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Primary effects of new leishmania major antigen on Balb/c mice Spleen.

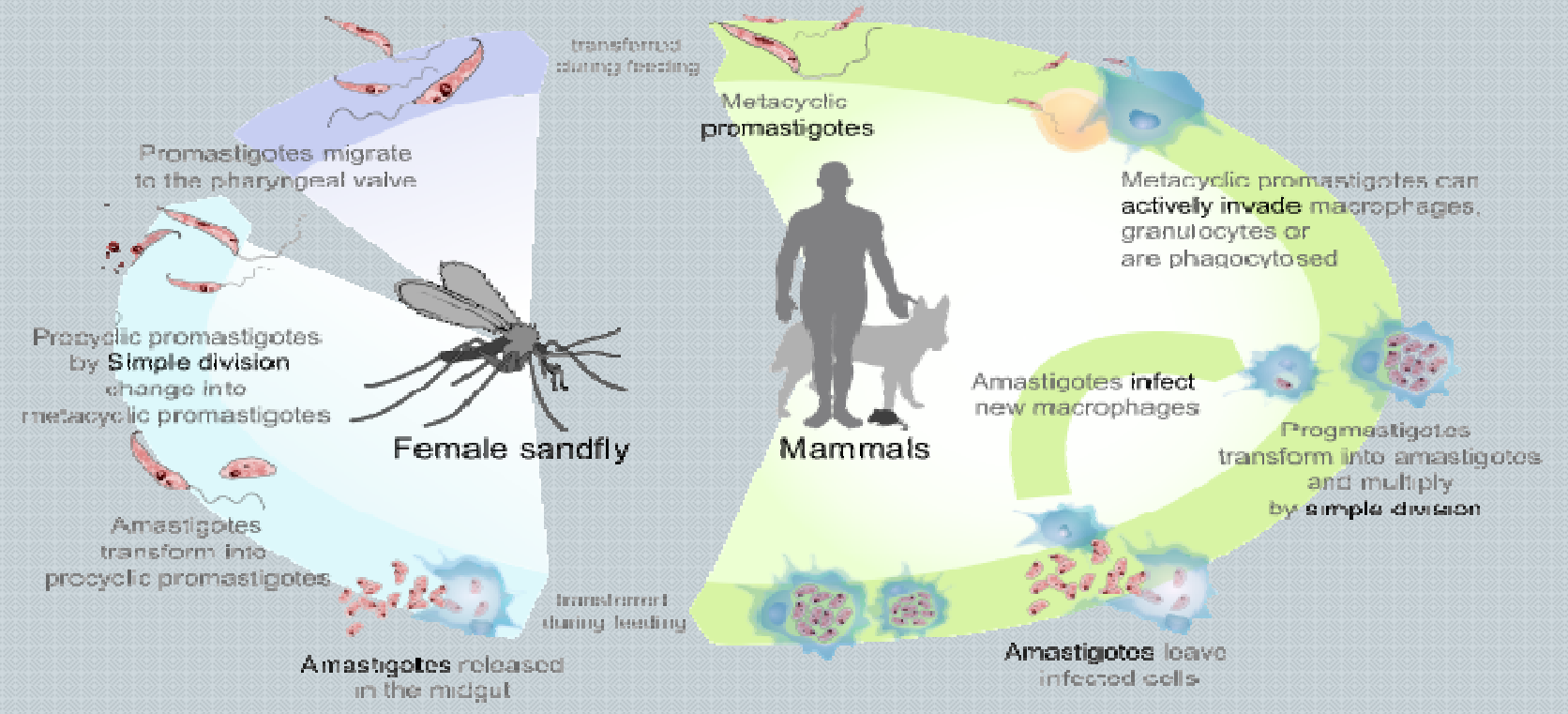
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Promastigotes of *Leishmania major*, 10×100, Giemsa stain.



