REPORT

SOME STUDIES ON HUMAN URINE AS PROMOTER FOR THE GROWTH OF *LEISHMANIA IN VITRO*

JAVEID IQBAL, MOHAMMAD JAMSHID*, BASHIR AHMED*, ISHRAT BUKHARI, SAJID BASHIR AND M. MASOOM YASINZAI**

Department of Pharmacy, University of Balochistan, Quetta, Pakistan
**University College of Pharmacy, University of The Punjab, Lahore, Pakistan*
***Institute of Bio Chemistry, University of Balochistan, Quetta, Pakistan*

ABSTRACT

Leishmaniasis, a parasitic disease caused by the protozoan *Leishmania* is endemic in many parts of tropic and sub tropic areas of the world. *In vitro* cultivation of parasite plays an important role in the study and treatment of disease. Traditionally media available do not meet the requirement for the bulk cultivation of *Leishmania* parasites, it requires fetal calf serum (FCS), that is very expensive and not easily available in the market.

It is reported that human urine has stimulatory effect on the growth of Leishmania while cultured *in vitro*. We undertook a detailed study of such an effect in old world Leishmania isolates causing cutaneous leishmaniasis. Different concentrations of urine are tried and it is found that 1% sterile urine in our undefined medium 1999 supplemented with foetal calf serum gives the maximum growth. It is observed that dated urine has less stimulatory effect on the growth of the parasites as compared to fresh urine. The effect of the urine of different groups is also observed and it is found that the urine of 60 year gives maximum growth.

Keywords: Leishmaniasis, human urine, FCS, cultivation, growth, medium.

**Corresponding author:* Prof. Dr. Javeid Iqbal, Department of Pharmacy, University of Balochistan, Quetta

INTRODUCTION

*Leishmania*sis is a parasitic disease caused by trypnosomatid protozoan of genus *Leishmania,* transmitted by the bite of infected female sandfly of genus *phlebotomus*. It is characterized by the intracellular parasitism of macrophages. Varying manifestation of disease occur ranging from simple, self healing *cutaneous Leishmaniasis,* through erosive *mucocutaneous* and rare diffuse cutaneous forms which rarely spontaneously cure, often fatal V*isceral Leishmaniasis* (Kala-azar), (Yasinzai *et al.,* 1996 and Baily, 1994). *In vitro* cultivation of the causative organism probably plays a more important role in the study and the treatment of the *Leishmania*sis then it in any group of the disease caused by the protozoa (Vouldoukis, 1986 and O'Daly, 1993). A variety of culture medias designed for *in vitro* maintenance and bulk cultivations of *Leishmania* promastigotes require inclusion of 10-30% heat inactivated foetal bovine serum (HIFBS) to support successful growth (Lemesre *et al.,* 1988 and O'Daly, 1993). With out HIFBS, these culture medias simply fail to support growth of *Leishmania* promastigotes and the culture dies off. Foetal bovine serum is not only the most expensive ingredient of these culture media but is also very difficult to obtain in many parts of the world where *Leishmania* is endemic (Evans, 1986). Other sera have been tried to replace expensive HIFBS but none of them could support the growth of the parasite and exhibited delerious effect on *Leishmania* culture Barral *et al* (1987).

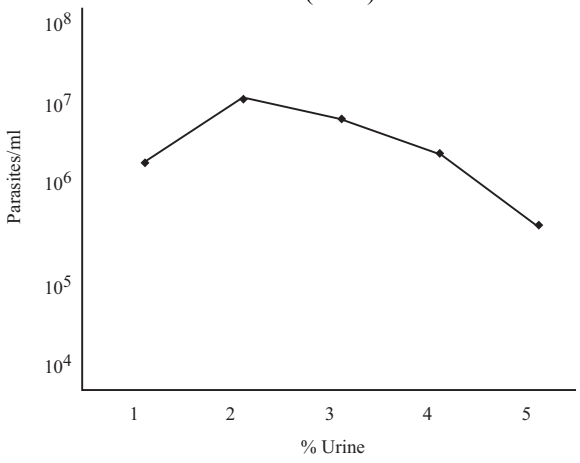

Fig. 1: Effect of urine on the growth of promastigotes.

