



Assessment of Antitheilerial Activity of the Aqueous Extract of *Kigelia africana* Unripe Fruits against *Theileria lestoquardi*

Hayat M. Farah^{1*}, Tigani H. El Amin², Abdel Rahim M. El Hussein¹
and Hassan E. Khalid³

¹Veterinary Research Institute, Animal Resources Research Corporation, P.O. Box 8067 (El Amarat), Khartoum, Sudan.

²Department of Pharmacology and Toxicology, Faculty of Veterinary Medicine, University of Khartoum, Sudan.

³Medicinal and Aromatic Plants Research Institute, National Centre for Research, Khartoum, Sudan.

Authors' contributions

This work was carried out in collaboration between all authors. Authors THEA, AME and HEK designed the study, and wrote the protocol. Author HMF managed the literature searches, and wrote the first draft of the manuscript. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/EJMP/2015/10213

Editor(s):

(1) Daniela Rigano, Department of Chemistry of Natural Compounds, University Federico II of Naples, Italy.

(2) Marcello Iriti, Plant Biology and Pathology Dept. of Agricultural and Environmental Sciences Milan State University, Italy.

Reviewers:

(1) Anonymous, St. Edmund's College, Shillong, India.

(2) Anonymous, University of the Punjab, Quaid-EAzam, Pakistan.

(3) Anonymous, Mediterranean Agronomic Institute of Bari, Italy.

(4) Anonymous, CONICET-Universidad Nacional de General San Martín, Argentina.

(5) Anonymous, Gaziosmanpasa University, Turkey.

Peer review History: <http://www.sciencedomain.org/review-history.php?iid=648&id=13&aid=6190>

Original Research Article

Received 19 March 2014
Accepted 25th July 2014
Published 23rd September 2014

ABSTRACT

Aims: To investigate the *in vitro* schizonticidal activity of aqueous extract of *Kigelia africana* unripe fruits (Um shotour) Lam. Benth. In Hook. against *Theileria lestoquardi*.

Study Design: *In vitro* screening of the aqueous extract of the fruits at different concentrations against *T. lestoquardi*.

Place and Duration of Study: University of Khartoum, Veterinary Research Institute, Medicinal and Aromatic Plants Research Institute, Khartoum, Sudan, between September 2007 and August 2009.

*Corresponding author: Email: hayatmahgoub@yahoo.com;

Methodology: Aqueous extract of *K. africana* fruits was screened for the first time to test *in vitro* activity against *T. lestoquardi* at different concentrations. Blood was collected separately from normal sheep and sheep infected naturally with *T. lestoquardi*. Normal Lymphocyte cells and lymphocyte cells infected with *T. lestoquardi* were isolated from heparinized blood with Ficoll-paque, grown in minimum essential medium and sub cultured continuously till passage 8 (5×10^4 cell/ml) which was used for the test. The parasite was identified with indirect fluorescent antibody test.

Results: The results revealed *in vitro* activities of 20%, 58% and 80% at concentrations of 500, 5000 and 10000 $\mu\text{g/ml}$, respectively. Lethal dose 50% (LC_{50}) was 2660.28 $\mu\text{g/ml}$. The extract activity significantly ($P < 0.05$) caused death of macroschizonts, decreasing the number of macroschizonts per cell, and significantly ($P < 0.05$) increasing the number of extra cellular macroschizonts at concentrations of 5000 and 10000 $\mu\text{g/ml}$. The number of dividing cells, and number of viable cells significantly ($P < 0.05$) decreased at concentrations of 5000 and 10000 $\mu\text{g/ml}$ compared with the control. Beside the activity, the highest concentration 10000 $\mu\text{g/ml}$ revealed some lymphoblast cells with vacuolated cytoplasm.

Conclusion: This study showed that aqueous extract of *K. africana* fruits has antitheatlerial effect on *T. lestoquardi* and could be an effective candidate for the treatment of malignant ovine theileriosis after *in vivo* confirmation. Further studies are recommended for phytochemical analysis and mode of action.

Keywords: *Kigelia africana*, *Theileria lestoquardi*, *in vitro* activity, aqueous extract.

1. INTRODUCTION

Theileria lestoquardi is a highly pathogenic parasite of sheep and goats. It occurs in south eastern Europe, North Africa, the Near and Middle East and southern USSR [1]. The parasite is transmitted by ixodid tick *Hyalomma anatolicum* [2].