Leishmaniasis life cycle



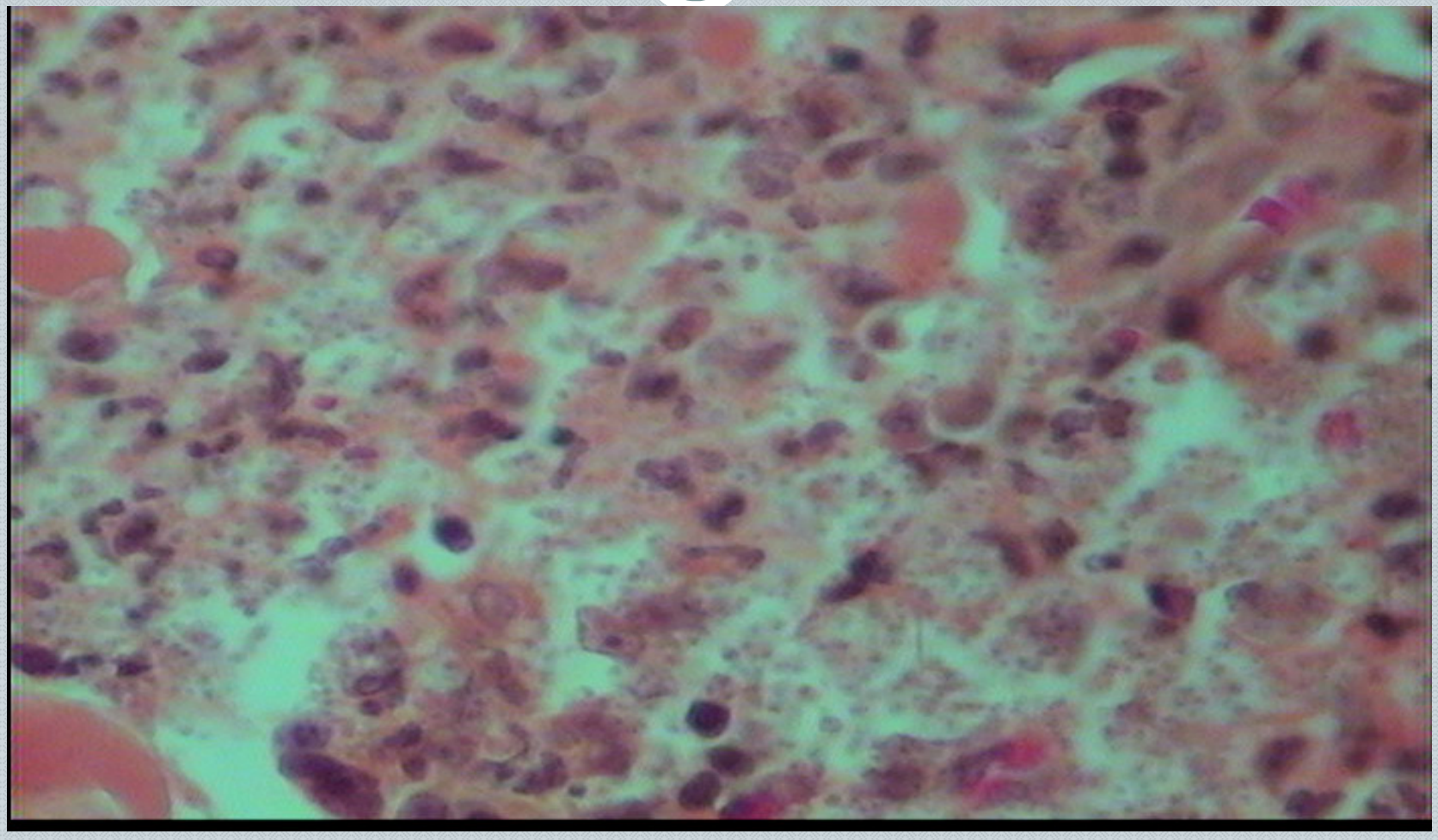
Cutaneous Leishmaniasis Sore(human)



Cutaneous Leishmaniasis Sore(dog)



Amastigotes of *Leishmania major ,free or within macrophages (cutaneous lesion)*, 4×100, H&E staining method