In vitro cultivation of *Leishmania* parasite plays an important role in parasitological diagnosis, identification, characterization of parasites and also its biochemical, immunological and chemotherapeutic studies. A count of $3x10^7$ to $3x10^8$ parasite/ml is required in such cases. To get such number of parasites there is always need for a better culture medium that can provide good parasitic density. Various culture media is used for this process (Chang *et al.,* 1983 and Ray *et al.,* 1980).

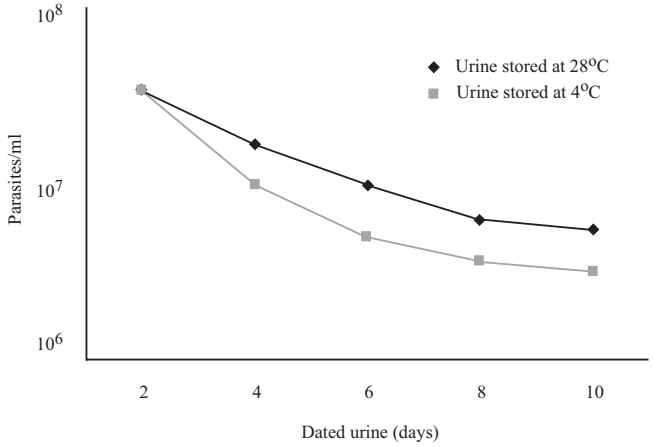

Fig. 2: Effect of dated urine on the growth of promastigotes.

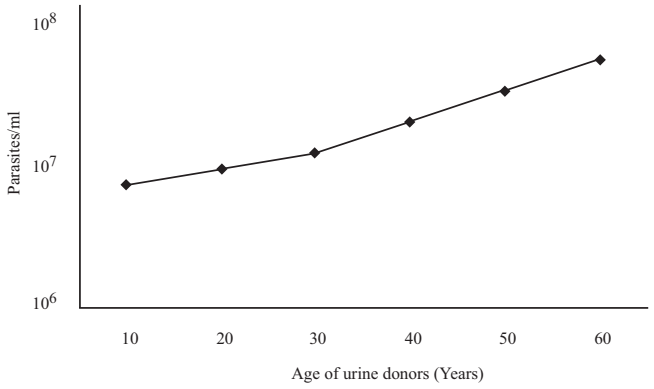

Fig. 3: Effect of urine from different age groups on growth of promastigotes.

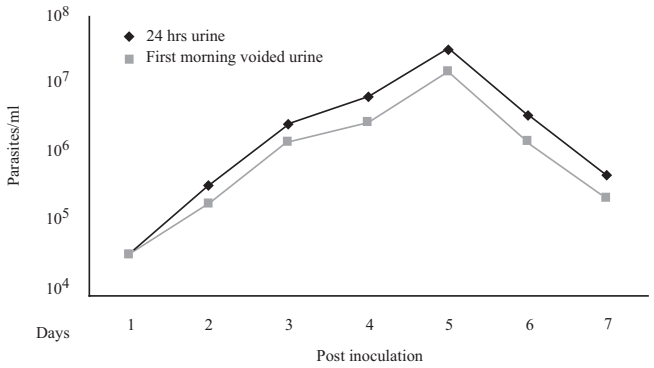

Fig. 4: Effect of 24 hrs and first morning voided urine on the growth of promastigotes.

In present work we studied the effect of human urine on the growth of the parasites *in-vitro*. It is observed that sterile human urine in a concentration of 1-2% increase the growth of parasites. Effect of time difference, different condition of storage of urine, age of the urine donor and sex etc, have also been studied.