T. lestoquardi causes malignant ovine theileriosis which is a serious infectious tick-borne protozoan disease. It is widely distributed in Sudan where 16.3% of sheep surveyed were found positive using schizont antigen in indirect fluorescent antibody test [3]. The disease causes economic losses in Khartoum state [2,4,5] and northern Sudan [6,7,8]. Additionally, an outbreak of malignant ovine theileriosis among goats showed that 16 out of 22 (72.7%) goats died in northern Sudan [9].

Treatment of malignant ovine theileriosis is mainly by buparvaquone [6]. This drug is very expensive and has a log plasma half life at least 7 days. Therefore, it is desirable to search for discovery of alternative drug which is effective, affordable, readily available, and has a short plasma half life. *K. africana* was selected for the first time to assess its schizonticidal activity against *T. lestoquardi* on the bases of its frequent use in the treatment of parasitic diseases by traditional healers. Previously, it was investigated as antimicrobial [10], antitrypanosomal [11].

K. africana belongs to the family Bignoniaceae. It is locally known in the Sudan as Umm Shutour. Elsewhere it is known as Sausage tree in London, Worm boom in Africa, Um Vunguta or Um fongothi in Zulu, Modukguhlu or Venda Muvevha in North Sotho, Balmkheera in India. It is found mostly in wet areas and spread abundantly across savannah and riverine area [12]. It is prevalent at stream bank and it is widely distributed in Central and Southern Sudan; South, Central and West Africa and India [13,14]. The plant is cultivated in other tropical countries and is used as ornamental tree in Australia, USA and parts of South East Asia.

Most of the studies on the biological activity and chemical constituents of the plant *K. africana* have been connected in some way or another to its traditional uses [15]. Chemical constituents of *Kigelia* are naphthoquinones, fatty acids, coumarins, iridoids, caffeic acid, norviburtinal, sterols and flavonoids [16].

The aim of this study was to screen the aqueous extract of *K. africana* unripe fruits for discovery of its *in vitro* activity against *T. lestoquardi*.

2. MATERIALS AND METHODS

2.1 Plant Collection

K. africana unripe fruits were collected in summer from banks of Blue Nile River in

Khartoum State. The plant was identified and authenticated by Dr. Hayder Abdelgader at the Medicinal and Aromatic Plants Research Institute, National Centre for Research, Khartoum, Sudan. Voucher specimen has been deposited in the herbarium museum of the Institute. The fruits were air dried in the shade, coarsely powdered using mortar and pestle, and kept in polythene bags.

2.2 Preparation of Cell Culture

2.2.1 Isolation and identification of *Theileria lestoquardi*

Two heparinized blood samples were separately collected from normal sheep, and sheep naturally infected with *T. lestoquardi*. Non-infected lymphocyte cells and lymphocyte cells infected with *T. lestoquardi* were isolated from blood with Ficoll-Paque [17]. The isolate was grown in GMEM with glutamine; calf serum (20%); antibiotics (Penicillin, 50 IU/ml; Streptomycin, 50 µg/ml); and antifungal (Nystatin, 50 IU/ml) using standard procedure [18]. The identity of the parasite was verified using indirect fluorescent technique (IFA) as described previously [3]. The isolated cells continuously multiplied and repeatedly sub cultured till passage 8. Non-infected cells had limited multiplication and cannot go for further passages.

2.3 Preparation of Plant Aqueous Extract

One gram (1.0 g) of the coarsely powdered fruits was soaked in 10 ml of deionized boiling water for 8 h at room temperature (24°C) with interval shaking [19]. The extract was filtered through cotton wool. The filtrate was sterilized through 0.22 µm Millipore filter (Millipore, U.K. Ltd., London). The filtrate (100000 µg/ml) - referring to its starting point - was diluted immediately to give concentrations of 50000, 5000 and 2500 µg/ml.

2.3.1 Screening of aqueous extract of *K. africana* fruits

Screening of the fruits aqueous extract was done as mentioned previously [19]. Cell suspension (2.7 ml) at concentration of 5×10^4 cell/ml was dispersed into flat bottom, polystyrene, tissue culture plate (Falcon, Corning Glass works, New York), in which clean sterile cover slips were placed. A volume of 0.3 ml from each concentration of aqueous extract was separately added to cell suspension to give final concentrations of 10000, 5000, 500 and 250

µg/ml, respectively, in a total volume of 3.0 ml. Deionized water (0.3 ml) was added to each of untreated control wells. The experiment was replicated twice.