Leishmaniasis Diagnosis

- **1-*L. major* should be considered chronic lesions in endemic area**
- **2-similar lesions : histoplasmosis, sporotrichosis, lobomycosis, lupus vulgaris, *Mycobacterium ulcerans*, syphilis, cutaneous sarcoidosis, and leprosy should all be considered as well**
- **3-Identify amastigotes in a Wright-Giemsa-stained touch preparation or through isolation of the parasites in cultures.**

Treatment

- **Because the host's immune system tends to resolve infection after 3–6 months, treatment of the lesions generally focuses on limiting tissue damage and necrosis. A number of different treatments have yielded results of varying effectiveness in the treatment of *L. major* caused cutaneous leishmaniasis.**

- **-Fluconazole given in 200 mg doses over the course of 6 weeks**

- **-Topical application of 15% paromomycin and 12% methylbenzethonium**

- **-Intralesional injections of 0.5-2.0mL of 100 mg/ML antimony**

Prevention

- interrupting the sand fly life cycle
- removing or treating pathogen reservoirs
- Avoiding sand fly bites where *L. major* is endemic
- use of DEET containing insect repellent, application of insecticides to clothes and bedding, as well as using mosquito nets to cover beds
- Sand flies usually bite between dusk and dawn
- Patients who have recovered from *L. major* infections develop high- level immunity to the pathogen
- Though a vaccine does not yet exist that can prevent cutaneous leishmaniasis

Primary effects of new leishmania major antigen on Balb/c mice Spleen.

- **Materials and Methods:**
- One hundred and twenty young adult female and male Balb/c mice
- **Culture and Isolation of *Leishmania Parasites:***
- *Leishmania promastigotes of L. major (WHO) strain were provided by the Tehran*University of Medical Sciences
- were grown in NNN medium and RPMI 1640 supplemented with 5% - 10%FCS
- Harvested parasites were washed three times with normal saline solution (0.9%) or PBS .
- counted in a Neubar chamber and kept at -70°c until use. accumulated parasites diluted to a concentration of 5.92×10^{10}

Materials and Methods:

- ***Vaccine Preparation***

- 5.92×10^{10} parasite dilution and divided into 5 batches those each other used one
- Inactivation procedures : freeze and thaw, autoclave, or inactivation at 56°C.
- At least antigen processed
- The dose of the vaccine certified according to 100 or 200 µg/0.1 mL *Leishmania protein* per dose

Materials and Methods:

- ***Vaccine Preparation(continued)***

- *The content of protein in* each dose was estimated by the Lowry method
- two injection doses (100, 200 µg/mL) were admistered
- The vaccine was stored at 4°C until use
- Just before injection, BCG Vaccine 2×10^5 CFU live BCG /0.1 ml and 400 mg alcoholic extract of Teucrium polium/0.1 ml dissolved in 1 ml distilled water and 2.5 mg/0.1 ml of used for each injection dosage solution (100, 200 µg/0.1ml) .

Materials and Methods:

Injection Groups

- LT received 100-200 µg /0.1 ml of the crude cocktail antigen plus alcoholic extract of Teucrium polium as adjuvant
- LB received 100-200 µg /0.1 ml of the crude cocktail antigen preparation plus BCG as adjuvant
- LBT received 100-200 µg/0.1 ml of the same antigen preparation plus alcoholic extract of Teucrium polium and BCG as adjuvant
- Control had not received antigen injection
- All three groups received antigen subcutaneously and they were received two booster injection with one week interval

- ***Challenge***
- A week after the last booster, all animals including control group were challenged with 3×10^5/0.1 ml live Leishmania major promastigotes.

- ***Protectivity***
- The protective response was evaluated by the challenge effects which notice almost daily for 70 days over all mice. Evaluation was composed of lesion induction, and rate of survival .


