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Evaluation of antiviral and cytotoxic potential of ethanolic extract of *Acacia nilotica* against *peste des petits ruminants* virus *in vitro* cell culture

R RAHEEL[1], M ASHRAF[2], A JAVEED[3], R BASHIR[4], N MUNIR[5] and I ALTAF[6]

University of Veterinary and Animal Sciences, Lahore 54000, Pakistan

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Acacia nilotica is an important medicinal plant (Rehman *et al.* 2011). Peste des petits ruminants virus (PPRV) is a negative sense RNA virus that causes *peste des petits ruminants* (PPR) disease. MTT [3–(4,5-dimethylthiazol–2-yl)-2,diphenyltetrazolium bromide] colorimetric assay is a commonly used *in vitro* cell culture technique to study cytotoxic effects of test substances on different cell lines in terms of cell viability. Researchers should explore new antiviral agents to eradicate this notifiable virus (Hussain *et al.* 2008, Abubakar *et al.* 2011). The present study was designed to evaluate cytotoxicity and antiviral activity of ethanolic extract of leaves, pods and bark of *Acacia nilotica* against PPRV on vero cell line by using MTT assay.

Leaves, pods and bark of *Acacia nilotica* (*desi kikiar*) were collected and correctly identified from the Department of Botany, Government College University, Lahore, Pakistan. Ethanolic extracts of each shade dried powdered part of the plant were obtained by Soxhlet apparatus. The extracts were dried by a rotary evaporator until semisolid extracts were obtained. Semisolid extracts were dried completely in a safety cabinet in petridishes. Two fold serial dilutions of 200, 100, 50, 25, 12.5, 6.25, 3.125 and 1.56 µg/ml were made for each dried extract in cell culture medium (M199).

Vero cell line and characterized PPRV were obtained from WTO Quality Operation Laboratory (QOL), University of Veterinary and Animal Sciences, Lahore, Pakistan. Tissue culture infective dose ($TCID_{50}$) of the virus was determined by Reed and Muench method (Maanen and Terpstra 1989). The Vero cell line was propagated in 96 well plate by adapting method as reviewed by Freshney (2011). Cytotoxicity was performed by MTT assay described by Twentyman and Luscombe (1987). Cell survival percentage (CSP) was calculated by following formula as given:

$$CSP = \frac{\text{Mean OD of test - Mean OD of negative control}}{\text{Mean OD of positive control}}$$

The results were analyzed by using analysis of variance (ANOVA) and Post Hoc test.

The results of cytotoxic and antiviral activity of each part of the plant are represented in Table 1. Both the leaves and pods of the plant were cytotoxic at the concentration range of 50 to 200 µg/ml as at this range CSP was below 50%. However, the bark was cytotoxic only at 200 µg/ml. The results showed dose dependant toxicity. Ethanolic extract of both the leaves and bark were showing

Table 1. Antiviral and cytotoxic activity of ethanolic extract of *Acacia nilotica*

Activity studied	Conc. used (µg/ml)	Mean cell survival percentage± standard deviation		
		Leaves (n=3)	Pods (n=3)	Bark (n=3)
Antiviral activity	1.56	64±4.00^{c}	59±3.51^{c}	30±0.57^{a,b}
	3.125	66±4.04^{c}	61±1.52^{c}	32±2.51^{a,b}
	6.25	63±3.05^{c}	62±0.57^{c}	33±1.15^{a,b}
	12.5	61±3.51^{c}	64±4.50^{c}	35±1.52^{a,b}
	25	59±2.51^{c}	59±4.50^{c}	38±2.30^{a,b}
	50	45±6.50	45±1.52	51±1.52
	100	38±1.52^{b,c}	28±2.51^{a,c}	54±3.51^{a,b}
	200	2±0.57^{b,c}	23±5.50^{a,c}	45±3.21^{a,b}
Cytotoxic activity	1.56	71±1.53^{c}	73±4.61	78±4.50^{a}
	3.125	69±2.52^{c}	70±3.51	75±2.52^{a}
	6.25	66±2.52^{c}	68±2.64	72±3.51^{a}
	12.5	64±3.51	65±1.15	70±1.53
	25	60±2.51^{c}	61±1.53^{c}	69±3.51^{a,b}
	50	46±1.53^{c}	49±0.57^{c}	65±1.53^{a,b}
	100	41±1.52^{c}	30±3.51^{c}	63±3.79^{a,b}
	200	5±4.33^{b,c}	25±1.15^{a,c}	48±2.08^{a,b}

a, signification variation from leaves at the 0.05 level; b, significant variation from pods at the 0.05 level; c, significant variation from barkat the 0.05 level.

Present address: [1,2,3](rizwana_raheel@yahoo.com, muhammad.ashraf @uvas.edu.pk, aqeel.javeed@uvas.edu.pk), Department of Pharmacology and Toxicology. [6](imran.altaf@uvas.edu.pk), Microbiology Department, WTO- QOL (Quality Operations Lab), University of Veterinary and Animal Sciences. [4,5](rasheedasbs@yahoo.com, neelma.munir@yahoo.com), Department of Biotechnology, Lahore College for Women University, Lahore, Pakistan.

antiviral activity at the concentration range of 1.56 to 25 µg/ml as at this range CSP was above 50%. The antiviral concentrations of the bark were 50 and 100 µg/ml. The antiviral activity was in the order of leaves > pods > bark. Pakistan has a rich heritage of medicinal plants (Hussain *et al.* 2008). Viral diseases especially trans-boundary animal diseases including PPR are causing an enormous economic loss to livestock sector in Pakistan (Hussain *et al.* 2008). It is mandatory to search new antiviral agents against PPRV due to high mortality rate and less effective treatment (Kitazato *et al.* 2007). PPRV is an RNA enveloped virus. The F (Fusion) glycoprotein of the virus facilitates it to pass through cell membranes of the host and enter into its cytoplasm.

The cytotoxicity profile studied in this research is in accordance with Kalaivani *et al.* (2011) who studied the cytotxic potential of different extracts of *Acacia nilotica* leaves and reported cytotoxicity of the extracts was in a dose dependent manner. Kalaivani (2011) reported cytotoxic potential of gallic acid and other polyphenols at higher concentrations which might be due to their pro-oxidant effect. Similarly in the present study, these might be polyphenols due to which the extracts exerted their cytotoxic effect at higher concentrations. Literature has reported antiviral activity of *Acacia nilotica* leaves against Hepatitis C virus (Rehman *et al.* 2011). A plant may produce its activity against the virus due to synergistic action of different antiviral agents present in it. In the present study, it might be these substances which, due to their synergistic action, exerted their antiviral effect.

The present study demonstrated that leaves, pods and bark of *Acacia nilotica* might contain substances that are effective against PPRV. However, it is required to isolate and characterize the active compounds in the plant and to investigate the mechanism by which these substances might exert their antiviral effect.

SUMMARY

The present study was designed to determine the cytotoxic and antiviral potential of ethanolic extract of *Acacia nilotica* leaves, pods and bark against PPRV by using MTT [3–(4,5–dimethylthiazol–2-yl)–2,5-diphenyltetrazolium bromide] colorimetric assay. Antiviral property of ethanolic extract of leaves was higher as compared to pods and bark at its non-cytotoxic concentrations. This plant might be a good source to isolate effective antiviral agents against PPRV.

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