Cover slips were used for microscopic description of lymphoblast cells and macroschizonts. Slides for determination of mean number of lymphoblast cells with dead macroschizonts per 50 lymphoblast cells, mean number of alive and dead macroschizonts per cell in 10 untreated control and 10 treated cells, respectively, mean number of lymphoblast cells with extra cellular macroschizonts per field, mean number of dividing cells (binucleated and multinucleated) per field while mean number of viable cells was counted by haemocytometer. Six slides were prepared from each concentration. Partial cytotoxicity was determined by microscopic examination to observe the degenerative changes of the lymphoblast cells and viable cell count. Graphical methodology is illustrated (Fig. 1).

2.4 Statistical Analysis

The results in this study were analyzed using the computer program Statistical Packages for Social Science (SPSS) Version 10. The statistical analysis was done using ANOVA. The data are expressed as mean \pm SD. The results with $P < 0.05$ were considered significant. Lethal dose, 50% (LC_{50}) were calculated from linear regression equation $y = a + b x$, where, x is the log transformation of the concentration.

3. RESULTS

3.1 Activity of Aqueous Extract of *Kigelia africana* Fruits

The activity of aqueous extract of *K. africana* unripe fruits at concentration of 250 µg/ml was 0%, but significantly ($P < 0.05$) changed at concentrations of 500, 5000 and 10000 µg/ml (Table 1). LC_{50} of *K. africana* was 2660.28.

3.2 Effect of Extract on Macroschizonts and Lymphoblast Cells

The activity of *K. africana* aqueous extract resulted in degenerated macroschizonts (Fig. 2), and significantly ($P < 0.05$) decreased mean number of macroschizonts per lymphoblast cell, mean number of dividing cells (binucleated and multinucleated) at concentrations of 500, 5000

and 10000 µg/ml, but significantly (P<0.05) increased mean number of lymphoblast cells with extra cellular macroschizonts at concentrations of 5000 and 10000 µg/ml (Table 2).

The number of infected untreated control lymphoblast cells at 0 h was 5×10⁴ cell/ml, but did not (P>0.05) significantly increase to 6.25×10⁴ cell/ ml after 48 h of incubation. However, the number significantly (P<0.05)

decreased to 4×10⁴ and 3.25×10⁴ cell/ ml at concentrations of 5000 and 10000 µg/ ml, respectively (Table 3).

Partial cytotoxicity of *K. africana* at concentration of 10000 µg/ml resulted in loss of cell aggregates, formation of cells debri. Some lymphoblast cells revealed vacuolated cytoplasm (Fig. 3).