LT-100


LT-100
86-03-30-6149

Materials and Methods:

- ***White Pulp Size Measurement:***
- the animals survived from post challenging were euthanized using diethyl- ether, necropsied and spleen was removed and fixed in 10% buffered formaldehyde solution.
- The fixed spleen tissues were processed in a tissue processor, paraffin blocks were made and 4-5 microns tissue sections were prepared and stained with Harris Hematoxyline and Eosine method.
- The expansion rate of the spleen white pulp size was evaluated using a light microscope with an eye- piece graticule.

Results

- Differences between the LT,LB ,LBT and control group were statistically significant(P=0.001).
- Compared to control group, the spleen white pulp size increased in the groups LBT and LB but not in LT group.
- The higher expansion rate of the spleen white pulp size was found in the LBT group. differences were significant statistically.
- p values for LT, LB, and control groups were 0.02, 0.036 and 0.005
- Among the groups LT, LB and LBT, the lower spleen white pulp size was seen in the LT group
- there was a significant difference between LBT and LT groups (P<0.002) and LB (0.036) and control (0.005) and differences were significant. The SWPs expansion rates differed in the female and male for LT, LB , LBT and control groups.
- Largest SWPs were seen in the female Balb/c LBT group
- smallest SWPs were seen in the male Balb/c LT group
- higher survival rate was seen in LT group followed by live L.major challenge
- protection rates 70 days post challenging in the LT,LB,LBT and control groups were 50.0% , 25.0% ,30.0% and 20.0 % respectively
- lower survival rate was seen in control group challenged by live L.major promastigotes.