MATERIAL AND METHODS

The parasites were maintained in disposable culture tubes (Corning) and were cultured at 22-23°C in semi defined liquid mediumAJM-1, which does not need foetal calf serum. AJM-1 was prepared as follow:

$CaCl_2$	0.015 g	KCl	0.05 g
K_2HPO_4	0.05 g	Glucose	0.2 g
$MgSO_4$	0.01 g	Bacto peptone	0.2 g
$MgCl_2$	0.01 g	Beef extract	0.35 g
NaCl	8.0 g	Yeast extract	0.05 g
$NaHCO_3$	0.01 g	L-peroline	0.2 g
$Na_2 HPO_4$	0.02 g	Phenol red	0.001 g
$Fe NH_4 (SO_4)_2$	0.005 g		

Solid ingredients were mixed in 750 ml of deionized distilled water, pH was adjusted at 7.5 with 1 M NaOH/HCl and volume was made up to 1000 ml with deionized water. The medium was autoclaved at 121°C for 15 minutes and cooled at room temperature. For avoiding contamination, gentamycine was added to a final concentration of 25µgml and stored at 4°C in dark.

Fresh human urine was obtained and made sterile by passing through 0.22µM membrane. Nine culture tubes were taken, to each culture tube was added 2 ml of the culture medium and inoculated with 10^6 parasites per ml, 0.1, 0.2, 0.4, 0.6, 0.8, 1.0, 2.0, 4.0 and 6.0% of sterile human urine was added to the final concentration in all tubes except the control, the tubes were placed in an incubator at 22-23°C for up to 72 hrs. Parasites were counted after 72 hours with the help of hamocytometer. For each study, the test was done in triplicate and mean of three readings was taken.

RESULT

Effect of percentage on growth of promastigotes

The effect of varying concentration of sterile human urine in the culture medium on growth of promastigotes is shown in figure 1; it can be seen that the growth of the parasites was maximally enhanced by 1.0 to 2.0% of human urine, on the other hand the concentration higher then 4% exhibited no stimulatory effect on the growth of the parasites.

Effect of dated urine

The effect of dated urine was observed using same urine stored at 4°C as well as -20°C for up to 6 days. The results obtained is graphically shown in figure 2. It was seen that the fresh urine was most suitable and it maximally supported the growth of the parasites. It was also observed that urine retained its growth promoting activity at -20°C better, then when stored at 4°C.

Effect of different age groups

Urine was obtained from different age groups and its effect was observed. The result is graphically shown in figure 3. As it is clear from the figure, that urine from age group 60 supported the maximum growth.

Effect of sex

Urine was taken from male and female donors and its effect was observed. Results are graphically shown in figure 4. It was found that urine from female donor supported the growth of parasites better than male urine.

Effect of spot and 24 hrs urine

The effect of spot in 24 hrs collected urine was observed. The results are graphically presented in figure 5. 24 hr urine supported the growth of the parasites better then the spot urine.

DISCUSSION

It was observed that human urine promote the growth of *Leishmania* promastigotes *in vitro* (Howard *et al.,* 1991). The major constituent of the urine includes proteins, amino acids, etc. urea, uric acid are unlikely to induce any stimulatory effect on the growth of the parasite, however the presence of glucose at concentration of 100-200 mg/24 hr and amino acids at concentration of 1.5g/24 hr can play a major role as growth promoting factor. Studies have shown that promastigotes of various species of *Leishmania* can use several carbohydrates as respiratory substrate (Mukkada, 1977) including glucose, fructose, mannose, glactose, etc, though the glucose is implicated as a major carbohydrate substrate for the growth. All enzyme of Embden–Mayerhoff pathway, the oxidative and non oxidative segments for the hexose monophasphote shunt and a complete tricorboxylic acid cycle have been identified in *Leishmania* however it may also be noted that carbohydrate are of secondary importance (Mukkada, 1977) as growth substrate for *Leishmania*. Several studies demonstrated the role of amino acids as primary growth substrate (Mukkada, 1985 and Krassner *et al.,* 1972). It is reported that promastigotes takes up proline at the rate 5 times faster than that of glucose (Krassner *et al.,* 1972). As in the urine, amino acids are present at a concentration of 1.5g/24 hr they may be the major growth promoting factor in urine.