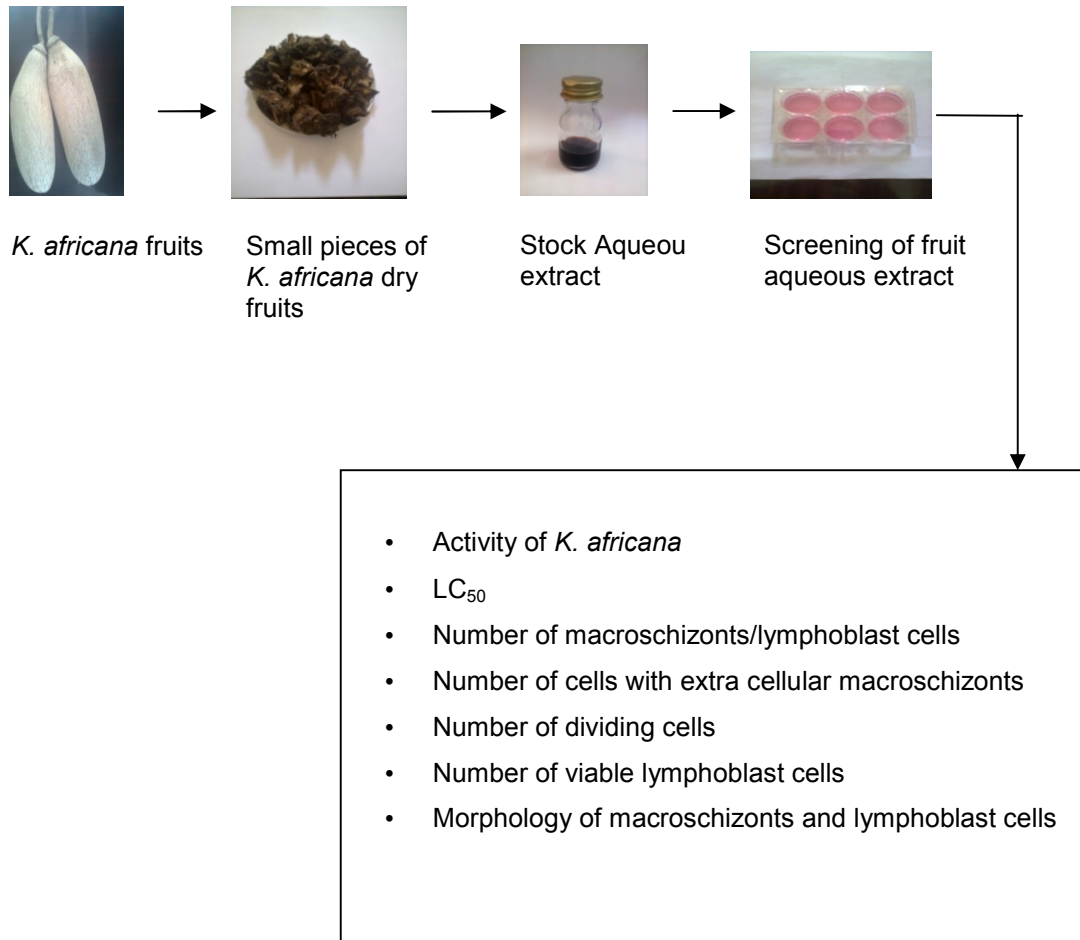


Fig. 1. Graphical methodology

Table 1. Mean *in vitro* activity of aqueous extract of *Kigelia africana* fruits against *Theileria lestoquardi*

Concentration µg/ml	Number of cells with Dead macroschizonts	Total number of cells	Activity (%)
0	0.00±0.00	50.00±0.00	0.00±0.00
250	0.00±0.00	50.00±0.00	0.00±0.00
500	10.00±0.63*	50.00±0.00	20.00±1.26*
5000	29.00±0.63*	50.00±0.00	58.00±1.26*
10000	40.00±0.89*	50.00±0.00	80.00±1.79*

Results are mean ± SD, statistical significance of difference from corresponding control values: *P<0.05

Table 2. Mean effect of aqueous extract of *K. africana* fruits on number of *T. lestoquardi* macroschizonts /cell, cells with extra cellular macroschizonts and number of dividing cells after 48 hours of *in vitro* exposure

Concentration µg/ml	No. of macroschizonts/ Cell	No. of cells with extra cellular macroschizonts	No. of dividing cells	
			Binucleated	Multinucleated
0	21.23±0.14	2.50±0.55	34.00±0.63	17.50±1.05
250	21.23±0.08	2.50±0.55	34.00±0.89	17.50±1.22
500	20.50±0.09	3.17±0.41	22.00±0.89*	13.00±0.89*
5000	19.50±0.23*	5.50±0.84*	10.00±0.89*	6.50±0.55*
10000	18.70±0.14*	8.33±0.82*	6.00±0.63*	3.00±0.63*

Data are expressed as mean ± SD, *P<0.05: Significantly different from control by ANOVA (n=6).



Fig. 2. *T. lestoquardi* lymphoblast cell with degenerated macroschizonts (arrow) after 48 hours of *in vitro* exposure to aqueous extract of *K. africana* unripe fruits at concentration of 5000 µg/ml, Giemsa's stain (x1000)



Fig. 3. *T. lestoquardi* lymphoblast cell with vacuolated cytoplasm (arrows) after 48 hours of *in vitro* exposure to aqueous extract of *K. africana* unripe fruits at concentration of 10000 µg/ml, Giemsa's stain (x1000)

Table 3. Effect of aqueous extract of *K. africana* fruits on number of viable cells after 48 hours of *in vitro* exposure

Concentration µg/ml	No. of viable cells x 10 ⁴ /ml	
	Before 48 h	After 48 h
0	5.00±0.00	6.25±0.35
250	5.00±0.00	6.25±0.35
500	5.00±0.00	5.25±0.35
5000	5.00±0.00	4.00±0.00*
10000	5.00±0.00	3.25±0.35*

The data are expressed as mean ± SD. *P<0.05: Significantly different from control by ANOVA, n=6.