Spleen White Pulp size- LB. dose100 .Balb/c

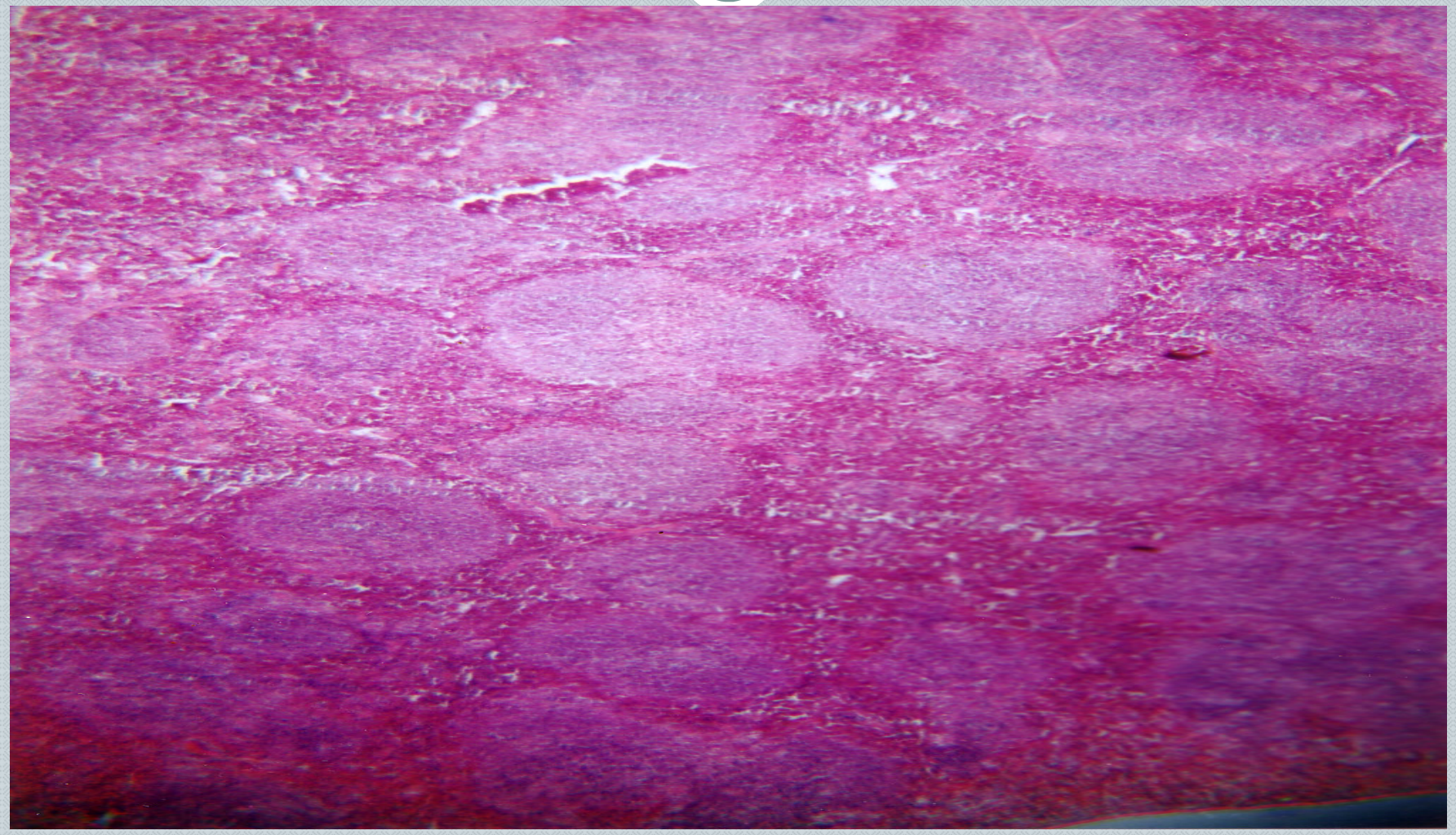


Figure1: The effects of the crude cocktail L. major antigen preparation on spleen white pulp size in the groups LT, LB and LBT challenged with the live L. major ptomastigotes compared to control group. Differences between LBT groups and three groups of LT, LB and control groups is significant statistically (P<0.05), but differences between three groups of LT,LB and control group is not significant(p>0.05).