It is also found that urine obtained from 60 year of age group supported the maximum growth; this was probably because in this age the function of glomrulus is not as efficient as in young ages as well as reabsorbence of threshold substances (water, glucose, aminoacids etc) of glomrulus filtrate in the capillaries of renal tubules before entering the loop of Henle does not take place as efficiently as in young ages (Kanwae, 1984 and Donadio, 1982) which result in comparatively elevated level and may play a role in enhanced growth of the parasite as compared to young aged urine.

It is also found that female urine supported comparatively better growth as compared to male urine. It might be due to

the fact that female urine normally contains 2-6 epithelial cells (intact or partially destroyed), (Brenner, 1983). These cells come from urogenetial tract and are considered normally if not accompanied with pus cells or RBCs. In urine these cells provide denatured proteins (mainly nucleoprotein) which can enhance the growth of parasites.

REFERENCES

Baily GG (1994). Visceral *Leishmaniasis*: More prevalent and more problematic. *J. Infect*., **29**(3): 241-247

Barral NM, Roters SB, Sherlock I and Reed SG (1987). Destruction of *Leishmania* mexicana amazonensis, promastigotes by normal human serum. *Am. J. Trop. Med. Hyg.,* **37**(1): 53-V.

Brenner BM and Reactor FC (eds) (1983). The Kidney, 2nd ed. Philadelphia, W.B. Saunders Co.

Chang KP and Fish WR (1983).*Leishmania: In vitro c*ultivation of protozoan parasites. ed. Jensen JB. CRC PRC Press, Coca Raton, Florida, pp.111-153.

Donadio JV and Holley KE (1982). Membrane prolifrative glomerulonephritis. *Semin*. *Nephrol*. **2**: 204.

Evans DA (1986). An inexpensive easily available replacement for foetal calf serum in media for the *in vitro* cultivation of *Leishmania* spp. *Z. Parasitenkd.,* **72**: 567-572.

Howard MK Pharoah MM, Ashall A and Miles MA (1991). Human urine stimulates growth of *Leishmania in vitro*. *Trans Royal Soc. of Trop. Med. Hyg.*, **85**: 447-479.

Kanwar YS (1884). Biopsychology of Glomrulus filtration and protein urea. *Lab Invest.* **51**: 7.

Krassner SM and Flory B (1972). Proline metabolism in *Leishmania* donovani promastigotes. *J. Protozol.,* **19**: 682-685.

Lemesre JL, Darcy F, Kweider M, Carpon A and Santoro F (1988). Requirement of defined cultivation conditions for standard growth of *Leishmania* promastigotes *in vitro. Acta. Tropica.,* **45**: 99-108.

Mukkada AJ (1977). Energy metabolism *Leishmania*sis. Chang *et al.* (editors), Elsevier, Amsterdam, pp.49-65.

Mukkada AJ (1985). Energy metabolism in *Leishmania* in human parasitic disease, *Leishmania*sis, Chang KP and Bray RS (editors). Elsevier Science Publishers, Amsterdam, New York and Oxford, pp.49-65.

O'Daly JA (1993). A comparison of molecular biology of Tryanosomes and *Leishmaniae*, and its impact on the development of methods for the diagnosis and vaccination of *Leishmania*sis and Chagas diseases. *Biol. Res.,* **26**(1-2): 219-224.

Ray R and Ghosh AC (1980). Cultivation of *Leishmania donovani in vitro* in high yielding liquid culture medium. *Indian J. Med. Res.,* **71**: 203.

Vouldoukis I, Alfred C, Monjour L, Mazier D, Brandicourt O, Ploton I, Tselentis Y, Nzuzi KK and Gentilini M (1986). Cultures systems for productions of promastigotes and amastigotes forms of *Leishmania*. Application to serological diagnosis and therapeutic trials. *Ann. Parasitrol. Hum. Como.,* **61**(2): 147-154.

Yasinzai MM, Iqbal J, Kakar JK, Ali SA, Ashraf S, Naz R, Nasimullah M, Nagi AG and Salam A (1996). *Leishmaniasis* in Pakistan: revised. *J. Col. Phys. Surg. Pak*., **6**: 70-75.

Received: 26-1-2006 – Accepted: 3-4-2006