4. DISCUSSION

The results of the present study indicated that *K. africana* aqueous extract had a high level of schizonticidal activity against *T. lestoquardi*. This activity could be due to the actions of chemical constituents of the fruit extract which have antimicrobial effects on *T. lestoquardi* macroschizonts. This result was in agreement with previous studies which showed that fruit extracts of the plant had antimicrobial activity [10,16]. Furthermore, the plant aqueous extract reduced the rate of multiplication of cells which was clearly shown by decrease in number of binucleated cells. This may be due to the effect of the extract on the protein synthesis of macroschizonts. This result is in line with previous study which reported that *Theileria* parasite lives in perfect balance with its host cell, replicating within it and stimulating its multiplication as it is located in the Golgi apparatus [20]. The number of viable cells in infected untreated control did not significantly ($P<0.05$) increase. However, the number of *T. annulata* infected cells per ml increased by 3-folds [21]. This may reflect differences in growth rates due to inherent genetic and/or environmental conditions between the two studies [19]. Increase in number of lymphoblast cells with extra cellular macroschizonts could be due to the effect of the extract on fragility of the cell membrane.

Beside the high level of activity of *K. africana* aqueous extract there was partial cytotoxicity at concentration of 10000 µg/ml. This was evident by the cytopathic changes in the morphology of some of the lymphoblast cells and significant ($P<0.05$) decrease in the number of viable cells. This result showed the effect of toxic compound (s) in the extract, because parasitized

lymphoblast cells multiplied continuously, were repeatedly sub cultured and did not have cytopathic changes attributed to the parasite. The lymphoblast cells have not shown any significant change during passage and in infected untreated control of the experiment. This confirms that partial cytotoxicity is due to the extract and not the parasite. This result is in agreement with other study which showed *in vitro* cytotoxicity through the inhibition of the mammalian cell line growth [22,23]. On the other hand, *in vivo* study indicated that aqueous and methanol extracts of the fruit had been found to possess non-fatal toxicity effects [24]. Additionally, *K. africana* unripe fruits showed hepato and renal toxicity at concentration above 6000 g/ kg body weight without mortality of wistar albino rats (Farah et a, unpublished data).

5. CONCLUSION

Since the aqueous extract of *K. africana* unripe fruits showed high *in vitro* activity against *T. lestoquardi*, fractionation, isolation, structure determination and identification of the active compounds are recommended. *In vivo* studies to confirm these results are, also, recommended. Partial toxicity of the effective extract to the infected cells is an interesting observation that can be extended to antitumor research since *T. lestoquardi* infected cells are tumor-like transformed cells.

CONSENT

Not applicable.

ETHICAL APPROVAL

Not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Dolan TT. Theileriasis: A comprehensive review. Rev Sci Tech off Int Epiz. 1989;8(2):11-38.
2. Latiff AA, Abdulla HM, Hassan SM, Zubeir N, Morzria SP, Osman AM. Theileriosis of sheep in the Sudan. In: Tropical