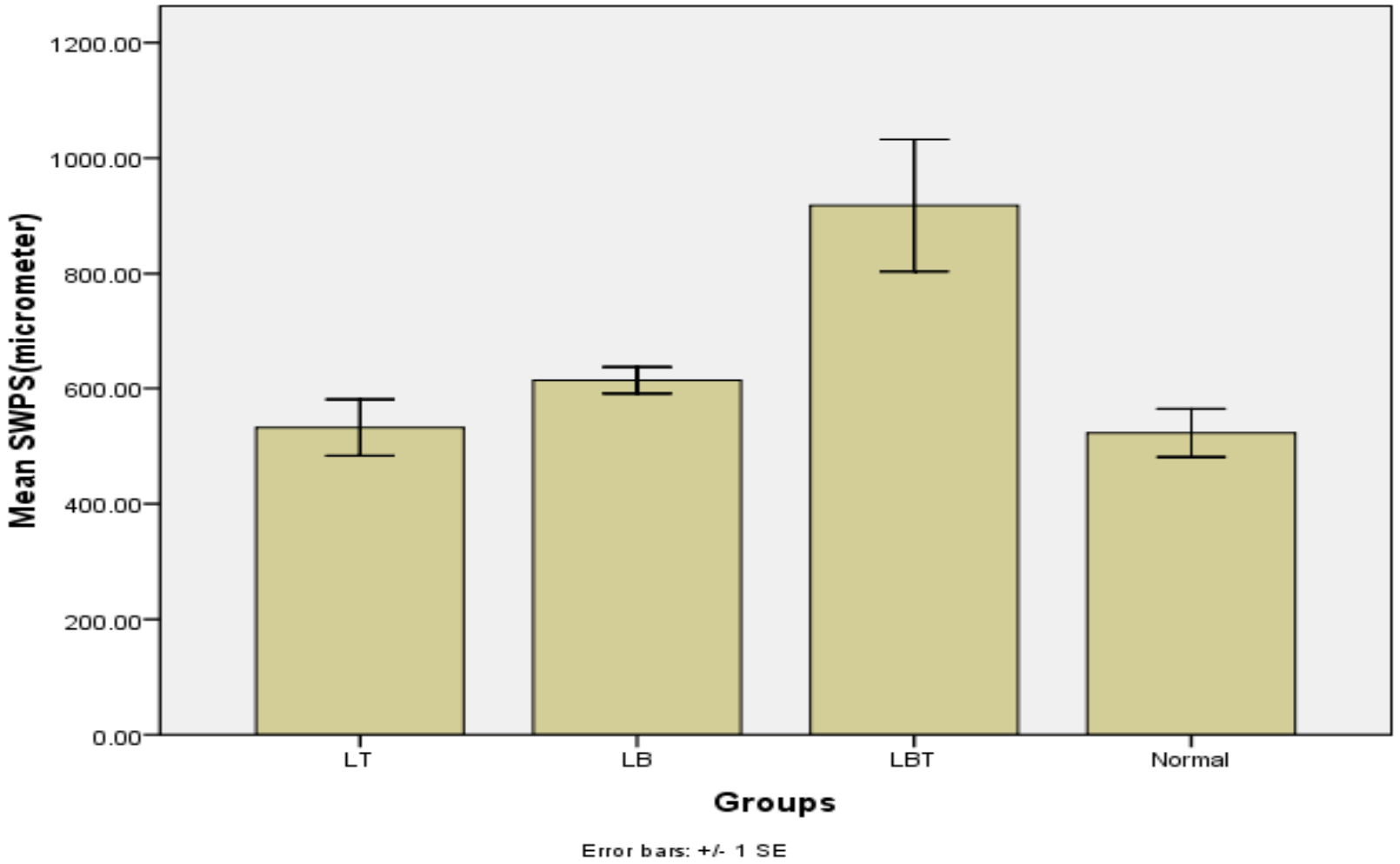


Table1:ANOVA

The results of analysis variance show a significant differences in white pulp size between Balb/c mice (P<0.001).

	Sum of Squares	df	Mean Square	F	Sig.
SWPS(micrometer) *					
Groups Between Groups(Combined)	639993.436	3	213331.145	7.362	.001
Within Groups	608538.611	21	25978.029		
Total	1248532.047	24			

Discussion

- Our research method (combination of freezing and thawing ,autoclaving ,heat inactivation ,chemical inactivation) offer many leishmania epitopes
- It could be expected that some of these epitopes could induce such an immune response which capable to protect the vaccinated subjects
- It seems that ,when BCG and alcoholic extract of T.polium are used together ,they show a remarkable synergistic effects

Discussion(continued)

- The crude cocktail antigen preparation plus alcoholic extract of Teucrium polium and BCG or plus BCG alone is not recommended for the provisional vaccine in spite of their potency in both cell mediated or humoral immunity induction.
- The live BCG could produce mycobacterial infection particularly in the immune compromised or immunodefficient subjects.
- The differences between two groups of female and male Balb/c mice were statistically significant

Discussion(continued)

- We expect that the new antigen formula must show the following characteristics in order to use as a satisfactory vaccine:
- Firstly, could induce a reasonable immune response which activate macrophages therefore it enable macrophages to inhibit propagation or destroy intracellular amastigotes at the early stages of infection, Secondly, could inhibit transformation of promastigote organisms to intracellular forms or amstigotes at the site of the parasite entrance Thirdly, to induce a safe and potent immune response which could help clinically infected individuals ;to eradicate intracellular form of leishmania organisms,
- Fourthly ,from our point of view the side effect of the antigen preparation must be negligible or tolerable when it is administrated ,since induction of high levels of cytokines including inflammatory cytokines or activation of a large proportion of lymphocytes will have a grave consequences.

Conclusion:

- LT preparation is safer than those supplemented with the live BCG as adjuvant since they could produce mycobacterial infection particularly in the immune deficient individuals.
- The crude cocktail antigen preparation at doses of 100-200 µg/0.1ml which is adjuvanted with alcoholic extract of Teucrium polium could induce an acceptable and satisfactory immune response in the Balb/c mice. To evaluate the preparation as a provisional vaccine against Leishmania major infection ,the author recommends further investigations conducted in other susceptible species including canine species.
- Finally mass production of antigen preparation as a vaccine must be feasible commercially

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for your
attention