no statistically significant difference (*P*=0.588). The mean (± standard error) time to complete cure—defined as at least a 90% reduction in induration or wound diameter compared with baseline—was 8.25 ± 0.61 wk in Group A and 8.47 ± 0.37 wk in Group B, showing no significant difference (*P*=0.784).

No cases of disease recurrence were observed at 1 or 3 months of follow-up after treatment completion in either group. No serious adverse events were observed during the treatment period.

Discussion

In the present study, substantial differences were found between the intervention and control groups regarding age and occupation. At first glance, these differences are potential confounding factors that could influence treatment outcomes. However, a 2012 study reported no significant association between patients' age, sex, occupation, or body weight and the therapeutic success rate in treating CL lesions (25). Therefore, it is unlikely that these demographic differences had a substantial impact on the study results.

Notably, no cases of treatment failure were observed in our study, whereas previous reports have documented treatment failure rates of up to 22% (25, 26). In other to Previous studies from Iran have demonstrated both clinical and molecular evidence of resistance to meglumine antimoniate among *L. tropica* isolates (12-14). Therefore, topical herbal products with anti-leishmanial activity may represent useful adjunctive options in regions where antimonial responsiveness is declining.

By the end of week 12, the complete cure rate was 95.2% in the Group A, compared with 83.3% in the Group B. Although this difference did not present statistical significance, it may still be clinically meaningful and warrants attention. Both groups received MAT as the first-line standard therapy. Previous studies have reported an approximate success rate of 80% for MAT monotherapy (25, 27). In addition, cryotherapy alone has been shown to achieve cure rates ranging from 77% to 95% in different studies (28, 29). Given these findings, it is plausible that the cotton boll extract contributed to lesion healing in the intervention group. The absence of statistical significance may be attributed to the relatively small sample size of this pilot clinical trial, which limits its statistical power.

To date, no other clinical trials evaluating the use of cotton boll extract in the treatment of CL are available. The crude cotton boll extract effectively inhibited the growth of *Leishmania* parasites in vitro. In a subsequent study, they reported significant therapeutic effects of this product in an experimental model (19, 20). In their animal study, a 35% concentration of cotton boll extract was used. In contrast, in the present study, due to the hydroalcoholic maceration extraction method, the estimated extract concentration was approximately 10%. This lower concentration may have influenced the observed therapeutic efficacy and partly explained the absence of statistically significant differences between the two groups.

Changes in induration size and wound diameter over the course of treatment showed a similar pattern of reduction in both groups (Fig. 4).

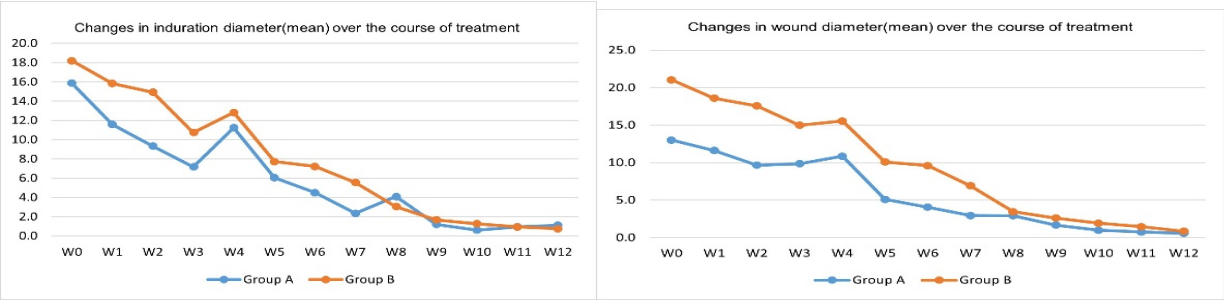

Fig. 4: Induration size(left) and wound diameter (right) changes over the course of treatment

Interestingly, a transient, unexpected increase was observed around week 4 in both groups, further supporting the hypothesis that the cotton boll extract may act as an adjunctive therapy, exerting effects comparable to those of cryotherapy on the overall healing process. Although these differences were not statistically significant, the parallel trends suggest a potential supportive role for the cotton boll extract in the management of CL.

In this study, the sample size in both intervention and control groups was relatively small, which may have limited the statistical power to detect significant differences between treatments.

Conclusion

Based on the above findings, and given that cryotherapy, recommended as an adjunctive treatment alongside MAT compounds in current CL treatment guidelines, is a painful procedure requiring specialized equipment and trained personnel, the replacement of this method with less invasive and more user-friendly alternatives warrants serious consideration. The crude cotton boll extract formulation, given its easy availability, low cost, and lack of need for specialized skills or equipment, appears to have the potential to serve as a suitable alternative adjunctive therapy to cryotherapy. However, confirmation of this potential requires further and more robust clinical evidence. Accordingly, it is recommended that larger-scale clinical trials be conducted using higher concentrations of cotton boll extract and exploring different topical formulations, such as creams or ointments with longer skin residence time, to more accurately evaluate the therapeutic efficacy and clinical applicability of this herbal product in the treatment of CL.

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Conflict of interest

The authors declare that there is no conflict of interests.

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