- theileriosis in the Sudan. Proceedihgs of a Workshop Held at the Sudan Veterinary Association Residence, Khartoum, Sudan. 1994;66-72.
3. Salih DA, El Hussein AM, Hayat M, Taha KM. Survey of *Theileria lestoquardi* antibodies among Sudanese sheep. Vet Parasitol. 2003;111:361-367.
 4. Nagwa ZG. A survey of sheep piroplasmosis in Khartoum Province (Sudan). M Sc Thesis, University of Khartoum, Sudan; 1986.
 5. Tageldin MH, Zakia AM, Nagwa EG, El Sawi SAS. An outbreak of theileriosis in sheep. Sud J Trop Anim Hlth Prod. 1992;24:15-16.
 6. El Husseinl AM, El Ghali AA, Mohammed SA. Efficacy of buparvaquonane in the treatment of malignant theileriosis of sheep in Ed-Damer Province, N state, Sudan: A field trial. Sud J Vet Res. 1993;12:14 -17.
 7. El Ghali A, El Hussein AM. Diseases of livestock in Ed damer Province, El Nil state, Sudan: A two year retrospective study. Sud J Vet Sci Anim Husb. 1995;3437-45.
 8. Ahmed BM. Studies on epizootiology of *Theileria lestoquardi* (*Nomen novem*) in River Nile State. Sudan. M Sc Thesis, Nile Valley University, Sudan; 1999.
 9. Taha KM, Salih DA, Ahmed BM, Enas KA, Ali AM, El Hussein AM. First confirmed report of outbreak of malignant ovine theileriosis amog goats in Sudan. Parasitol Res. 2011;1525-1527.
 10. Latiff AA, Abdulla HM, Hassan SM, Zubeir N, Morzria SP, Osman AM. Theileriosis of sheep in the Sudan. In: Tropical theileriosis in the Sudan. Proceedihgs of a workshop held at the Sudan Veterinary Association Residence, Khartoum, Sudan. 1994;66-72.
 11. Peter O, Magiri E, Auma J, Magoma G, Imbuga M, Murilla G. Evaluation of *in vivo* antitrypanosomal activity of selected medicinal plant extracts. JMPR. 2009;3(11):849-85.
 12. Gabriel OA, Olubunmi A. Comprehensive scientific demystification of *Kigelia africana*. Afr J Pure and Applied Chem. 2009;3(9):158-164.
 13. El Ghazali GEB, Wai'l EA, Awatif, A/ B El Egami, Aisha ZI Al Magboul, Abeer A/ Daim Hamad. *Kigelia africana* (Lam.) Benth in Hook. Aromatic Plants of the Sudan, El Shima Press. 2004;48.
 14. Sangita S, Harmeet K, Bharal V, Ripudaman, Singh SK. *Kigelia africana* (Lam.) Benth. – An overview. Natural Prod Radiance. 2009;8(2):190-197.
 15. Akunyili DN, Houghton PJ, Raman A. Antimicrobial activity of the stem bark of *Kigelia pinnata*. J. Ethnopharm. 1991;35:173-177.
 16. Grace OM, Light ME, Lindsey KL, Mulholland DA, Van Staden J, Jäger AK. Antibacterial activity and isolation of active compounds from fruit of the traditional African medicinal tree *Kigelia africana*. South Afr J Bot. 2002;68:220-222.
 17. FAO. Gradient method with ficoll-paque. Tick and Tick-borne Diseases Control. Food and Agriculture Organization of the United Nations, Rome. 1984;2:523-525.
 18. Jura WGZO, Brown CGD, Kelly B. Fine structure and invasive behavior of the early developmental stages of *Theileria annulata in vitro*. Vet Parasitol. 1983;12:121-122.
 19. Farah HM, El Amin TH, Khalid HS, Hassan SM, El Hussein AM. *In vitro* activity of the aqueous extract of *Gardenia ternifolia* fruits against *Theileria lestoquardi*. J Med Plants Res. 2012;6(41):5447-545. DOI: 10.5897/JMPR 12.621.
 20. Peter O, Magiri E, Auma J, Magoma G, Imbuga M, Murilla G. Evaluation of *in vivo* antitrypanosomal activity of selected medicinal plant extracts. JMPR. 2009;3(11):849-85.
 21. MCHardy N. *In vitro* studies on action of menoctone and other compounds on *Theileria annulata*. Ann Trop Med Parasitol. 1978;72(2):501-511.
 22. Khan M, Mlungwana S. Short report: Sitosterol, a Cytotoxic sterol from *Markhamia zanzibarica* and *Kigelia africana*. Fitoterapia. 1999;70:96-97. DOI: 10.1016/S0367-326x(99)00005-2.
 23. Houghton PJ. The sausage tree (*Kigelia pinnata*): Ethno botany and recent scientific work. South Africa J Botany. 2002;68:14-20.
Available:
<http://dx.doi.org/10.4314%2Fsaib.v68i.1.2.0488>.

24. Shama IY, Marwa IA. *Kigelia africana* fruits' extracts anti-hepatotoxicity effect on male wistar rats liver destruction induced by CCl₄. Asian J Med Sci. 2013;5:26-32.

© 2015 Farah et al.; This is an Open Access article distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/4.0>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Peer-review history:

The peer review history for this paper can be accessed here:

<http://www.sciencedomain.org/review-history.php?iid=648&id=13&aid=6190>